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The Acquisition of Dangerous Biological Materials: Technical Facts Sheets to Assist Risk Assessments of 46 Potential BW Agents

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International Biological Threat Reduction

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Abstract

Numerous terrorist organizations have openly expressed interest in producing and deploying biological weapons. However, a limiting factor for many terrorists has been the acquisition of dangerous biological agents, as evidenced by the very few successful instances of biological weapons use compared to the number of documented hoaxes. Biological agents vary greatly in their ability to cause loss of life and economic damage. Some agents, if released properly, can kill many people and cause an extensive number of secondary infections; other agents will sicken only a small number of people for a short period of time. Consequently, several biological agents can potentially be used to perpetrate a bioterrorism attack but few are likely capable of causing a high consequence event. It is crucial, from a US national security perspective, to more deeply understand the likelihood that terrorist organizations can acquire the range of these agents.

Few studies have attempted to comprehensively compile the technical information directly relevant to the acquisition of dangerous bacteria, viruses and toxins. In this report, technical fact sheets were assembled for 46 potentially dangerous biological agents. Much of the information was taken from various research sources which could ultimately and significantly expedite and improve bioterrorism threat assessments. By systematically examining a number of specific agent characteristics included in these fact sheets, it may be possible to detect, target, and implement measures to thwart future terrorist acquisition attempts. In addition, the information in these fact sheets may be used as a tool to help laboratories gain a rudimentary understanding of how attractive a method laboratory theft is relative to other potential acquisition modes.

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Acronyms

ABSL	Animal Biosafety Level
AIDS	Acquired immune deficiency syndrome
APMV-1	Avian paramyxovirus-1
BSE	Bovine Spongiform Encephalopathy
BSA	Bovine serum albumin
BSL	Biosafety Level
BW	Biological Weapon
CAMs	Chorioallantoic membranes
CCHF	Crimean-Congo hemorrhagic fever
CDC	Centers for Disease Control and Prevention
CNS	Central nervous system
CSF	Cerebrospinal fluid
CSFV	Classical Swine Fever virus
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DRC	Democratic Republic of the Congo
DMEM	Dulbecco/Vogt minimal essential medium
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EEEV	Eastern Equine encephalitis virus
ELISA	Enzyme-Linked ImmunoSorbent Assay
FMD	Foot and mouth disease
FMDV	Foot and mouth disease virus
GG	Genogroups
GI	Gastrointestinal
H5N1	Influenza A virus subtype H5N1
HA	Hemagglutination
HEPA	High efficiency particulate air filter
HIV	Human immunodeficiency virus
HPAI	Highly pathogenic avian influenza virus
HUS	Hemolytic-uremic syndrome
ID50	Infectious Dose to 50 Percent of Exposed Individuals
MDR	Multidrug resistant
MEM	Minimal Essential Medium
NDV	Newcastle Disease virus
OIE	World Organization for Animal Health
PAPR	Positive air pressure respirator
PBS	Phosphate-buffered saline
PCN ^R	Penicillin-resistant
PCR	Polymerase chain reaction
PPE	Personal protective equipment
PrP	Protease resistant protein
PSP	Parasitic shellfish poisoning
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid

RT-PCR	Reverse transcription polymerase chain reaction
RVF	Rift Valley Fever
SAN	Specific antibody negative
SARS	Severe acute respiratory syndrome
SARS-CoV	Severe acute respiratory syndrome coronavirus
Sd1	<i>Shigella dysenteriae</i> 1
SDS	Sodium dodecyl sulfate
SEB	Enterotoxin B-producing strains
SMAC	Sorbitol-MacConkey agar
SPF	Specific pathogen free
TB	Tuberculosis
TCBS	Thiosulfate-citrate-bile salts-sucrose agar
TSS	Toxic shock syndrome
TSST-1	Toxic shock syndrome toxin
US	United States
USAMRIID	US Army Medical Research Institute of Infectious Diseases
UTI	Urinary tract infection
vCJD	Variant Creutzfeldt-Jakob disease
WHO	World Health Organization
XDR	Extensively drug resistant

Agent: Abrus precatorius plant (source of abrin toxin)

Environmental Factors

1) Geographic Distribution:

Abrus precatorius plants (often called rosary or jequirity pea plants) grow as invasive, slender, hardy tropical vines that climb on trees and bushes. The vines can grow 50 feet or more, but are dormant during winter months. The plant typically grows in tropical and subtropical regions all over the world. It is indigenous to Indonesia and particularly prevalent in tropical parts of Southeast and South Asia, in India, Sri Lanka, Thailand, and the Philippines. In addition, the plant is present in parts of southern China and Africa. In the United States and its protectorates, *A. precatorius* is now very common in Florida, Hawaii, Alabama, Georgia, Arkansas, Puerto Rico, and the Virgin Islands. The plant produces flowers that are small, pale, and violet or pink in color. The seeds develop within pea-shaped pods that split when they mature. The peas are bright scarlet with characteristic black tips that are very easy to identify which are often used to produce jewelry and beads. Powdered abrin toxin is yellowish-white.

2) Disease Symptoms (human and animal):

The symptoms of abrin poisoning are identical to those of ricin poisoning. Initial symptoms after ingestion include excessive salivation, burning of the mouth, vomiting, dehydration, and diarrhea (may become bloody). Fever may also develop, as well as low blood pressure. In severe cases, hallucinations and seizures may develop, followed by death. Within a few hours of inhaling abrin, symptoms often include respiratory distress, fever, cough, nausea, and tightness in the chest. Heavy sweating may follow. Fluid may accumulate in lungs, making breathing difficult. The skin might turn blue. Finally, low blood pressure and respiratory failure may occur, leading to death. Skin and eye exposure to abrin mist or powder results in redness and pain.

3) Strain Information:

Numerous plant varieties, but little information on the relative seed toxicity of plant strains.

4) Reservoirs/Vectors:

N/A

5) Agent Sources:

Only the seeds of rosary pea plants contain toxin. Plants typically produce large numbers of seeds.

6) Stability:

Plants are hardy, invasive and resistant to weather fluctuation. Toxin is very stable and soluble in water; can survive for extended periods of time in the environment, even in very hot or cold conditions.

Laboratory Factors

1) Security Classification:

US Select Agent [laboratories with over 100 mg of toxin must abide by regulatory requirements (registration, etc.)]. Plant material is unregulated.

2) General Research Volume:

Relatively low volume of laboratory work (PubMed).

3) Survey Information:

Only 6/722 (0.83%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain this toxin.

4) Past Weapons Activity:

There are no known past attempts by state BW programs to weaponize abrin toxin.

5) Commercial Sources:

The seeds of numerous plant varieties are available for purchase online from a number of commercial sources. From one source, 36+ seeds (labeled as poisonous) can be purchased for US\$3.00 and 1,000 seeds can be purchased for US\$27.00. Another source offers ten seeds for US\$4.00, 100 seeds for US\$28.50, and 1,000 seeds for US\$230.00. Toxin can also be purchased in small amounts within the United States, but is subject to the confines of the Select Agent Rule.

6) General Growth Conditions:

There is a significant amount of information available online for growing plants. *Abrus precatorius* plants are hardy and can grow well within a range of conditions; under the right conditions the plant can grow very quickly. Plants are best grown in full sun (or partial shade) in moist but well-drained soil. Methods for isolating and purifying toxin from seeds are widely available in the open literature, however many of the popular recipes (e.g. Poisoner's Handbook; Al-Qaeda's manual) are inadequate, and past attempts to isolate ricin (which requires a very similar extraction process) have largely all been unsuccessful.

Safety Factors

1) Risk of Disease Contraction:

Accidental exposure to abrin is unlikely; abrin seeds are toxic only if the hard outer shell is chewed, releasing the toxins before swallowing. No other part of the plant is poisonous. Abrin may be inhaled if the toxin is in the form of a mist or a powder. Skin exposure occurs through direct contact with abrin particles or droplets. Abrin may also be ingested via contaminated food or water. Very small amounts of toxin can kill a person if ingested or inhaled. Depending on the route of exposure and dose received, death by abrin poisoning could occur within 36 to 72 hours of exposure. The fatal dose of abrin toxin is approximately 75 times lower than that of ricin.

2) Countermeasures (PPE and Medical):

BSL2 laboratory containment and practices should be used for any work with purified toxin or with potentially contaminated clinical, diagnostic, or postmortem samples (abrin may retain toxicity in lesion fluids, secretions, and tissues). PPE such as laboratory coats, gloves, and protective masks (respirator if aerosols are produced) should be used. A biosafety cabinet or chemical fume hood (with an exhaust HEPA/charcoal filter) should be used for activities that may generate aerosols. If abrin exposure is suspected (there is no reliable test to confirm abrin poisoning), remove clothing, wash site of exposure, and dispose of clothes. It is critical to get abrin off or out of the body as quickly as possible. No antidote currently exists, so abrin poisoning must be treated with supportive medical care. The type of care depends on various factors including the route of exposure. Care often includes giving patients intravenous fluids, medications to treat low blood pressure and seizure, and/or flushing the stomach.

Agent: African swine fever virus

Environmental Factors

1) Geographic Distribution:

African swine fever is a disease of wild and domestic pigs. The highest disease incidence is reported in the equatorial region of Africa to the northeastern edge of South Africa (the Transvaal). The virus is prevalent in most countries of sub-Saharan Africa; epizootics are reported often in the eastern and southern regions of Africa in the countries of Madagascar, the Democratic Republic of the Congo, Kenya, Zambia, Tanzania, South Africa, Mozambique, and Senegal. In addition, severe epidemics have been reported historically in Brazil (1978-1981), Haiti (1978-1984), Malta, and the Dominican Republic as well as in parts of Europe (Iberian Peninsula, Belgium, Holland, France, and Italy). However, the disease is believed to be eradicated from most of South America and the Caribbean, and with the exception of Africa, is probably endemic only in feral pigs in Sardinia, Italy.

2) Disease Symptoms (human and animal):

A range of syndromes may characterize African swine fever, depending on the form of disease. Clinical symptoms and post-mortem signs are very similar to classical swine fever in domestic animals. Infections with very virulent strains result in acute disease. Symptoms include: high fever, appetite loss, occasional vomiting and diarrhea (sometimes bloody), and hemorrhages in skin and internal organs. Most animals that contract the disease die (mortality rate is close to 100%); some survivors never develop symptoms but carry the virus for life. Less virulent strains cause milder disease: slight fever, reduced appetite, and lethargy. Infection in warthogs and bushpigs is difficult to detect as the symptoms are subclinical. The virus does not infect humans.

3) Strain Information:

Isolates can vary greatly in virulence and in propensity to cause serious symptoms. Highly pathogenic strains cause ~100% mortality; some strains may cause infections that are not apparent or difficult to diagnose (seroconversion only).

4) Reservoirs/Vectors:

The virus can infect all known porcine species, although African swine fever is persistent in African warthogs and bushpigs which are considered the natural reservoirs. Soft ticks (*Ornithodoros*) may act as vectors.

5) Agent Sources:

The virus is present in all tissues and body fluids of sick and dead animals; however the blood, when collected in the early febrile stage, contains particularly high levels of virus. Ticks may also be a potential virus source.

6) Stability:

The virus is resistant to environmental conditions and is particularly stable within protein-rich environments: virus can survive and remain infectious for 15 weeks in putrefied blood, 70 days in blood on wooden boards, 11 days at room temp feces (three hours at 50 °C), 18 months in pig blood at 4 °C, 150 days in meat, 140 days in salted, dried hams, several years in frozen carcasses, and at least a month in contaminated pig pens. The virus can be inactivated at a pH of <3.9 or >11.5, or by ether, chloroform, sodium hydroxide, and formalin.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Moderate amount of laboratory research (PubMed)

3) Survey Information:

No survey information

4) Past Weapons Activity:

The Soviet Union conducted BW research on African swine fever virus.

5) Commercial Sources:

Little information available

6) General Growth Conditions:

The virus can be isolated from blood (in an anticoagulant such as EDTA or heparin), spleen, tonsil, kidney, and lymph nodes. It is also detectable in many other tissues. Tissue suspensions are normally prepared by homogenization in buffered salt solution or tissue culture medium (with antibiotics); suspensions can then be clarified by centrifugation and used to directly inoculate pigs or tissue culture cells. The virus is often grown in primary cell cultures of pig leukocyte or bone marrow cells; porcine alveolar macrophages and blood monocyte cultures can also be used. PCR methods have been developed for detecting the virus in various tissues. PCR testing on tonsil scraping samples can detect the disease a few days before the onset of symptoms.

Safety Factors

1) Risk of Disease Contraction:

Humans are not susceptible to infection with the African swine fever virus. The disease is highly contagious in swine, both by direct contact between animals and indirect contact with infectious materials. Infection routes may include direct contact between swine leading to exposure of mucus membranes, eyes, and skin abrasions with infected fluids or via the bites of infected soft ticks. The most common route however, is ingestion of contaminated meat or other infectious material.

2) Countermeasures (PPE and Medical):

In the United States (and other countries where the virus is not present) laboratory work is carefully restricted to a minimal number of specialized, highly secure facilities. In endemic areas, BSL3 facilities are recommended for any laboratory work with the virus; ABSL3 facilities for any work with infected animals. There is no effective treatment (drugs, vaccines, etc.) for swine; current control programs include rapid detection/diagnosis followed by culling.

Agent: *Amanita* genus mushrooms (sources of *alpha amanitin* toxin)

Environmental Factors

1) Geographic Distribution:

A number of species within the *Amanita* genus of mushrooms produce alpha amanitin toxin; *Amanita phalloides* (commonly known as the Death Cap mushroom) is the most well-known toxin-producing species. Death Caps are pale and whitish in color with a slight metallic green-yellow tint. They also exhibit a ring around the stem and emit a sweet aroma. The mushroom is similar in appearance to some edible species; it may be responsible for as many as 90% of mushroom poisoning deaths worldwide. Death Caps are native to Europe specifically from the southern coastal regions of Scandinavia in the north, to Ireland in the west, east to Poland and western Russia, and south through the Balkans into Italy, Spain, and Morocco and Algeria in northern Africa. A similar type of mushroom has also been reported further east into Asia but this has yet to be confirmed as *A. phalloides*. This mushroom species is also present within the United States (it is the most common cause of domestic mushroom poisoning) although its distribution is not well defined. These mushrooms appear to grow well in the cool coastal regions of the West Coast, particularly in the Pacific Northwest, also on the East Coast, especially in the Blue Ridge Mountains. *A. phalloides* appears to be spreading rapidly in California—Point Reyes National Seashore is considered a “hotspot.” The Destroying Angel mushroom (*Amanita bisporigera*) also produces significant amounts of toxin and has a wide growth range.

2) Disease Symptoms (human and animal):

The first symptoms of toxin ingestion are painful abdominal cramps and watery diarrhea; poisoning may rapidly lead to dehydration. These symptoms usually regress within a few days, then typically on the 4th to 5th day the toxin starts to have severe effects on the liver and kidneys, leading to total system failure in both. Jaundice is often apparent, and poisoning may lead to seizures and coma. Death may occur as soon as one week after ingestion.

3) Strain Information:

There are several alpha amanitin producing species in the *Amanita* genus of mushrooms. The Death Cap and Destroying Angel mushrooms are well-known species that produce significant amounts of toxin.

4) Reservoirs/Vectors:

N/A

5) Agent Sources:

Mushrooms of the *Amanita* genus produce alpha amanitin toxin; the *Galerina autumnalis* and *Conocybe filaris* mushrooms also produce the toxin. A gram of Death Cap mushroom yields approximately 0.2-0.4 mg of toxin; less than 0.1mg/kg can kill a person.

6) Stability:

The alpha amanitin toxin is very stable; it is normally not destroyed or deactivated by cooking, canning, freezing, drying, or other means of food preparation.

Laboratory Factors

1) Security Classification:

Not a Select Agent

2) General Research Volume:

Moderate volume of research (PubMed); the toxin is sometimes used as a reagent to study transcription.

3) Survey Information:

No survey information, but a small number of research laboratories appear to study the toxic effects of alpha amanitin.

4) Past Weapons Activity:

No known research by state BW programs

5) Commercial Sources:

Alpha amanitin is sometimes used as reagent in the study of transcription; the toxin may be purchased in small amounts of pure powder from commercial sources such as Sigma-Aldrich and Roche. In addition, a number of mycological repositories appear to store *Amanita* species—the Mushroom Research Institute of Japan (Kiryu, Japan) is an example of a laboratory/repository that contains alpha amanitin-producing species.

6) General Growth Conditions:

Amanita mushrooms grow best in cool, moist environments. The fruiting period of these species is typically in late summer to autumn, with mushrooms appearing after summer and autumn rainfall.

Safety Factors

1) Risk of Disease Contraction:

Most alpha amanitin poisoning occurs when *Amanita* species, particularly *A. phalloides*, are mistaken for edible species and eaten. Approximately 0.1 mg/kg of toxin is enough to kill an adult.

2) Countermeasures (PPE and Medical):

BSL2 laboratory containment and practices should be used in handling toxic material. A biosafety cabinet or a chemical fume hood (with exhaust HEPA/charcoal filters) should be used for any aerosol-generating activities (manipulation of powder samples, large quantities, etc.). PPE such as disposable gloves should be worn when handling poisonous material; protective masks and goggles should also be considered when handling powdered toxin. If poisoning occurs, treatment is mainly supportive; drinking large amounts of liquid to quickly purge the toxin from the body may partially alleviate some of the symptoms. The most reliable method of treatment is stomach pumping immediately following ingestion; however, this treatment is generally ineffective at the onset of symptoms.

Agent: *Bacillus anthracis*

Environmental Factors

1) Geographic Distribution:

Anthrax is endemic in many countries throughout the world. Outbreaks occur only sporadically in most places, but several countries have high rates of disease. The *World Anthrax Data Site* categorizes 14 countries as being “hyperendemic” for anthrax, these are: Turkey, Tajikistan, Myanmar, Niger, Chad, Ethiopia, Zambia, Zimbabwe, Togo, Ghana, Cote d’Ivoire, Liberia, Sierra Leone, and Guinea – all but three are in Africa. A large number of countries in Africa and Asia are considered “endemic”; very few countries are considered anthrax-free. The United States has sporadic cases of disease in animals and very rarely do humans contract the disease naturally.

2) Disease Symptoms (human and animal):

Clinical manifestations vary greatly and typically depend on the route of infection (cutaneous, gastrointestinal, and inhalation). Cutaneous symptoms include the formation of a red macule on the skin that becomes papular and vesiculated, then ulcerates and develops into a blackened, depressed eschar. The surrounding lymph glands may swell. Little pain is normally associated with the lesion. The case fatality rate is approximately 5-20%, if untreated. GI symptoms include abdominal pain, vomiting, bloody diarrhea, fever, and septicemia. In these cases, the fatality rate ranges from 25-60%, if untreated. The initial symptoms of inhalational anthrax infection often resemble the common cold or other respiratory diseases, but inhalation eventually results in massive bacteremia and secondary pneumonia in many cases. The respiratory distress quickly worsens, fever develops, and shock may occur. Inhalational anthrax is usually fatal. In humans, ~95% of all cases are cutaneous, ~5% are inhalation, <1% are gastrointestinal. The disease is usually fatal in livestock such as cattle, sheep, goats, and horses. *B. anthracis* infections spread so rapidly that it can be difficult to diagnose in live animals as sudden death frequently occurs. Animal disease symptoms include high temperatures, bloody discharge, and swelling in the neck and shoulder areas. Swine and dogs are more resistant to the acute disease but develop extensive swelling in the neck area.

3) Strain Information:

Strains found in the environment are vastly different in pathogenicity and virulence; only a small percentage are very dangerous to people. Several dozen genetically distinct strains of *B. anthracis* have been described. Plasmid composition adds to the genetic variety significantly. Bacilli can carry numerous copies of two different plasmids—pX01 and pX02. It appears that strains may carry up to 243 copies of pX01 and up to 32 copies of pX02. Strains with more copies of pX02 often develop severe disease. Strains lacking the pX02 plasmid, such as the Sterne strain, are avirulent. However, the genetic content of the bacteria’s chromosome also dictates virulence – e.g., the Ames strain, which possess only two pX02 plasmids, is extremely dangerous.

4) Reservoirs/Vectors:

Herbivorous animals, including cattle, sheep, goats, and pigs, are the natural reservoirs. Other mammals, birds, and humans also contract the disease.

5) Agent Sources:

Spores may be isolated from spore-contaminated soil, as well as the blood and tissues of gravely ill or recently deceased animals, and/or contaminated meat or animal products (hides, etc). The carcasses of animals that recently died of anthrax are highly infectious and excellent sources of spores. In humans, bacilli may be isolated from vesicular fluid swabs in cutaneous cases, stool and blood in GI cases, sputum and blood in inhalation cases.

6) Stability:

Bacillus anthracis spores are extremely resistant to environmental duress including drying, heat, and sunlight. They are also resistant to numerous disinfectants (2% glutaraldehyde, formaldehyde, and 5%

formalin). Spores may remain viable in soil, animal skins, hides, and wool for decades. One estimate suggests that spores will survive for 40 years or more in dry soil. The spores can survive in milk for ten years, filter paper for 41 years, silk threads for 71 years, and in pond water for two years.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Relatively large volume of research (PubMed)

3) Survey Information:

51/722 (7.06%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *B. anthracis*.

4) Past Weapons Activity:

A number of countries, including Canada, Germany, Iraq, Japan, the Soviet Union, the United Kingdom, and the United States had weaponized anthrax. France and South Africa had also conducted BW research. Iran, Syria, and North Korea are currently suspected of having active BW programs and may have also weaponized the agent.

5) Commercial Sources:

Little information available

6) General Growth Conditions:

Fresh blood (collected during the acute stages of disease to within a few days of death and prior to antibiotic treatment), sputum, and tissue samples taken from sick or recently deceased animals (or humans) may be cultured. If GI anthrax is suspected, rectal or stool samples may yield bacteria. Isolation of bacilli from soil, old carcasses, or processed specimens such as hides is more complicated. The soil surrounding decaying animal carcasses may also harbor spores. *B. anthracis* grows readily on many types of routinely used media at 35-37 °C. From blood, sputum, and vesicular fluid swabs, the bacterium will grow well on sheep blood agar plates, MacConkey agar, and chocolate agar. In approximately 24 hours, 2-5 mm nonhemolytic, grey-white colonies that are flat with circular/irregular edges will appear, often exhibiting comma-shaped projections ("Medusa head" appearance). Simple stains, microscopy, and PCR can be used to identify *B. anthracis*. PCR can be used (or small animals can be inoculated) to detect whether a strain has the virulence plasmids.

Safety Factors

1) Risk of Disease Contraction:

Humans can contract cutaneous anthrax when spores enter the body through broken skin. Gastrointestinal anthrax occurs by ingesting spores, often in contaminated, undercooked meat. Inhalation anthrax develops when spores of less than 5 µm in diameter become lodged in the lungs. Human-to-human transmission is extremely rare. The infectious dose is estimated to be between 8,000 and 50,000 bacilli via inhalation.

2) Countermeasures (PPE and Medical):

B. anthracis should be isolated and identified using BSL2 containment and practices; biological safety cabinets should be used to manipulate any cultures. BSL3 facilities should be used for any work with spores and larger quantities of the agent. PPE should include a mask (or respirator) capable of filtering out spores, gloves, a laboratory coat, and face shields or goggles. In the event of infection, antibiotics must be administered early to be effective. Penicillin is generally the drug of choice; erythromycin, ciprofloxacin, doxycycline, and vancomycin are also effective. Many countries immunize herbivorous animals with live attenuated vaccines. Human vaccines are much less common, and may be administered to those in high risk occupations.

Agent: MDR *Bacillus anthracis*

Environmental Factors

1) Geographic Distribution:

Anthrax occurs globally and is treated frequently with β -lactams, particularly penicillin. As a result, naturally occurring penicillin-resistant (PCN^R) strains have been isolated periodically, although the vast majority of isolates are susceptible. Naturally occurring PCN^R *B. anthracis* strains have been documented since approximately 1972; an isolate from a deceased dairy cow in Japan was among the first PCN^R resistant strains found. However, it is very rare for a strain isolated from the environment to exhibit resistance to multiple antibiotics.

2) Disease Symptoms (human and animal):

Clinical signs are identical to those of nonresistant strains of *B. anthracis*.

3) Strain Information:

MDR *B. anthracis* strains are very rare in nature; most drug-resistant strains have been constructed—or selected for—in laboratories. However, only a handful of MDR strains are known to have been constructed. Antibiotic resistant *B. anthracis* is generated *in vitro* by multiple passages, or sequential selection, on increasing concentrations of antibiotics to select for mutations that permit growth on antibiotic-containing medium.

4) Reservoirs/Vectors:

Presumably, herbivorous animals, including cattle, sheep, goats, and pigs, are the natural reservoirs. Other mammals, birds, and humans also contract the disease. Currently, the only known macrolide-resistant, ciprofloxacin-resistant, doxycycline-resistant or other antibiotic resistant strains of *B. anthracis* are contained within a handful of laboratories.

5) Agent Sources:

Presumably the same as wild type *B. anthracis*, although naturally occurring multidrug resistant (macrolide-resistant, ciprofloxacin-resistant, doxycycline-resistant or other antibiotic resistance) strains of *B. anthracis* are extremely rare in nature.

6) Stability:

Wild type and MDR strains of *B. anthracis* would likely have similar stability attributes.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Only a handful of laboratories, principally in developed countries, are known to be generating antibiotic resistant strains.

3) Survey Information:

No survey information available

4) Past Weapons Activity:

Though many countries have performed research or attempted to weaponize *B. anthracis*, it is unclear if similar attempts have been made with antibiotic resistant *B. anthracis* strains. Evidence suggests that a

research facility in Oblensk, Russia generated a vaccine strain (STI-1) resistant to several antibiotics; some have suggested that this research was for BW purposes.

5) Commercial Sources:

Little information is available but few commercial sources are expected. The few laboratories that have generated MDR strains are certain to retain stocks. Furthermore, large, established culture collections may have PCN^R resistant strains.

6) General Growth Conditions:

In most cases, the growth protocols and conditions used for MDR strains would likely be similar to those used for nonresistant strains, with an important exception: antibiotic resistance needs to be continually selected for, with the addition of appropriate antibiotics to the media. Consequently, MDR strains may grow at a slower rate than nonresistant strains.

Safety Factors

1) Risk of Disease Contraction:

The risk of infection would be expected to be very similar (if not identical) to that of nonresistant strains; individuals may contract the cutaneous, gastrointestinal, and pulmonary forms of anthrax. People are very unlikely to contract MDR *B. anthracis* from the environment, as all known strains are present in laboratories. Laboratory personnel could be at risk of accidentally contracting the disease while handling the agent.

2) Countermeasures (PPE and medical):

The countermeasures would be similar to those used for naturally occurring *B. anthracis*. Cultures of MDR strains should be handled in BSL3 facilities (or above); the same PPE should be used. MDR *B. anthracis* is much more difficult to treat with antibiotics, particularly if the causative strain is resistant to the most effective or commonly available antibiotics. The efficacy of vaccines should be unaffected by drug resistant anthrax.

Agent: Bovine Spongiform Encephalopathy prion

Environmental Factors

1) Geographic Distribution:

Bovine spongiform encephalopathy (BSE) was first recognized in England in 1986, but cases have since been detected in indigenous cattle populations throughout most of Europe including Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Ireland, Israel, Italy, Japan, Liechtenstein, Luxembourg, the Netherlands, Poland, Portugal, Slovakia, Slovenia, Spain, and Switzerland. More recently, the disease has also been detected in Asia and North America although there are very few cases. England has accounted for >90% of cases worldwide; as of January 2004, 180,000 cases have been confirmed from more than 35,000 different herds. However, the incidence has gone down dramatically due to prevention measures. In some countries, BSE has been linked to the import of infected cattle or feed containing contaminated meat-and-bone meal from other countries. In some countries, BSE appears to have risen indigenously. The peak incidence is often in cattle between four and five years of age; the disease has no predilection for any particular breed.

2) Disease Symptoms (human and animal):

BSE is a disorder that causes progressive neurological degeneration in cattle. Symptoms are very similar to those of scrapie, a neurological disease in sheep. The clinical course of BSE varies, but can extend for several months. The disease is invariably fatal. Early clinical signs are subtle and nonspecific; later signs are somewhat variable but very distinct. Aggregation (in the form of fibrils) of a disease-specific isoform of the host-encoded PrP membrane protein (PrP^{res} prion) causes lesions in the CNS (brain and spinal cord). Lesions are characterized by sponge-like changes visible with an ordinary microscope; leading to neurological defects and changes in behavior. Animals display temperamental changes such as apprehension, aggression, and hyper-reactivity. Later during the course of disease, abnormal posture, uncoordination, and weakness are common. Animals also lose weight and produce less milk. No diagnostic tests have been developed to detect the BSE agent in live animals. A similar disease, Variant Creutzfeldt-Jakob disease (vCJD), is a rare and fatal human neurodegenerative condition also caused by CNS lesions that lead to spongy degeneration of the brain. vCJD has been convincingly linked to BSE exposure. Patients normally experience psychiatric symptoms early in illness, most commonly depression, sometimes schizophrenia-like psychosis. Unusual sensory symptoms, such as "stickiness" of the skin are experienced initially by approximately half of all cases. Neurological signs develop as the illness progresses, including unsteadiness, difficulty walking, and involuntary movements. Before death, patients are completely immobile and mute.

3) Strain Information:

Strains or isolates of the scrapie agent typically exhibit different incubation periods and patterns of neuropathological change when administered to mice. For quite some time, only one BSE strain was known, however recent reports strongly suggest that other BSE strains exist.

4) Reservoirs/Vectors:

Cattle are the reservoir. The causative agent of BSE is a modified form of a genetically encoded, highly conserved bovine membrane protein.

5) Agent Sources:

BSE has been found in brain tissue, the spinal cord, and the retina of the eye. Additional studies have suggested that BSE may also be present in the small intestine, tonsil, bone marrow, and dorsal root ganglia.

6) Stability:

Aggregates of the BSE isoform of PrP are protease resistant and very stable. The prions are extremely resistant to heat, ultraviolet light, ionizing radiation, normal sterilization processes, and common disinfectants that normally inactivate viruses and bacteria. High concentrations of either sodium

hypochlorite or 2N sodium hydroxide for approximately one hour, or autoclaving at high temperatures is required for disinfection.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

In general, large volume of research (PubMed)

3) Survey Information:

No survey information

4) Past Weapons Activity:

No reports of any state BW research

5) Commercial Sources:

Little information available, but likely most common in high containment United Kingdom and European laboratories

6) General Growth Conditions:

The only reliable diagnostic methods are direct examination of spongiform encephalopathy morphological features by histopathological examination of brain tissue and the observation of PrP^{res} fibrils using electron microscopy and/or immunohistochemical labeling. These methods require that the obex (a portion of the brain stem) be extracted and carefully sectioned. Rapid immunoassays have recently been developed for quick screening, but must be used on the brain tissue of slaughtered animals – they cannot screen live animals. The presence of BSE and the overall level of infectivity are assessed by injecting laboratory animals (mostly mice) with homogenates made from suspicious brain material, and then following the symptoms. These tests may take up to 700 days. Brain tissue should be removed quickly after slaughter. There are currently no *in vitro* methods for the isolation of BSE agent. Currently, only animal models such as mice and macaques can be used to "grow" the agent. Portions of infected brains containing PrP^{res} are homogenated in glucose solution (20% v/v if intracerebrally administered, 2% if intraperitoneally). Animals can also be fed infected material.

Safety Factors

1) Risk of Disease Contraction:

The primary cause of BSE in cattle is the ingestion of food supplemented with contaminated meat-and-bone meal. Experimentally, BSE can also be transmitted by parenteral or oral exposure to infected bovine brain tissue; however BSE is not a contagious disease. Evidence suggests that exposure to the BSE agent causes the vCJD in humans, possibly by ingesting contaminated beef. There is no scientific evidence suggesting that milk and dairy products carry the BSE agent.

2) Countermeasures (PPE and Medical):

Materials contaminated with the causative agent of BSE should be handled in BSL3 facilities. There is no evidence that the agent can be aerosolized, so HEPA filtration is not necessary. Penetrating injuries (needle sticks, etc.), contamination of abraded skin, and ingestion should be avoided. Use of standard masks, gowns, and disposable gloves significantly minimize the risk of working with BSE. There is no treatment or cure in the event of infection.

Agent: *Brucella suis*

Environmental Factors

1) Geographic Distribution:

B. suis is present worldwide but the overall prevalence is generally low. *B. suis* biovar 1 and 3 strains are more common than biovar 2 strains; strain prevalence is much higher in South America and Southeast Asia, and is also elevated in some European and African countries that border the Mediterranean, the Middle East, Central Asia, India, and Mexico. *B. suis* biovar 1 strains are also found in the southern United States and Australia, although the prevalence is low. Biovar 2 strains are generally located in a broad range between Scandinavia and the Balkans.

2) Disease Symptoms (human and animal):

Biovars 1 and 3: Initial infection leads to bacteremia, then acute onset of systemic disease. In pigs, bacteria colonize the reproductive organs, leading to chronic inflammatory lesions in both sexes (placenta and fetuses in females, testis in males). Lesions may form in other organs also; bones and joints are often affected, leading to lameness and sometimes paralysis. The most common manifestation in females is abortion and the birth of dead piglets. Symptoms of human infection include acute onset of fever, headache, weakness, profuse sweating, chills, arthralgia, and localized suppurative infections. The human mortality rate is low but recovery can take a long period of time.

3) Strain Information:

B. suis species are distributed into five biovars; only biovars 1, 2, and 3 infect pigs (2 differs from 1 and 3 in host range, distribution, and pathology); only biovars 1 and 3 strains cause severe disease in humans.

4) Reservoirs/Vectors:

Pigs are particularly susceptible to *B. suis* and are considered the natural hosts; wild and feral pigs are normally the reservoirs. Humans are susceptible to biovar 1 and 3 strains. Various biovars are capable of infecting cattle, goats, sheep, deer, caribou, elk, and dogs. There are no known vectors.

5) Agent Sources:

Sources include: blood, select tissues (lymph nodes, testes, and spleen), placentas, fetuses, urine, uterine discharges, and semen. Bacteria also colonize the bovine udder and are shed in milk.

6) Stability:

The organism survives in carcasses and organs for up to 135 days, on paper for 32 days, soil for 125 days, and blood at 4°C for 180 days. The bacterium is susceptible to numerous disinfectants such as 1% sodium hypochlorite, 70% ethanol, glutaraldehyde, and formaldehyde, and ultraviolet irradiation.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Small volume of research on this organism overall (PubMed)

3) Survey Information:

58/722 (8.03%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *Brucella abortus*, *melitensis* or *suis*. It is not known what percentage of these contain *B. suis* specifically.

4) Past Weapons Activity:

Canada, Iraq, the Soviet Union, and the United States have all conducted BW research on *Brucella* sp. Some reports indicate that Egypt may have conducted research as well.

5) Commercial Sources:

Little information available

6) General Growth Conditions:

B. suis can be cultured readily from uterine discharges, the aborted fetuses, and birth products of infected animals, and from the lymph nodes, testes, spleen, and other select organs of carcasses. Culturing from milk is improved by centrifugation; the pellet can be plated directly. However, the best samples are aborted fetuses, vaginal secretions (vaginal swabs are an excellent source), and fetal membranes. Agent isolation is usually conducted on solid media because bacterial colonies exhibit characteristic growth, simplifying identification. A range of commercial basal media works (*Brucella* medium base or tryptone-soy agar); the addition of 5% serum may improve isolation and culture maintenance. Other basic media such as blood agar, serum-dextrose agar, and glycerol dextrose agar also work. Carbon dioxide is not necessary for growth. Selective media (like Castaneda's medium) have been developed for culturing bacteria from heavily contaminated samples. Colonies are typically visible after two days of incubation, and are invariably round and smooth in appearance exhibiting a pale honey to white color. *B. suis* cultures look exactly like other *Brucella* species. Any variation from this morphology (rougher colonies, etc.) usually signals a change in virulence as well. A host of phages and antibodies are often used to type strains. PCR is more commonly used now to distinguish *B. suis* from other *Brucella* species although it cannot distinguish among the biovars.

Safety Factors

1) Risk of Disease Contraction:

Human infection typically occurs by ingestion or inhalation of the agent; direct contact of the agent with skin abrasions and mucus membranes may also lead to transmission. Human illness is usually limited to those in close contact with pigs, as well as laboratory workers. Infection often occurs through contact with the tissues, blood, urine, vaginal discharge, aborted fetuses of infected animals; or the ingestion of the raw milk or cheese of infected animals. The growth of large quantities of bacteria is a significant hazard—most laboratory infections occur through inhalation of aerosols. Human-to-human transmission does not appear to occur.

2) Countermeasures (PPE and Medical):

BSL2 containment and practices should be used when handling potentially contaminated clinical materials; BSL3 or higher should be used for any amplification of the organism and for all manipulations of cultures or experimental animal studies. Laboratory coats, gloves, and masks should be worn whenever direct contact with infectious materials is unavoidable. Any procedures that may generate aerosols should be conducted in a biosafety cabinet. Antibiotic therapy is available and usually consists of a combination of doxycycline and streptomycin; tetracyclines are also effective. Some strains are resistant to penicillins and cephalosporins, but prophylaxis does not appear effective. Vaccines are not available for use in humans, but in animals the *B. Suis* strain 2 vaccine (China) is sometimes used. Some *B. melitensis* and *B. abortus* vaccines may be partially effective, although no product has gained general acceptance.

Agent: Burkholderia mallei

Environmental Factors

1) Geographic Distribution:

B. Mallei has largely been eliminated from many regions of the world, although it is still endemic in enzootic foci in some Middle Eastern countries (Turkey, Syria, Iraq, and Iran), South Asia (Pakistan and the Indian subcontinent), Southeast Asia (Burma, Indonesia, Philippines), parts of China and Mongolia, and Africa. The Balkan states and former Soviet republics may also still have *B. mallei*. Infection with *B. mallei* cause the disease, Glanders, which is sporadic in Europe and the Americas.

2) Disease Symptoms (human and animal):

The clinical signs can be nasal, cutaneous (also called Farcy), or pulmonary which can all be present in the same animal. Glanders typically results in the formation of nodules and ulcers in lungs, and subcutaneous vesicles filled with exudates in mucous membranes, particularly in nostrils, which rupture into ulcers. Submaxillary lymph nodes are firm. Equids develop characteristic grayish-yellow viscid discharge from nostrils (frequent snorting due to impeded breathing). As the disease progresses, ulcers in the nose increase in number, enlarge or become confluent, extend in depth and sometimes completely perforate the septum; therefore, nasal discharge is often streaked with blood. Acute illness in equids includes a high fever, weight loss, and respiratory signs like breathing difficulties, pneumonia, cough, and swollen nostrils. Ulcers form in lungs and death typically occurs quickly. Chronic disease includes pulmonary symptoms and cutaneous symptoms, but symptoms are milder. Horses may survive several years as carriers. Farcy is more of a local infection where multiple nodules form in the skin and ulcerate, discharging yellow, oily pus. In humans, common clinical signs typically include fever, myalgia, headaches, chest pain, sensitivity to light, excess tear production, diarrhea, and muscle tightness.

3) Strain information:

A large number of strains likely exist in nature, but little information is available in the literature. Some studies have shown a great degree of variation in the infectiousness of laboratory strains.

4) Reservoirs/Vectors:

Equines, particularly horses, mules, and donkeys are the reservoirs. Humans are accidental hosts; the organism can also infect camels, goats, dogs, and cats.

5) Agent Sources:

In live animals, the agent may be isolated from wound/nodule exudates or blood samples (collected only during first three days infection). At autopsy, exudates from nasal passage and the upper respiratory tract may also provide bacteria. Bacteria are numerous in smears from fresh lesions, but few are present in older lesions. It may also be possible to culture sputum and urine, although this will be less effective.

6) Stability:

The bacterium is rather hardy and can remain active for up to six weeks in horse stables. At room temperature, bacteria can survive and remain virulent up to 30 days. The agent is deactivated by many disinfectants: 1% sodium hypochlorite, 70% ethanol, and 2% glutaraldehyde. *B. Mallei* is sensitive to desiccation, ultraviolet irradiation, and heat greater than 55°C.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Generally rather low volume of research (PubMed)

3) Survey Information:

31/722 (4.29%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *B. mallei*.

4) Past Weapons Activity:

Germany used rudimentary techniques to disseminate *B. mallei* during WWI; Japan weaponized and used *B. mallei* during World War II. The Soviet Union also weaponized *B. mallei* and the United States conducted BW research.

5) Commercial Sources:

Little information is available however old stocks likely exist in many research/clinical laboratories worldwide.

6) General Growth Conditions:

B. mallei bacteria are best isolated from unopened, uncontaminated lesions. Bacteria may be first identified in stained smears taken from exudates. Bacterial growth is slow, but can be improved with enrichment of glycerol. Optimal growth is at 37°C under aerobic conditions on standard culture media supplemented with 1-5% glucose and/or 5% glycerol enrichment media. After approximately 48 hours, bacteria are normally visible as confluent, smooth, moist colonies that are viscid and somewhat creamy in color. Longer incubation results in thicker growth that is dark brown and tough. Bacteria also grow well on glycerol potato agar and in glycerol broth. A selective media (nutrient agar containing 4% glycerine + polymyxin E, bacitracin, actidione, donkey/horse serum, trypton agar) has been developed for isolation from contaminated samples. Guinea pigs are very susceptible to infection and are commonly used to test for virulent bacteria. ELISAs and PCR are typically used for diagnostic testing.

Safety Factors

1) Risk of Disease Contraction:

Humans mainly contract the disease from direct contact with the highly infectious nasal and skin secretions of infected equines. Disease transmission occurs frequently by ingestion, inhalation of aerosols, and direct or indirect contact with skin lesions and abrasions. Human-to-human transmission of *B. mallei* is possible but unlikely. Handling laboratory cultures is a high risk activity.

2) Countermeasures (PPE and Medical):

Any laboratory work with infectious body fluids, tissues, or cultures should be confined to BSL3 facilities. Masks should be worn to prevent the inhalation of aerosolized bacteria. Gowns, gloves, and eye protection (preferably face shields) should be used to prevent any contact with infectious droplets. If working with infected animals, puncture-resistant Kevlar gloves should be worn. Severe disease must be treated aggressively with multiple systemic antibiotics: most strains are sensitive to antibiotics such as ceftazidime, imipenem, doxycycline, minocycline, ciprofloxacin, and gentamycin, but treatment may require a long period of time. Strains are often resistant to tetracyclines. Vaccines are unavailable.

Agent: Burkholderia pseudomallei

Environmental Factors

1) Geographic Distribution:

Melioidosis is primarily a disease of the tropics and subtropics. Infections often peak in periods of heavy rainfall/flooding. The disease is endemic throughout Southeast Asia; the highest incidence is reported in Vietnam, Cambodia, Laos, Thailand, Malaysia, Myanmar, followed by Australia. The bacterium is also present in Africa, India, and the Middle East; isolated cases occur in the Americas and are usually associated with foreign travel. *B. pseudomallei* bacteria are so prevalent in some of these countries that the bacillus is a common contaminant of laboratory media. The prevalence is also extremely high in farmers. In one study, isolates were acquired from 50% of the rice paddies in Thailand.

2) Disease Symptoms (human and animal):

B. pseudomallei is mostly an opportunistic pathogen. The bacterium may infect healthy individuals but is typically asymptomatic. However, it may cause local, acute, chronic, and pulmonary disease. Local symptoms include formation of nodules or ulcers at the site of infection followed by fever and muscle aches. The infection may also possibly spread to the bloodstream in immunocompromised people. Bloodstream symptoms vary and may include shock, fever, diarrhea, headache, and skin lesions. Pulmonary infection is largely nonspecific and may include fever, headache, sore muscles, chest pain, and severe cough; subsequent visceral abscesses often occur, followed frequently by death. Chronic symptoms include visceral abscesses in the joints, viscera, lymph nodes, skin, brain, liver, lung, bones, and spleen. Disease symptoms also vary greatly in animals. Melioidosis is often misdiagnosed as typhoid, tuberculosis or malaria.

3) Strain Information:

There are likely thousands of *B. pseudomallei* strains, but little is known about strain variance. Some experimental evidence suggests the infection severity of single strains can vary greatly depending on the host species, even between closely related mice strains.

4) Reservoirs/Vectors:

There is no real animal reservoir. Bacteria live primarily in surface water and soils. However, many animals are susceptible to infection and include mammals, birds, and reptiles. Agriculturally-important animals such as pigs, goats, sheep, and water buffalo are often infected. There are no known vectors.

5) Agent Sources:

The organism may often be isolated from agricultural fields in the tropics, particularly in Southeast Asia. Bacteria can also be isolated from infected individuals, usually from sputum, blood, wound exudates, and various tissues. Swabs of nasal discharge or lesions can also be cultured.

6) Stability:

Bacteria survive for months, maybe years in soil and water. The agent is susceptible to 1% sodium hypochlorite, 70% ethanol, glutaraldehyde, and formaldehyde.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Moderate amount of research (PubMed). Large post-Amerithrax funding increase in United States has motivated more laboratories to conduct research.

3) Survey Information:

34/722 (4.71%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *B. pseudomallei*.

4) Past Weapons Activity:

There is little evidence of any past state BW activity, although the Soviet Union is believed to have conducted BW research on this organism. The United States had also conducted research.

5) Commercial Sources:

Little information is available. However, the organism is a common source of infection in Southeast and has been studied for a long time.

6) General Growth Conditions:

Many protocols have been developed for culturing and selecting isolates from environmental samples. Soil samples are normally suspended in selective nutrient broth and subsequently streaked on Ashdown media (not available commercially but can be made easily). Growth occurs under aerobic conditions and there are many tests for identifying colonies in culture. Colonies are often wrinkled in appearance. Other protocols are available for isolation from water and clinical samples such as pus, aspirates, respiratory secretions, and blood. Generally, samples are first incubated for several hours in trypticase soy broth (recipe available online), then streaked onto selective media such as Ashdown. Commercially available diagnostic tests are available for the identification of the organism although many of these show some cross-reactivity with *B. mallei* and *B. thailandensis*. PCR can be used to specifically detect *B. pseudomallei* in variety of samples and the protocols are freely available.

Safety Factors

1) Risk of Disease Contraction:

The organism may cause disease in healthy individuals. However *B. pseudomallei* is mostly an opportunistic pathogen. Most infections are asymptomatic. The route of entry is a key factor in the manifestation of disease; it is most often acquired through contact of contaminated soil or water with compromised, abraded skin; ingestion or inhalation of bacteria is also possible. Infections also occur through direct or indirect contact of mucus membranes with the lesion discharge of infected animals. Person-to-person transmission is rare, though may occur via contact with blood or bodily fluids.

2) Countermeasures (PPE and Medical):

BSL3 containment and practices should be used for any handling of infectious body fluids and tissues, and also for any activity that may potentially produce aerosols or droplets or when large quantities of infectious materials are produced. Laboratory coats and gloves should always be worn when direct contact with infectious materials is unavoidable (necropsy, etc.). Wearing high boots can greatly reduce the chance of infection in field work. Most forms of melioidosis are nonfatal and completely curable with early treatment. However, many strains are resistant to penicillin, erythromycin, gentamicin, and rifampicin. Concurrent treatment with multiple drugs (meropenem/imipenem, ceftazidime, trimethoprim-sulphamethoxazole, and doxycycline) is often necessary. Antibiotic treatment often takes several months. It is sometimes difficult due to the continual development of drug resistance leading to relapses. There is no effective vaccine available.

Agent: Chlamydophila psittaci

Environmental Factors

1) Geographic Distribution:

Chlamydiosis is a disease that occurs sporadically worldwide, although the natural incidence is unknown. The disease is often associated with pet birds, pet shops, and aviaries. Humans in high risk occupations—e.g. workers at turkey or duck farms—often contract the disease. There are an average of 50-200 cases per year in the United States but the incidence can vary greatly from year to year.

2) Disease Symptoms (human and animal):

In birds, clinical signs may vary significantly according to the specific host species, the age of the infected birds, and the particular bacterial strain. Generally the disease is acute. Symptoms include conjunctivitis, loss of appetite and weight loss, ruffled feathers, nasal secretions and sneezing, diarrhea, and yellowish, sometimes bloody stools. Pneumonia may follow. Often birds (especially older psittacine birds) will exhibit no symptoms but will continue to shed the agent for long periods of time. The disease is usually quite severe in ducks. In humans, disease presentation can vary from asymptomatic to acute, systemic disease with fever, chills, headache, photophobia, respiratory symptoms, often interstitial pneumonia, cough, and myalgia. Encephalitis is possible. Clinical symptoms range from asymptomatic to severe, often lethal atypical pneumonias with multi-organ failure. In other mammals, chlamydiosis may cause pneumonia and abortion.

3) Strain Information:

Strains are distributed among six known avian serovars (A-F) and two mammalian serovars. The mammalian strains do not appear to circulate among birds and very few outbreaks have involved these strains. Strains from psittacine birds appear to be particularly virulent to people but all strains are typically considered transmissible to humans. Serovar D strains (largely present in turkeys) appear to cause particularly high morbidity and mortality in birds, and very severe disease in humans.

4) Reservoirs/Vectors:

Birds, particularly psittacine (of the parrot family), are the natural reservoir. However, the disease has been detected in over 130 bird species (only 57 of which were psittacine). Many different birds, including parakeets, parrots, pigeons, turkeys, ducks, and ostriches can carry the agent. Most of these birds may be asymptomatic but continue to shed the virus. Animals and humans are also susceptible.

5) Agent Sources:

The feces and nasal discharge of infected animals often carry high titers of the agent. The organism may also be present in the blood, tissues, and eggs of infected birds. In acute cases, nasal discharge, blood, and tissue samples may be used. If diarrhea occurs, excrement and cloacal swabs can be used. Infected birds may shed the virus intermittently or continuously for several weeks or even months. The blood, sputum, and tissues of infected humans carry the agent.

6) Stability:

C. psittaci elementary bodies are capable of surviving for months in the environment under the right conditions and are very resistant to drying. The virus is known to survive for 52 hours in infected egg fluids, a few days in bird droppings, two months in bird feed, 15 days on glass, and 20 days in straw. The virus may survive for up to a year in turkey carcasses. The agent is susceptible to many disinfectants including 1% sodium hypochlorite, 70% ethanol, glutaraldehyde, and formaldehyde.

Laboratory Factors

1) Security Classification:

Not a US Select Agent

2) General Research Volume:

Some laboratory based work—moderate to high amount (PubMed); sometimes grown in reference laboratories.

3) Survey Information:

35/722 (4.85%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *C. psittaci*.

4) Past Weapons Activity:

The Soviets and the United States conducted research on *C. psittaci*.

5) Commercial Sources:

Little information is available, but some reference laboratories will likely have samples.

6) General Growth Conditions:

The isolation and identification of the agent in culture is typically used to diagnose the disease definitively. Isolation may be achieved by inoculating animals, chicken embryos, or, most conveniently, cell lines (typically buffalo green monkey, Vero, McCoy, HeLa or L cells). Contaminant bacteria may sometimes make *C. psittaci* isolation difficult but pretreatment with antibiotics can help. Samples are usually homogenized in antibiotic solution and the supernatant used for inoculation. Infection of cells is enhanced by centrifuging (500-1500g for 30-90 minutes at 37°C) the inoculum onto the cell monolayer. The addition of cyclohemimide and other cell division inhibitors also enhances infectivity. The organism is grown in McCoy cells at 38°C in monolayer cultures in Eagle's minimum essential medium containing 10% fetal calf serum (MEM) and antibiotics noninhibitory to *C. psittaci*. Protocols are available for growth and isolation in chicken embryos. Cell cultures should be observed for several days using a variety of staining methods: Gimenz, Giemsa, Ziehl-Neelson or Machiavello's. Fluorescent antibodies can also be used to detect bacteria in culture. Serovar-specific antibodies and PCR methods have also been developed to serotype isolates.

Safety Factors

1) Risk of Disease Contraction:

Transmission to humans is normally through contact with the excretions of infected animals, primarily through inhalation of the desiccated droppings and secretions of infected birds. The bite of an infected bird can also transmit the disease. Diseased birds that are asymptomatic (often psittacines and pigeons) are the most important source of human infection. Transmission from infected ducks and turkeys to humans occurs frequently during slaughter. Person-to-person transmission is rare, but could occur through droplet production during coughing. *C. psittaci* is one of the most commonly reported laboratory-acquired infections.

2) Countermeasures (PPE and Medical):

BSL2 containment and practices are recommended for any handling of potentially contaminated material, and necropsy of infected birds. Particular care must be taken when handling dead infected birds as the feathers may harbor residual infectious feces and nasal secretions. For laboratory activities that result in large volumes of the agent, or have an increased potential to produce droplets or aerosols, BSL3 containment and practices should be employed. Laboratory coats and gloves should be worn whenever handling infectious material to prevent any contact with skin. It is important to use respiratory protection for any work with infected live birds. A number of antibiotics are effective: doxycycline is often the favored antibiotic, as well as tetracycline. Erythromycin, azithromycin, or clarithromycin are also used but are not always effective. A number of isolates have been resistant to penicillin.

Agent: Classical swine fever virus

Environmental Factors

1) Geographic Distribution:

The classical swine fever virus (CSFV) is generally present throughout East and Southeast Asia, the Indian subcontinent, China, Southern Mexico, South and Central America, and East and Central Africa. Most Western European domesticated swine are disease free (although the virus is still present in some wild boar populations), but foci remain in Eastern Europe. Many countries have eradication programs. The United States, Canada, Japan, New Zealand, and Australia are completely free of the disease.

2) Disease Symptoms (human and animal):

Disease symptoms can vary greatly depending on the virus strain and host susceptibility. However, the clinical signs are indistinguishable from African swine fever. The disease can present as acute or chronic infection—both are often fatal. Acute form involves high fever, anorexia, lethargy, hemorrhagic skin lesions, conjunctivitis, and constipation followed by diarrhea, vomiting, dyspnea, and coughing. Acutely sick animals often huddle together. A few days later, hemorrhaging may occur, whereby the abdomen, thighs, and ears develop a purple discoloration. Diagnosis may require the necropsy of a few animals in order to observe internal lesions. Convulsions are often seen later before death, between one and two weeks after the onset of symptoms. Chronic form involves intermittent fever, anorexia, and stunted growth. Hemorrhagic lesions are less severe. Concurrent infections are possible. Mortality approaches 100%, particularly in young pigs. A congenital form also exists; congenitally infected piglets shed the virus for months. A variety of reproductive defects are also possible, and less virulent isolates may only cause poor reproductive behavior; more virulent strains may result in abortion or stillbirth. The large variability of symptoms makes laboratory work necessary for definitive diagnosis.

3) Strain Information:

Only one serotype is known, but isolates vary widely in virulence. Very virulent strains cause acute, obvious disease (hemorrhagic lesions, neurological symptoms) and high mortality; less virulent strains cause chronic, mild or asymptomatic infection. The majority of strains are of moderate virulence.

4) Reservoirs/Vectors:

Pigs and wild boars are the natural reservoirs; domestic pigs are easily infected.

5) Agent Sources:

Virus sources include blood, all tissues, secretions and excretions of sick and dead animals. From live animals, whole blood (collected in EDTA or heparin) is preferred, but tonsil biopsies are also sometimes used. Spleen, kidneys, lymph nodes (pharyngeal and mesenteric), and the distal section of the ileum can also be used. Samples should be kept cold but not frozen.

6) Stability:

The virus is rather fragile in nature, but very stable in protein-rich environments. The virus can survive for months in refrigerated meat (years in frozen meat), and two weeks in contaminated pens and on fomites. Classical swine fever virus is partially resistant to heat (56 °C) and survives well in cold conditions. A pH <3, >11 inactivates the virus. It is also sensitive to ultraviolet light and drying, and is susceptible to ether, chloroform, 2% sodium hydroxide, 1% formalin, sodium carbonate, and strong iodophors.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Relatively high volume of laboratory based work/research (PubMed)

3) Survey Information:

17/722 (2.35%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain classical swine fever virus.

4) Past Weapons Activity:

No known past weapons research

5) Commercial Sources:

Little information is available

6) General Growth Conditions:

ELISAs, direct immunofluorescence using monoclonal antibodies, or RT-PCR can be used to detect the virus in whole blood or tissues. The virus can be grown and isolated in a variety of cell lines—PK-15 cells cultured on cover slips are best—using readily available protocols. A 2% suspension of infected tonsil tissue from deceased pigs can be used for inoculation; spleen, kidney, lymph node suspensions can also be used. Tissue suspensions (20% w/v) are prepared by grinding 1-2 g of material (mortar and pestle typically used); and adding Hanks' balanced salt solution (or Hanks' minimal essential medium) with 1 ml of glutamine-antibiotic stock solution. The suspension is centrifuged for clarification, and then supernatant added to PK-15 monolayer growing in Eagle's minimal essential medium (with 5% fetal bovine serum). After a few days, the cultures can be checked for infection. Whole blood (in EDTA or heparin) from live, clinically diseased animals can also be used to inoculate cells. Blood is first frozen and thawed (to lyse red blood cells); 300 µl is used to inoculate PK-15 monolayer for one hour. The monolayer is subsequently washed with Hanks' solution and the cells checked for virus three to four days later.

Safety Factors

1) Risk of Disease Contraction:

Humans are not susceptible to the classical swine fever virus, although the disease is highly contagious in swine. Infection results from contact between swine that exposes the mucus membranes, eyes, and skin abrasions with virus, although ingestion of the virus is the most common infection route. In addition, aerosol spread has been observed but only within confined spaces.

2) Countermeasures (PPE and Medical):

In the United States (and other areas where the virus is not present) laboratory work is carefully restricted to just a handful of specialized, highly secure facilities. In endemic regions, BSL3 facilities are recommended for any laboratory work with virus; ABSL3 for any animal work. No treatment is available currently for infected pigs. Current control programs should include rapid detection/diagnosis, preventive culling, followed by emergency vaccination. Vaccines are available that treat the symptoms but do not prevent infection.

Agent: *Clostridium botulinum*

Environmental Factors

1) Geographic Distribution:

C. botulinum spores are widely distributed in nature, particularly in anaerobic environments such as soil, water, aquatic sediments, the GI tracts of fish and mammals, and in the gills and viscera of mollusks and shellfish. Botulism occurs sporadically in humans worldwide and is often associated with the consumption of contaminated foods. Infant botulism is the most common form of the disease in the United States (serotype A is more common west of the Mississippi; serotype B is more common east).

2) Disease Symptoms (human and animal):

There are three forms of botulism: foodborne, wound, and infant. All are generally characterized by the same set of symptoms. Generally, botulism is characterized by acute flaccid paralysis. Individuals initially experience paralysis of the facial and neck muscles, often experiencing double or blurred vision, drooping eyelids, slurred speech, difficulty swallowing, dry mouth, and muscle weakness. Paralysis may later occur in the arms, legs, and respiratory muscles; death may result from respiratory failure. The neurological signs are sometimes preceded by nausea, abdominal cramps, vomiting, or diarrhea in foodborne botulism. Infant botulism occurs principally in infants under one year of age; the clinical severity can vary greatly.

3) Strain Information:

A number of genetically diverse strains of *Clostridium* species can produce neurotoxin. Seven serotypes (A through G) of *C. botulinum* are recognized based upon the antigenic specificity of the toxin produced by each strain. Serotypes A, B, and E primarily cause human botulism (occasionally F). The WHO reports that the current mortality rate ranges from ~5% (type B) to 10% (type A). Serotypes C and D cause most of the cases of botulism in mammals and birds. Although type G has been isolated from soil in Argentina, it has never been known to cause an outbreak.

4) Reservoirs/Vectors:

C. botulinum is widely distributed in nature in soil, water, and aquatic sediments. Bacteria can also inhabit the intestinal tract of animals. In humans, the bacteria generally only colonize the intestinal tracts of infants and the immuno-compromised. Bacteria may contaminate food and agricultural products if sterilization and preservation procedures are poor. The animals most commonly affected by botulism are wild fowl and poultry, cattle, horses, and some rare cases, certain species of fish.

5) Agent Sources:

Environmental samples such as soil and water may harbor the organism. Appropriate human specimens for isolation include feces, enema fluid, gastric aspirates or vomitus, tissue or exudates, and postmortem specimens. The feces of animals with botulism may also harbor bacteria. Improperly prepared canned or bottled food products may also contain toxin-producing bacteria.

6) Stability:

Spores found in the environment are extremely resilient, capable of surviving up to two hours at 100°C. However, spores are destroyed after 15 minutes in moist heat at 120°C and the toxin is destroyed after boiling for ten minutes. The bacteria are susceptible to a large number of disinfectants including 1% sodium hypochlorite and 70% ethanol; the toxin is inactivated by 0.1% sodium hypochlorite or 0.1N NaOH.

Laboratory Factors

1) Security Classification:

US Select Agent [laboratories with over 0.5 mg of toxin must abide by regulatory requirements (registration, etc.)]

2) General Research Volume:

Relatively large volume of research (PubMed)

3) Survey Information:

49/722 (6.79%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *C. botulinum*. Forty-two out of 722 (5.82%) contain botulinum toxins.

4) Past Weapons Activity:

The US BW program first produced botulinum toxin during World War II; the toxin was eventually weaponized shortly thereafter. The Soviet Union also produced large quantities of botulinum toxin for weapons use. Iraq also weaponized botulinum toxin, using it to arm a number of missiles and bombs. Canada, France, South Africa, and the United Kingdom conducted BW research on the agent. Currently, Iran, North Korea, and Syria are believed to have either already developed or are in the process of developing weapons containing botulinum toxin. Aum Shinrikyo apparently isolated the *C. botulinum* strain they used in their attacks from soil samples collected in northern Japan.

5) Commercial Sources:

Little information is available

6) General Growth Conditions:

Feces can be suspended in PBS, diluted, and plated directly on solid media. Initial treatment of the sample with heat (80 °C) or ethanol can select against non-spore forming bacteria. *C. botulinum* will grow well when directly inoculated onto commercially available anaerobe blood agar or phenylethyl alcohol blood agar (PEA); *Brucella* agar (with 5% sheep blood), Columbia agar, or brain heart infusion agar (with yeast extract, vitamin K, and hemin). Cultures should be incubated for one to two days at 35-37 °C under anaerobic conditions. Colonies are grey-white with circular/irregular edges and are β -hemolytic. The agent can also be enriched in certain broth cultures that are grown under optimal conditions for toxin production which may contain up to 2×10^8 mouse LD₅₀/ml. Various microbiological tests and PCR can be used to screen colonies or specimens. Virulence can be tested in mice.

Safety Factors

1) Risk of Disease Contraction:

Foodborne botulism occurs following ingestion of toxin-containing foods, most frequently home-prepared and home-preserved foods contaminated with bacteria. Botulism can also occur when puncture wounds become infected with the organism, which grow and produce toxin, releasing it into the bloodstream. Infant botulism occurs predominantly in infants under the age of one after an initial ingestion of spores. The bacteria colonize the intestinal tract and produce toxin. Contaminated honey is suspected to be the cause of most infant botulism. Bacteria may also colonize the GI tract of adults with poor immune systems. Contact of toxin with the skin, eyes, or mucous membranes of the respiratory system can also cause botulism. The intravenous lethal dose of toxin is estimated to be 0.1 to 0.5ng/kg; 0.2 to 1 μ g/kg if ingested. Person-to-person transmission does not occur.

2) Countermeasures (PPE and Medical):

BSL2 containment and procedures should be used whenever materials suspected of containing toxin are handled. BSL3 conditions and biosafety cabinets should be used for any work where aerosols may be generated, toxin is produced or purified. PPE should include laboratory coats, gowns, and gloves. Therapy for botulism is still largely limited to supportive care and passive immunization with antitoxin. Supportive care consists of fluid and nutritional support, assisted ventilation (in cases of respiratory failure), and the use of antibiotics to combat secondary infections, and complications. Antitoxins are available that are effective against all toxin types, but must be administered early. A pentavalent (A to E) toxoid (available from the CDC) can be used for immunization.

Agent: *Clostridium perfringens* (epsilon toxin)

Environmental Factors

1) Geographic Distribution:

C. perfringens spores are present worldwide in anaerobic environments (sometimes at a high incidence), particularly in soil and water, and are a common cause of food poisoning and wound infections/gangrene in humans. *C. perfringens* is the clostridial species that is most commonly isolated from human clinical specimens (this excludes feces). However, the bacteria subtypes that produce epsilon toxin are rarely isolated from humans.

2) Disease Symptoms (human and animal):

C. perfringens can cause a variety of different types of disease in humans, depending on the strain subtype. Type A strains can cause food poisoning, wound contamination, and gas gangrene; type C strains can cause necrotizing enteritis (Pigbel disease). Type B and D strains produce epsilon toxin, causing edema and hemorrhage in the brain, heart, spinal cord, and kidneys of animals; these subtypes are also capable of infecting wounds. The toxin induces necrosis and death of brain tissue. Natural infection with type B strains can cause severe gastroenteritis in young calves, foals, lambs, and piglets. Symptoms may include listlessness, recumbency, and blood-tinged diarrhea. In calves, symptoms include diarrhea, dysentery, abdominal pain, and convulsions; calves may die very quickly. Type D strains are known to cause enterotoxemia in sheep and goats, and rarely, in cattle and young horses. Lambs normally die very suddenly without exhibiting clinical symptoms. Some may show excitement, uncoordination, or convulsions; diarrhea sometimes occurs. Adult sheep exhibit similar symptoms and die within 24 hours. There are three forms in goats—peracute, acute, or chronic—with symptoms that may include diarrhea with blood, neurologic signs, and sudden death.

3) Strain information:

There are five subtypes of *C. perfringens* (A through E). Collectively they produce approximately 15 different toxins; each subtype can produce a number of toxins. Types B and D specifically produce epsilon toxin. Type B can also produce alpha and beta toxins; type D can also produce alpha toxin. Type A strains, which cause the vast majority of foodborne gastroenteritis in the United States, do not produce epsilon toxin.

4) Reservoirs/Vectors:

C. perfringens is typically a part of the normal flora of animals and humans, and a common cause of disease. Spores are frequently found in soil, dust, and water. Type B and D strains normally cause disease in cattle, sheep, goats, pigs, and horses; they rarely infect or are isolated from humans.

5) Agent Sources:

Clostridium perfringens strains B and D can be isolated from infected wound exudates and the stool of infected individuals. Bacteria are normally not uniformly distributed in lesions, so samples should be taken from multiple sites. Fresh fecal samples (within 24 hours of being passed) are preferred for culturing *C. perfringens*. Food suspected of being tainted can also be used for culture.

6) Stability:

C. perfringens spores are quite resistant to stress, surviving for long periods of time in soil and approximately 330 days in tainted meat. The spores are destroyed by moist heat (121°C for 15 minutes), but are capable of surviving normal cooking temperatures (although the epsilon toxin is heat labile). Spores are moderately susceptible to 1% sodium hypochlorite and prolonged exposure to glutaraldehyde.

Laboratory Factors

1) Security Classification:

US Select Agent [laboratories with over 100 mg of toxin must abide by regulatory requirements (registration, etc.)]

2) General Research Volume:

Moderate amount of laboratory work (PubMed)

3) Survey Information:

46/722 (6.37%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *C. perfringens*, although it is unknown what percentage of these strains produce epsilon toxin.

4) Past Weapons Activity:

Iraq weaponized and produced *C. perfringens* epsilon toxin; Japan and South Africa conducted BW research on epsilon toxin.

5) Commercial Sources:

Little information is available. Epsilon toxin poisoning is rare in humans, so public health and human diagnostic laboratories are likely to have few specimens. Epsilon toxin poisoning is more common in animals, so veterinary diagnostic and reference laboratories may have more contaminated specimens.

6) General Growth Conditions:

Feces may be suspended in PBS, diluted, and plated directly on solid media for bacterial isolation. Initial treatment with heat (80 °C) or ethanol can select against non-spore forming bacteria. *C. perfringens* will grow well when directly inoculated onto commercially available anaerobe blood agar or phenylethyl alcohol blood agar (PEA), *Brucella* agar (with 5% sheep blood), Columbia agar, or brain heart infusion agar (with yeast extract, vitamin K, and hemin). Cultures should be incubated at 35-37 °C under anaerobic conditions at pH 5.5-9.0. *C. perfringens* will form colonies after an overnight incubation (sometimes in less than six hours). Colonies are grey-white with circular/irregular edges and are β -hemolytic. The agent can also be enriched in certain broth cultures. *C. perfringens* epsilon toxin is produced during sporulation. PCR assays have been developed for genotyping *C. perfringens* isolates and for detecting whether the epsilon toxin gene is present. ELISAs can be used to detect toxin. Mouse neutralization tests and an MDCK cell cytotoxicity assay can be used to assess toxicity.

Safety Factors

1) Risk of Disease Contraction:

C. perfringens usually infects humans after consumption of contaminated food or after infection of open wounds. The high incidence of *C. perfringens* in soil is thought to be of little clinical significance. Epsilon toxin-producing strains can be transmitted to humans in tainted food and water, as well as by aerosols. Person-to-person transmission does not occur.

2) Countermeasures (PPE and Medical):

The toxin should generally be handled in BSL2 facilities. Staff should wear laboratory coats, gloves, and goggles. There is no antidote for epsilon toxin; treatment mostly includes supportive care with consistent fluid replacement (potassium loss is a feature of epsilon toxin poisoning) and monitoring of electrolytes. Antibiotics may help alleviate infection; clindamycin and rifampin may help suppress epsilon toxin production. A human vaccine is not available for *C. perfringens* infection in humans. In animals, a toxoid vaccine may help prevent enterotoxemia.

Agent: *Coxiella burnetii*

Environmental Factors

1) Geographic Distribution:

Q fever is a global zoonosis. Often times, very large outbreaks of Q fever occur. The actual incidence is unknown but is likely greater than reported. Explosive epidemics have occurred in stockyards, meat packing plants, and agricultural research facilities. Men are twice as likely to get the disease as women.

2) Disease Symptoms (human and animal):

Infection can be symptomatic or asymptomatic. Approximately 40-50% of infected individuals exhibit noticeable symptoms. Numerous factors including inoculum size, route of infection, host factors, and the strain will effect the clinical expression of Q fever. Acute febrile disease presents abruptly with high fever, severe headache, malaise, myalgia, sore throat, chills, sweats, cough, vomiting, diarrhea, abdominal pain, sometimes hepatitis (70%) and/or pneumonia (45%). A rash may develop in a small number of cases, and recovery often takes several months. The clinical disease presentation varies from country to country. The prevalence of fever, hepatitis, and pneumonia can be quite different. For example, pneumonia is the major symptom in Canada, Spain, and the United Kingdom, while hepatitis is more common in most other parts of world, including France and Australia. Individuals who are immunocompromised may develop a chronic form of the disease (less than 1% of acutely infected patients) which often leads to endocarditis. This disease form is difficult to diagnose. In animals, there are normally no clinical signs, although the disease may cause abortions in goats and sheep. Diagnosis is typically by serology.

3) Strain Information:

The organism undergoes phase variation: phase one is virulent and isolated in nature; phase two is avirulent and progressively selected for after numerous passages in the laboratory. Strains can vary greatly in antigenic and genetic properties, as well as in virulence in experimental animals.

4) Reservoirs/Vectors:

C. burnetii is an obligate intracellular bacterium that has been detected in a large variety of arthropods (ticks, lice, mites), fish, birds (chickens, ducks, geese, turkeys), and animals (wild and livestock). Primary reservoirs appear to be cattle, sheep, goats, and ticks. Ticks (>40 species in 12 genera on five continents) can act as vectors to animals.

5) Agent Sources:

Blood, a variety of tissues, and placentas from infected humans. Blood should be collected in EDTA. Present in the milk, urine, feces, and tissues of infected animals. Very high numbers of bacteria are shed in amniotic fluids and placenta during birthing. Infected sheep placentas may contain millions of bacteria/gram; milk may contain 100,000/gram.

6) Stability:

Spores can survive for months, even years in the environment (30 days in dried sputum, up to 120 days in dust, approximately 49 days in dried guinea pig urine, 586 days in tick feces, one month in meat, 42 months in milk at 4-6°C, and 12-16 months on wool at 4-6°C). The organism will likely remain stable during collection and transport in the field. The agent is resistant to heat, desiccation, ultraviolet light, and many common disinfectants.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Some (low volume) academic work (PubMed); limited biodefense work

3) Survey Information:

16/722 (2.22%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *C. burnetii*.

4) Past Weapons Activity:

The Soviets and the United States produced *C. burnetii* weapons.

5) Commercial Sources:

Little information is available.

6) General Growth Conditions:

C. burnetii isolation can be achieved by inoculating clinical specimens in embryonated egg yolk sacs, animals, or cell cultures. For eggs, clinical samples should first be homogenized in PBS with antibiotics (streptomycin and penicillin or gentamycin), centrifuged for clarification, and then inoculated onto five day-old egg yolk sacs. Yolk sacs can be harvested ten to 15 days later. Staining of smears or PCR can be used to detect the organism. For cell culture, shell-vial assay cell culture systems (human embryonic lung fibroblasts; commercially available microculture systems) are efficient at isolating bacteria from a variety of samples, including blood (5 ml samples), bone marrow aspirates, kidneys, and placentas from patients with acute disease; cardiac valves, vascular grafts, and aneurisms from chronic cases. In one study, only 11/66 (17%) of blood samples taken from untreated acute cases yielded bacteria; 9/17 (53%) yielded bacteria from chronic cases. Typically, blood samples are diluted 1:2 with Eagle's minimal essential medium. Tissue samples are homogenized in PBS and diluted 1:2 in Eagle's MEM. Centrifugation of sample suspension onto confluent layer of cells enhances infection; six day incubation should follow. Positive cultures may yield organisms for up to three months as long as the culture medium is changed every week. For animals, inoculation of mice or guinea pigs is best to select for and isolate *C. burnetii* from samples containing other bacteria (placentas, vaginal discharges, feces, or milk). Sick animals with appropriate symptoms can be killed and clinical samples (spleen extracts in 10% suspension) used to inoculate chicken eggs or culture cells. Gimenez stain can be used for detection; direct immunofluorescence, ELISAs, or PCR can be used to identify organism in clinical samples and in cell cultures.

Safety Factors

1) Risk of Disease Contraction:

C. burnetii is extremely infectious—only a few organisms (~10) are required for human infection. The principle route of transmission to humans is by inhalation of dust particles containing contaminated materials (barnyard dust, etc.). Infection can occur through direct contact with animals (wool), birth products, fertilizer, and raw milk. Individuals in certain occupations—veterinarians, meat processors, dairy workers, and livestock farmers—are at particularly high risk. Other forms of transmission such as tick bites and human-to-human contact are rare. Q fever is the second most commonly reported laboratory infection.

2) Countermeasures (PPE and Medical):

BSL2 practices and containment are recommended for non-propagative laboratory procedures (serological examinations and staining of impression smears). BSL3 practices and containment are recommended for any amplification of the organism (inoculation, incubation, and harvesting of embryonated eggs or cell cultures), animal necropsy or manipulation of infected tissues. Experimentally infected animals should be contained in ABSL3 facilities. Laboratory staff should always wear gloves, gowns, eye protection, and masks or respirators when handling materials potentially containing the organism. Numerous *C. burnetii* strains are resistant to certain antibiotics. Doxycycline is often used for treatment, and quinolone antibiotics are also effective. A vaccine (Q-Vax) has been licensed in Australia but is unavailable in the United States commercially. An investigational Phase I, Q fever vaccine (IND) is available on a limited basis from USAMRIID for at-risk personnel.

Agent: Crimean-Congo hemorrhagic fever virus

Environmental Factors

1) Geographic Distribution:

The geographic range of the Crimean-Congo hemorrhagic fever (CCHF) virus is the most extensive of tickborne viruses that affect human health. Human CCHF has been observed in 38 countries, although the case incidence is generally rather low. In endemic areas, the incidence may be higher in the spring or summer. Over the past decade, human cases have occurred across a strip of Asia, extending into Eastern Europe, and large parts of Africa. Cases occur frequently in Bulgaria, the Balkans (yearly), the former Soviet Union (seasonal: May-June in Astrakhan; May-August in Rostov), the countries of Central Asia, the United Arab Emirates, Oman, Iraq (sporadic over entire year), Kuwait, Iran (sporadic year-round), Afghanistan, Pakistan, western China, Mauritania, Burkina Faso, the Democratic Republic of the Congo, Australia (particularly Tasmania), Sierra Leone, and South Africa. In 2006, over 240 cases were seen in Turkey. Many human infections occur in rural areas near livestock. The virus has been detected in domestic animals in several other countries (without any reported human infections), particularly in South, Central, and West Africa (Nigeria and Sudan experience many cases), the Middle East, and parts of the Mediterranean. The tick vectors have a larger geographic range than where the disease has so far been detected, so the range of disease may actually be larger than that described above.

2) Disease Symptoms (human and animal):

In humans, disease symptoms may include abrupt onset of fever, shivering, malaise, irritability, and aches in limbs and the back. Anorexia, nausea, and abdominal pain may also occur; vomiting is also common. Later the face and neck may become red and swollen. Hemorrhagic diathesis occurs in approximately 75% of patients on day four or five with bleeding of the skin over the entire body and mucosal membranes. Anemia, dehydration, shock, and multiple organ failure occur in fatal cases. Mortality is typically between 30-50%. In animals, infection is asymptomatic.

3) Strain Information:

There are at least three CCHF subtypes. Little is known about the variability in strain virulence, but the disease symptoms appear to be similar worldwide.

4) Reservoirs/Vectors:

Ticks, particularly *Hyalomma* species (>30) act as virus reservoirs and vectors to humans. Domestic animals such as horses, donkeys, goats, cattle, sheep, and pigs; and wild animals such as hedgehogs and rodents are also reservoirs. The virus may more frequently infect cattle than sheep or goats (although it is rather prevalent in Sudanese sheep). Ground birds like ostriches and chickens sometimes become infected (prevalence seems to be higher in ostriches).

5) Agent Sources:

The virus is present in blood, mucous secretions, and tissues and can also be isolated from infected ticks.

6) Stability:

The agent is a lipid-containing enveloped virus. It is susceptible to thermal treatment (56°C, 30 min) and disinfectants such as 1% hypochlorite and 2% glutaraldehyde; soaps and detergents also inactivate the virus. The virus is generally stable in blood for ten days at 40°C.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Few laboratories appear to conduct research on the CCHF virus (PubMed)

3) Survey Information:

5/722 (0.69%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain CCHF virus.

4) Past Weapons Activity:

The Soviet Union may have weaponized the CCHF virus.

5) Commercial Sources:

Little information available

6) General Growth Conditions:

Virus infectivity in cell culture systems is often low, but the virus can be isolated from blood and organ suspensions taken from severely affected patients (8-12 days after symptoms onset). Blood should be EDTA-treated and chilled after collection; RT-PCR can be used to detect the virus in samples. The virus can be difficult to isolate from infected ticks. The virus can be grown and harvested in cell culture or in mice. Vero cells are typically used, but LLC-MK2, BHK-21, and SW-13 cells are also effective. Vero cells are grown in Eagle minimum essential amino acid medium with Hanks' salt solution supplemented with 10% fetal bovine serum, as well as antibiotics. Infected Vero cells do not exhibit cytopathic effects. Immunofluorescence assays using monoclonal antibodies can typically detect the virus in culture after five days of inoculation. PCR assays have also been developed to detect the virus in culture; ELISAs are also available. Inoculation of mice is a more sensitive method for virus isolation but it is more time intensive. Traditionally, a blood sample from an acute-phase patient is inoculated intracranially or intraperitoneally into newborn mice. Virus particles can later be detected and isolated in mouse brains. In addition, potentially infected ticks can be pooled, homogenized, and suspensions injected into infant mice. The brains of sacrificed mice can then be homogenized, and the suspensions used to inoculate tissue culture cells or more mice.

Safety Factors

1) Risk of Disease Contraction:

The virus is highly infectious to humans; the infectious dose is between ~1-10 organisms. The virus is transmitted principally through tick bites. Direct exposure of infected blood or tissues with mucus membranes is another source of infection and may lead to more severe symptoms. Animal slaughter, castrations, branding, and birthing may be especially hazardous. Aerosol transmission cannot be ruled out. Person-to-person transmission can easily occur through direct contact with infected blood or body fluids. Nosocomial infections occur frequently, making patient care and diagnostic sample collection very hazardous.

2) Countermeasures (PPE and Medical):

Any work with this agent, including diagnostic tests, should occur in BSL4 laboratories. Infected individuals should be housed in intensive care units with negative pressure rooms. The use of flexible plastic hoods with battery-powered blowers is preferable for patient care. Barrier nursing, masks, gloves, and gowns are important in hospital settings. Treatment is limited primarily to supportive therapy. Ribavirin, if administered early, can ameliorate disease severity and is sometimes available in emergency situations. An inactivated vaccine was developed in Russia long ago but modern vaccines are unavailable.

Agent: *Cryptosporidium parvum*

Environmental Factors

1) Geographic Distribution:

C. parvum is present worldwide. The organism is found in drinking and recreational water and sewage globally. It is a very common cause of waterborne disease for humans and animals. *C. parvum* is also a very common infection of farm animals. There is often a seasonal variation in the incidence of human infection in developed and developing countries ascribed to waterborne transmission. In tropical climates, infections usually peak during the rainy season. Developing countries have a higher incidence (infection rate range of 3-20%) than the developed world (one to 4.5% infection rate). Infection rates are higher in AIDS patients (3-20% in the United States, 50-60% in Africa and Haiti), children, and other immunocompromised people (outbreaks occur frequently in daycare centers and nursing homes). A 1993 outbreak in Milwaukee infected 400,000 due to waterborne transmission, although approximately 10% of US infections are foodborne in nature.

2) Disease Symptoms (human and animal):

In humans, the principle disease symptom is profuse watery diarrhea. The illness also often includes dehydration, headache, weight loss, stomach cramps/pain, low-grade fever, nausea, malaise, and vomiting. Symptoms typically last for one to two weeks, although some infected individuals are asymptomatic. In mammals, cryptosporidiosis is usually a disease of newborn animals, sometimes causing large outbreaks of diarrhea in calves as calves excrete many more oocytes than adult animals. Other symptoms include dullness, anorexia, and fever. Animals usually recover within two weeks. Older infected mammals often do not show symptoms, even as they continually shed *C. parvum* oocytes. Serology is often used to confirm outbreaks.

3) Strain information:

C. parvum strains found in nature exhibit great genetic diversity, but the vast majority are likely capable of infecting humans. Some research has shown that isolates can differ greatly in their infectious dose, attack rate, and duration of diarrhea.

4) Reservoirs/Vectors:

Domestic and wild ruminants are the primary reservoirs. A host of small and large mammals (~150 species), poultry, fish, reptiles and humans are also susceptible.

5) Agent Sources:

C. parvum is a parasite that infects the small intestines, so feces and intestinal biopsies (terminal ileum) harbor the organism. *C. parvum* lives in the GI tracts of animals and humans. Millions of organisms are excreted in stools from the beginning of symptoms to several weeks after symptoms resolve. The organism is regularly found in the diarrhetic feces of calves. Almost all diagnoses are by analysis of stool specimens; feces should be collected during the acute stage of illness. Numerous samples should be collected two to three days apart as some carriers shed only small numbers of oocysts.

6) Stability:

A thick outer shell enables the oocysts to survive for long periods of time outside of the body (~2-6 months in moist environments). Oocysts are resistant to low temperatures, high salinity, as well as most disinfectants including 3% hypochlorite, iodophors, 5% formaldehyde, 5% ammonia, 10% formol saline, and 3% hydrogen peroxide. Oocysts are very resistant to chlorine-based disinfectants. One minute of boiling will kill the organism.

Laboratory Factors

1) Security Classification:

Not a Select Agent

2) General Research Volume:

Relatively high volume of laboratory work (PubMed)

3) Survey Information:

No survey information.

4) Past Weapons Activity:

No known past weapons research

5) Commercial Sources:

It is possible to purchase viable, infectious *C. parvum* oocytes from commercial sources (e.g. Waterborne, Inc, sells ~one million spores for US\$105; 1,000 million oocysts for US\$1765).

6) General Growth Conditions:

The organism is usually isolated from fresh stool samples. A large number of commercial diagnostic assays are available for the detection of *Cryptosporidium* oocysts or antigens, but most cannot differentiate between species. Oocysts can also be detected in fecal smears by microscopic analysis (direct immunofluorescence, or more commonly in developing countries, modified acid-fast staining because of the low cost and ease of use). Since oocytes of different cryptosporidium species are very similar, PCR is increasingly used to differentiate species. *C. parvum* is an obligate intracellular parasite, so it must be grown in animal tissue culture cells. However, according to the World Organization for Animal Health (OIE), there is no fully reproducible method for culturing *C. parvum* from body fluids or feces; *in vitro* cultivation is inefficient because of low parasite yields and oocyst production. Recent developments of several media formulations and procedures have improved overall culture success (MDCK, UltraMDCK, PC-1, UltraCHO or UltraCulture). Microscopy and PCR can be used to detect the organism in culture.

Safety Factors

1) Risk of Disease Contraction:

Fecal-oral route and airborne transmission are possible; the relative incidence of infection by each route is not well known. Disease is usually contracted by ingesting contaminated material (water or food). The infectious dose is typically small—around 50 to 100 organisms. Direct human-to-human (especially those with diarrhea) and animal-to-human (with farm animals) contact appears to be commonly responsible for transmission. Day care facilities and nursing homes have a high prevalence of illness.

2) Countermeasures (PPE and Medical):

BSL2 practices and containment should be used for *C. parvum* research. PPE should include simple latex gloves when handling contaminated material. Good hygiene with frequent and thorough hand washing could significantly help to prevent infection. People should also avoid swallowing untreated water, especially in developing countries. Symptoms are typically self-limiting and there are generally no real effective therapeutic agents. Most healthy people will recover without treatment, but a new drug, nitazoxanide, has been approved for treatment of diarrheal symptoms. There are no widely accepted vaccines for animals.

Agent: Eastern equine encephalitis virus

Environmental Factors

1) Geographic Distribution:

Eastern Equine encephalitis virus (EEEV) infections occur primarily in North America and have been diagnosed in Texas, parts of the eastern United States, mainly along the Atlantic and Gulf coasts, and in southern Canada. EEEV foci are also scattered across the northern portion of South America, and in Central America and the Caribbean. The virus is mostly limited to wet areas, generally in secluded swamps and coastal areas where mosquitoes thrive. The disease occurs primarily in mid-summer to autumn. The incidence increases in years with elevated temperatures and many mosquitoes. In addition, there is sometimes a fluctuation between avirulent and virulent strains each season. The degree of virulence relates to host factors during the given epizootic outbreak. Disease is relatively rare in humans—in the United States there have only been 200 cases from 1964 to the present with an average of four cases a year.

2) Disease Symptoms (human and animal):

In humans, EEEV infection often results in no apparent illness. In those who do develop illness, clinical signs range from mild flu-like illness with respiratory symptoms, to meningoencephalitis, stupor, tremors, coma, and death. The onset of illness may be very sudden and duration may be short. The mortality rate is approximately 30%; approximately half of those who survive infection will have some form of permanent neurological damage. The disease is unexceptionally lethal in horses, causing fever, lethargy, and anorexia before death. In very severe cases, neurological symptoms such as blindness, mental depression, and convulsions may occur before death. There are no obvious external signs.

3) Strain Information:

EEEV strains are divided into two geographic variants or topotypes (North American and South American) based on differences in genome sequence and antigen expression. The two topotypes differ greatly epidemiologically and in clinical disease expression. North American strains are highly conserved, constituting a unique major lineage, whereas South American strains constitute three major lineages with more antigenic and genetic differences. All the strains in North America and most from the Caribbean belong to the North American lineage, whereas those isolated from Central and South America constitute the three South American lineages. The North American strains cause severe disease in humans (mortality 30-80%) and equines (mortality 90-95%). Infection by South American strains can be fatal in equines but human cases are very rarely seen; serosurveys indicate significant amounts of human infection with no clinical disease symptoms.

4) Reservoirs/Vectors:

The virus is maintained in an enzootic transmission cycle between mosquitoes and birds, primarily circulating in mid-summer to autumn. Several species of mosquitoes (and mosquito eggs) act as reservoirs and disease vectors. Infected ticks can also act as vectors, albeit less important ones. Humans and horses are dead-end hosts.

5) Agent Sources:

EEEV infection produces viremia of very short duration. Passerine birds develop high virus titers in their blood, but viremia only lasts for two to five days. Viremia in infected horses and humans is also brief, and titers are too small to transfer virus to biting mosquitoes. Therefore, animal blood is not a viable source for culturing. However, the virus can be isolated from horse brains (and presumably from humans). Tissues should be collected and transported in a viral transport medium (or even on a moist sponge).

6) Stability:

EEEV is an enveloped virus that does not survive outside of a host. Viral infectivity is inactivated by heat (56°C for ten minutes). Virions are stable when stored at -40°C to -70°C but not at -20°C or above. Virions are sensitive to treatment with lipid solvents, detergents, ether, trypsin, chloroform, formaldehyde, heat, and β -propiolactone.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Some (low volume) laboratory work (PubMed)

3) Survey Information:

Only 7/722 (0.97%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain EEEV.

4) Past Weapons Activity:

The United States had conducted BW research on EEEV.

5) Commercial Sources:

Little is known

6) General Growth Conditions:

Specimens should be homogenized in a 10% suspension in PBS with bovine serum albumin, penicillin, and streptomycin; then centrifuged to clarify. A number of host systems for virus isolation and amplification have been developed. Newborn mice are a sensitive system. Intracranial injection will result in infection; brains can later be harvested for virus isolation. Chicken embryos can also be used, although it is a less sensitive system. Yolk-sacs of six to eight day old embryonated chicken eggs are inoculated, incubated for seven days, and then the embryo passaged for virus isolation. Virus can also be isolated in a number of cell lines, including Vero cells, rabbit kidney (RK-13) cells, and BHK-21 cells, or in primary cell cultures made of chicken or duck fibroblasts. Typically 1 ml of tissue suspension is inoculated on a monolayer of cells, allowed one to two hours to absorb, maintenance media is added, cultures are incubated for seven days, and a passage is made. Cytopathic effects are seen starting one week later. Recent studies indicate excellent growth of the virus recovered from patient cerebrospinal fluid in A549 and MRC-5 cell cultures. Immunohistochemical staining, ELISAs, and RT-PCR can be used to identify the virus in clinical specimens (brain tissue or viremic blood) or cultures. Commercial kits are available for the detection of the agent in specimens and mosquitoes. RT-PCR can also be used to test for presence of virus in mosquitoes, and has also been used to detect the virus in dead equines.

Safety Factors

1) Risk of Disease Contraction:

EEEV is transmitted through the bite of infected arthropods, mostly mosquitoes. Person-to-person (or animal-to-person) transmission does not occur.

2) Countermeasures (PPE and Medical):

BSL3 (or above) laboratory containment and practices should be employed for any work involving EEEV. ABSL3 is required for work with infected animals. PPE such as laboratory coats, gloves, gowns, and eye protection should be worn at all times. In the field, measures that protect against insect bites could help prevent EEEV exposure. There are no effective therapeutic drugs available; therapy is limited to supportive care with fluid replacement and electrolyte monitoring. Inactivated vaccines are available commercially for equines, but human vaccine is available only for researchers and military personnel. These vaccines are poorly immunogenic and require frequent boosters.

Agent: Ebola virus

Environmental Factors

1) Geographic Distribution:

Ebola is endemic to sub-Saharan Africa. The virus ecology appears to be largely linked with the rainforest ecosystem, and outbreaks are more prevalent during the rainy season. However, outbreaks occur only sporadically (and unpredictably) in humans. There have thus far been 18 recorded natural outbreaks of Ebola in humans; most outbreaks have occurred in the Democratic Republic of the Congo, Sudan, Gabon, and Côte d'Ivoire. The outbreaks have varied significantly in size, with disease often spreading rapidly within health-care settings with poor barrier nursing. The disease is much more prevalent in nonhuman primates. Ebola continues to devastate the population of lowland gorillas—over 5,000 have died of the disease. Ebola occasionally infects chimpanzees (only a handful of recorded incidents). The ecology appears to be more complex than that of Marburg.

2) Disease Symptoms (human and animal):

The onset of illness is sudden, with fever, headache, joint and muscle aches, sore throat, and weakness followed by diarrhea, vomiting, and abdominal pain. By around the fifth day of illness, a maculopapular rash develops on the arms, legs, and trunk. Mucous membrane hemorrhages appear; patients may have red eyes, hiccups, and delirium. Bleeding may develop in the GI tract, and in 40-50% of patients extensive internal and external hemorrhaging occurs. Patients may have vacant facial expressions, breathe rapidly, and eventually go into shock or coma before death. The Marburg virus causes very similar symptoms. The disease progression of Ebola is similar in primates (nonspecific early on, followed by extensive hemorrhaging).

3) Strain Information:

There are four genetically distinct species: Zaire, Sudan, Tai Forest (formerly Ivory Coast), and Reston. Zaire, Sudan, and Tai Forest are often fatal to humans and other primates, while the Reston strain kills nonhuman primates only. Several strains of the Zaire and Sudan species have been identified and all are deadly. Based on very limited data, the Zaire species may be more virulent than the Sudan species. Little is known about the Tai Forest species, as this virus has only infected two individuals thus far.

4) Reservoirs/Vectors:

The reservoir has not been definitively identified, but evidence suggests that three species of fruitbats (*Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquata*) show signs of asymptomatic infection in nature, and have a broad geographical range spanning parts of western and central Africa where outbreaks have occurred. In addition, people in these areas are known to eat bats. Similar bats are in Southeast Asia and may harbor the Reston subtype. Ebola virus has continually circulated in gorilla and chimpanzee populations. Evidence suggests dogs may be asymptotically infected and shed the virus.

5) Agent Sources:

The virus is present in blood, body fluids (sweat, urine, semen, stool, etc.), and tissues (skin, liver, spleen, lymph nodes, kidneys, lungs, and gonads). Virus particles have been isolated from the blood and secretions up to 61 days after the onset of illness. In nonhuman primates, the virus is found in mucous membranes, GI surfaces, and skin. Blood (serum) should be collected during the acute febrile stages of illness for virus isolation. Thus far it has not been possible to isolate the virus from wild bats; primate carcasses are infectious for a few days.

6) Stability:

The Ebola virus is relatively stable for a lipid-enveloped virus. It can survive for one to two weeks in blood specimens and in corpses. The virus is susceptible to 2% sodium hypochlorite, 2% glutaraldehyde, and formalin as well as ultraviolet irradiation and drying. It is inactivated by heating to 60°C for an hour.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Few laboratories with access/research. Moderate amount of research (PubMed)

3) Survey Information:

Only 4/722 (0.55%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain filoviruses (Ebola or Marburg). It is uncertain how many contain strains nonpathogenic to humans (such as Ebola Reston).

4) Past Weapons Activity:

The Soviet Union produced large quantities of Ebola for BW purposes until 1992. In 1992, Aum Shinrikyo attempted to acquire a sample of Ebola virus during an outbreak in Zaire but it is unclear if they were successful.

5) Commercial Sources:

Very few commercial sources have the Ebola virus.

6) General Growth Conditions:

Virus isolation in cell culture is the most reliable mode of disease diagnosis, but serology can be used to detect infection in blood. The virus can be isolated from human blood samples and tissues (clarified 10% homogenate) by inoculation of guinea pigs or by passage in cell culture. Ebola will grow vigorously in a variety of cell lines—Vero and Vero E6 cells are used frequently, but PK 15, MDCK, and BHK cells can also be used—upon inoculation with infected serum, or other bodily fluid or tissue extract. Virus propagation is optimal at 37°C in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin, streptomycin, and L-glutamine following readily available protocols. For virus propagation, DMEM with 2% fetal calf serum should be used. Cytopathic effects are typically seen about a week later. Electron microscopy can detect filovirus particles in clinical specimens and culture supernatants. Indirect immunofluorescence, immunohistochemistry, ELISAs, and RT-PCR can be used to specifically detect Ebola.

Safety Factors

1) Risk of Disease Contraction:

Ebola infection occurs through direct contact of mucous membranes or broken skin with the virus particles present in infected body fluids, tissues, and blood. Natural human infection is rare, overall, and most outbreaks are traced to a single index case of an individual handling an infected animal carcass. The disease is not transmissible during the incubation period or during the onset of symptoms. The disease has often spread during outbreaks among healthcare workers, especially in situations with poor barrier nursing. Family members who have prepared dead relatives for burial also spread the disease. The virus is highly infectious in the laboratory and has the potential to spread by small-particle aerosols generated during virus manipulation. Only ten virions or less are required to infect nonhuman primates via aerosol.

2) Countermeasures (PPE and Medical):

BSL4 facilities are recommended for any handling of potentially infectious material. Biosafety cabinets should be used for all procedures that may produce aerosols. In hospitals, infection control practices should be implemented that reduce contact with patients and the production of droplets. Medical staff should wear eye protection or face shields, impermeable gowns, shoe covers and caps, gloves, and N95 masks or powered respirators. The only treatment option at this time is supportive care with monitoring and maintenance of electrolyte balance, intravascular volume, and blood pressure to help prevent

hemorrhage and shock. Ribavirin appears to be effective *in vitro* but is probably ineffective as a prophylactic drug. New experimental vaccines appear effective in primates but are still being tested.

Agent: Escherichia coli O157:H7

Environmental Factors

1) Geographic Distribution:

E. coli O157:H7 is present wherever cows are raised, but the prevalence rates can vary and fluctuate considerably in agricultural settings. The disease is a recognized problem globally and is considered a very important food borne disease in developed countries. Less information is available in developing countries but the prevalence is expected to be higher. Cases are consistently seen throughout North America, Europe, South Africa, South America, and Australia; less is known about Asia but numerous cases have been reported in Japan, India, China, and Malaysia. In North America, the prevalence is highest during the spring and late summer. The CDC estimates that 73,000 cases of human illness occur per year in the United States alone.

2) Disease Symptoms (human and animal):

In adults, infection typically causes severe diarrhea (sometimes bloody due to hemorrhagic colitis) with abdominal cramps, little or no fever, and symptoms typically resolve in five to ten days. Young children and the elderly are more susceptible. Infection can cause urinary tract infection (UTI) and hemolytic-uremic syndrome (HUS) in which red blood cells die and the kidney eventually fails. A small proportion of people with HUS have immediate complications with lifelong implications such as blindness, paralysis, persistent kidney failure, and bowel inflammation. Neonates and infants with UTI or bacteremia may develop symptoms such as fever, hypothermia, jaundice, respiratory distress, apnea, poor feeding, vomiting, severe diarrhea, irritability, and lethargy. Meningitis may also develop with no obvious signs. Older children with UTI may develop fever, vomiting, abdominal cramps, and diarrhea (sometimes with blood or mucus).

3) Strain Information:

There are thousands of *E. coli* strains but most are innocuous. However some cause severe illness. Different *E. coli* subtypes can produce different kinds of diarrheal illness: enterotoxigenic, enterohemorrhagic or enteropathogenic. O157:H7 is one of the most dangerous strains, producing Shiga-like toxins and enterohemorrhagic illness. O157:H7 is not the only (but appears to be most common) Shiga-like-toxin-producing strain, others such as O26:H11, O111:H8, O103:H2, O113:H21, and O104:H21 produce similar disease. Strain EDL933 is a commonly studied reference strain for O157 that was isolated in a large human outbreak in 1982.

4) Reservoirs/Vectors:

The organism is found in the guts of ruminants. Cattle are a primary reservoir of enterohemorrhagic *E. coli* (common inhabitant of intestinal flora in healthy cows). Calves frequently shed bacteria at higher rates. Also, a variety of other animals including deer, raccoons, and birds shed the virus in their feces.

5) Agent Sources:

Cattle feces on ranges or pastoral areas and in the more confined spaces of the feedlot and dairy industries are excellent sources of agent. The agent has also been isolated from deer, swine, and dogs. Infected young children typically shed the bacterium in their feces for one to two weeks after the illness resolves.

6) Stability:

There is some degree of variation in strain stability. The organism is typically resistant to low pH (even as low as pH 1.5), and can survive for weeks in very dry conditions and desiccation (depending on the temperature). The bacteria can survive for months (perhaps years) in chilled/frozen foods, for at least 150 days in soil, 90 days in cattle feces, and at least four months in cattle drinking trough sediment. However, the organism quickly dies at high temperatures (70°C for two minutes), and the organism is sensitive to ultraviolet light/direct sunlight.

Laboratory Factors

1) Security Classification:

Not a Select Agent

2) General Research Volume:

Relatively high volume of academic research (PubMed)

3) Survey Information:

196/722 (27.15%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain this *E. coli* strain.

4) Past Weapons Activity:

There are no known past attempts to weaponize *E. coli* O157:H7.

5) Commercial Sources:

Little is known. High prevalence worldwide, large volume of research and the organism is not a Select Agent. Thus, many culture collections and diagnostic/clinical laboratories may have samples.

6) General Growth Conditions:

Many effective culture methods have been developed. A variety of basic, easy to make media can be used. Much of the media can be purchased pre-made commercially. *E. coli* is part of normal intestinal flora, so it is first necessary to detect the pathogenic varieties—this can be done with PCR. Also, unlike most *E. coli* strains, O157:H7 does not ferment sorbitol, so the samples can be cultured on sorbitol-MacConkey (SMAC) agar (reliable, cheap, easy to make). O157:H7 colonies are colorless, in contrast to bright pink colonies of non-sorbitol fermenting bacteria. Large amounts of O157:H7 are present in bloody diarrhea taken during the acute stage of human illness, resulting in heavy growth in culture. (In one study, 18/99 bloody stools but 0/944 non-bloody stools yielded isolates). *E. coli* O157:H7 can also be isolated from cattle manure or fecal samples by overnight enrichment in Luria-Bertani liquid broth containing cefixime, potassium tellurite, and vancomycin, followed by plating onto SMAC. Even more selective media has been developed for O157:H7 growth; much of it is available commercially. The optimum growth temperature is 37°C (range 8-46°C); neutral pH is optimal. The bacterium can grow in the presence or absence of oxygen.

Safety Factors

1) Risk of Disease Contraction:

The disease is transmitted from person to person (or animal to person) by the fecal-oral route. Nosocomial infections are also common. The disease can also spread by contaminated water (drinking and recreational) and food, often resulting in large outbreaks. Since *E. coli* is a common inhabitant of the bovine intestine, beef products and vegetables may become contaminated by animal waste. Dangerous strains are also commonly found in petting zoos on animal fur. Only a small number of organisms are required to cause the disease (the ID₅₀ is believed to be between ten and 100 bacteria). Any age group can be infected, but the majority of cases occur in infants (less than four years of age) and the elderly (greater than 65 years).

2) Countermeasures (PPE and Medical):

BSL2 containment, practices, and equipment should be used for any work with potentially infectious materials or cultures. Special care must be taken to prevent splashes. ABSL2 facilities should be used for any work with infected animals. PPE such as face shields, gowns, and gloves should be used. Infection can be prevented significantly by consistently and thoroughly washing hands. Most people recover without antibiotics or other specific treatment within five to ten days. In fact, the use of antibiotics is discouraged since there is evidence that it may help induce HUS. Anti-diarrheal agents, such as loperamide (Imodium), should also be avoided. For HUS, the treatment is typically supportive care, with the monitoring of fluids,

electrolytes, and dialysis, if needed. All individuals with bloody diarrhea should have their stool tested for *E. coli* O157:H7.

Agent: Exotic Newcastle disease virus

Environmental Factors

1) Geographic Distribution:

Newcastle disease virus (NDV) can be found on all continents, including Antarctica. However, the velogenic form of the virus, which causes exotic Newcastle disease, is endemic only to Asia, the Middle East, Africa, and Central and South America. North America and Europe are free of the disease.

2) Disease Symptoms (human and animal):

Different strains of the virus can produce a wide variety of symptoms among domestic and wild bird populations. Birds are often infected with multiple strains at once, complicating clinical diagnosis. In addition, different bird species often present different symptoms when infected with identical strains. Symptoms of velogenic infection can include nervous system depression, muscular tremors, drooping wings, dragging legs, lack of appetite, twisting of head and neck, circling, complete paralysis, sneezing, gasping for air, nasal discharge, coughing, increased respiration, and hemorrhagic intestinal lesions. Viscerotropic velogenic strains are highly pathogenic, frequently producing hemorrhagic intestinal lesions. Neurotropic velogenic strains are also very pathogenic, producing severe respiratory and nervous signs, followed by a high incidence of mortality. Mesogenic strains produce respiratory symptoms with occasional nervous signs, and low-level mortality. Infection with lentogenic strains present mild (or subclinical) respiratory symptoms. Serology is typically used to diagnose disease in flocks.

3) Strain Information:

Newcastle disease is caused by viruses of the APMV-1 serotype in the genus Avulavirus. There are eight other serotypes, some antigenically and genetically very similar to APMV-1. Strains are grouped into three pathotypes according to clinical signs produced in chickens: non-virulent (lentogenic), intermediate (mesogenic), and highly virulent (velogenic). Velogenic isolates can be further subdivided into neurotropic and viscerotropic types, based on the cell types they infect. Separation of strains into pathotypes can be difficult, and the symptoms caused by the different pathotypes can overlap considerably. In addition, harsh environmental conditions (crowding, etc.) and co-infection with other organisms can accentuate the severity of symptoms caused by infection with less pathogenic strains.

4) Reservoirs/Vectors:

Newcastle disease can affect many bird species. It is most severe in chickens, peafowl, guineas, pheasant, quail, and pigeons, but can also be found in a milder form in turkeys. Carrier states also exist in many other bird species. Carriers do not show clinical signs but some are capable of shedding the Newcastle virus for 400 days or more.

5) Agent Sources:

NDV is present in the feces of infected birds as well as secretions from the nose, mouth, and eyes. From live birds, the virus is typically cultured from tracheal and cloacal swabs, or whole feces. From dead birds, the virus can be cultured from oro-nasal swabs, pooled organ samples (lung, kidneys, intestine, spleen, brain, liver, and heart), and feces. Samples should be collected early during the course of disease, and placed in PBS (pH 7.0-7.4) containing antibiotics (penicillin, streptomycin, gentamycin, mycostatin, etc.).

6) Stability:

Newcastle disease virus is resistant to a wide pH range, and is most stable at low temperatures (up to 210 days in fluid suspensions at 1-2 °C, several days at 4 °C, and years if stored at -20 to -70 °C). It can survive in wet feces for 56 days at 37 °C, 94 days at 20-30 °C, 172 days at 11-36 °C, and 538 days at 3-6 °C. The virus is susceptible to high temperatures, direct sunlight, and drying.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Relatively high volume laboratory/academic research on Newcastle virus (PubMed)

3) Survey Information:

21/722 (2.91%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain velogenic NDV.

4) Past Weapons Activity:

Both the United States and the Soviet Union had conducted BW research on this agent.

5) Commercial Sources:

Little information is available. However, many collections and agricultural/veterinary reference laboratories would be expected to have samples throughout the developing world.

6) General Growth Conditions:

The preferred method of disease confirmation is virus isolation and characterization. Typically suspensions (10-20% (w/v) in an antibiotic solution) are made from tracheal or cloacal swabs, feces, or homogenized organs, are clarified and then used to inoculate the allantoic cavities of nine to 11 day-old embryonating fowl eggs. After an incubation of four to seven days at 37 °C, the allantoic fluid of any dead (or dying) embryo is tested for NDV HA. In addition, NDV infects a variety of mammalian cells lines but is particularly infectious to chick embryo fibroblasts or cell lines derived from rhesus kidney cells. Strain pathogenicity can be tested by inoculating chicken embryos and assessing the mean time to death—velogenic strains take 60 hours or less to kill all embryos; mesogenic strain take 60-90 hours; lentogenic strains take more than 90 hours. Pathogenicity can also be tested by intracerebrally injecting chicks. In addition, RT-PCR, and restriction enzyme analysis or sequencing can be used to assess the relative pathogenicity of isolates.

Safety Factors

1) Risk of Disease Contraction:

Humans are not susceptible to NDV infection. Direct contact between healthy birds and the excretions/secretions of infected birds is responsible for avian transmission. Vaccination and de-beaking crews, manure haulers, rendering-truck drivers, feed-delivery personnel, poultry buyers, egg-service workers, and poultry farm owners and their employees often inadvertently spread the disease.

2) Countermeasures (PPE and Medical):

Lentogenic NDV strains can be handled in BSL2 or ABSL2 facilities, but mesogenic and velogenic serotypes are typically handled in BSL3/ABSL3 laboratories. There is no effective treatment for Newcastle disease in birds. Both live and inactivated vaccines are widely in use—and administered prophylactically—but none are 100% effective at preventing disease. Newcastle disease can infect and cause death even in vaccinated poultry.

Agent: Foot-and-mouth disease virus (FMDV)

Environmental Factors

1) Geographic Distribution:

Foot and mouth disease (FMD) is endemic in Asia, Africa, the Middle East, most of South America, and parts of Europe. There is a high prevalence throughout Africa (except in the African nations of Zimbabwe, Namibia, Botswana, and South Africa). Significant outbreaks have also occurred in Japan, Korea, and Taiwan. FMDV is endemic in many South American nations, except for Chile, Uruguay, Argentina, Paraguay, and the southern states of Brazil. The World Organization for Animal Health (OIE) reports 61 countries are free of the virus. The United States is FMDV-free; all research occurs at the Plum Island Animal Disease Center.

2) Disease Symptoms (human and animal):

Foot and mouth disease affects cloven-hoofed livestock. The symptoms are indistinguishable from vesicular stomatitis (which only affects horses). General clinical signs include vesicular lesions, erosions and ulcers in the mouth, interdigital areas, on the muzzle, teats, and coronary band. In cattle the symptoms include slobbering, lameness, abortion, lethargy, nasal discharge, shivering, fever, lip smacking, and sores and blisters on the feet and mouth. Vesicles also form on the epithelium of lips, tongue, gums, nostrils, coronary bands, and interdigital space. Dairy cows exhibit reduced milk yields; young calves may die even before forming lesions. Sheep and goats show similar but much milder symptoms such as sudden acute lameness, unwillingness to move, a tendency to lie down, and vesicles on the hoof and the mouth. Infected swine display signs of acute lameness, fever, fatigue, constant squealing, and vesicles on the upper edges of the hoof, snout, nostrils, feet, coronary bands, interdigital spaces, udder, and tongue. In the rare case of human infection, symptoms may include fever, headache, malaise, excessive saliva, painful blisters in the oral cavity, and between fingers.

3) Strain Information:

There are at least seven immunologically distinct types of FMDV: O, A, C, Asia 1, SAT1 (Southern African Territories), SAT2, and the SAT3. Within these, at least 60 various subtypes have been identified. There is little virulence information on most strains. The O type appears to be the most ubiquitous, causing most African outbreaks. In Asia, the O, A, and Asia-1 strains are endemic to India, China, Bangladesh, Myanmar, Thailand, Laos, and Cambodia. Outbreaks of types A and O occur sporadically in the Middle East, Turkey, Iran, Israel, Saudi Arabia, and Kuwait.

4) Reservoirs/Vectors:

Most domestic and wild cloven-hoofed animals can be hosts, including cattle, sheep, goats, swine, water buffalo, bison, deer, elk, antelope, llamas, camels, giraffes, and elephants. Infected ruminants typically stop shedding virus after two weeks, but some may shed for six to 24 months. Rats and hedgehogs are also susceptible. Ticks and flies may carry the virus, although they are not proven causes of outbreaks.

5) Agent Sources:

Infected bovines release high titers of virus in their saliva, nasal fluid, lachrymal fluid, vesicular fluid, milk, and even their breath. FMDV is also present in urine and feces, although at lower titers. Viral loads drop four to five days after the onset of clinical disease. Fomites can also be viable sources of virus.

6) Stability:

FMDV is extremely stable in normal environmental conditions. The virus is most stable at 4°C at a neutral pH (it is very sensitive to basic or acidic pH), and under moist conditions. FMDV can remain active for 26 to 200 days in soil, on wood, hay, and straw for approximately 15 weeks, footwear and clothing up to 14 weeks, cow's hair for four to six weeks, and wool for about two weeks. Viable virus is capable of spreading at least 250 km over water and 60 km over land.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Relatively high volume of laboratory work/research (PubMed)

3) Survey Information:

27/722 (3.74%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain FMD.

4) Past Weapons Activity:

Germany had conducted BW research on FMDV. Iran is believed to have had a BW program which may still conduct research.

5) Commercial Sources:

Little is known about how many commercial culture collections may have the virus worldwide. There are many FMDV vaccine production facilities worldwide and many produce large volumes of inactivated virus vaccine.

6) General Growth Conditions:

Conventional virus culturing protocols are typically used to isolate, purify, and propagate FMDV. Fluid specimens are diluted in buffered cell culture media. Epithelial specimens should be ground with a mortar and pestle then immersed in buffered media. The solution is next clarified by centrifugation and inoculated into monolayer cell cultures of swine kidney cells, calf kidney cells or BHK-21 cells. After inoculation, Eagle's Maintenance Medium is added. If FMDV is present, cytopathic effects can be seen with a microscope. When the cytopathic effect is most pronounced, the supernatant nutrient fluid can then be harvested, centrifuged, and stored. Numerous diagnostic tests (immunofluorescence, ELISA, PCR) have been developed to detect virus in clinical specimens and culture.

Safety Factors

1) Risk of Disease Contraction:

FMD is technically considered a zoonotic disease. However, human infection is extremely rare: only 40 cases have been documented between 1921 and 1969. Even upon contraction, clinical symptoms are rarely seen. Laboratory infections are also extremely rare. Disease transmission is extremely efficient between animals, and occurs by direct contact between animals or with contaminated fomites (shoes, clothing, tires, and equipment). Aerosols also often spread the disease rapidly.

2) Countermeasures (PPE and Medical):

In the United States and other places where the virus is not endemic, FMDV work is restricted to just a few highly secure locations. Where endemic, laboratory work should occur in BSL3 facilities; infected animals should be kept in ABSL3 laboratories. Strict decontamination procedures should be adhered to. Basic PPE (gloves, laboratory coats) should be worn by staff. Vaccines are available widely for animals, however vaccination against one subtype often does not necessarily protect against others, and immunity is short-term requiring additional vaccinations two to three times a year. Thus, effective vaccination can be quite difficult. Prevention and control in endemic regions involves quarantine and vaccination. In FMD-free regions, rapid detection and slaughter of all animals within three km of outbreak is necessary. Carcasses should be burned. No vaccine or specific treatment is available in the event of human infection.

Agent: *Francisella tularensis*

Environmental Factors

1) Geographic Distribution:

F. tularensis is present widely throughout the Northern Hemisphere. It occurs throughout North America, and in many parts of continental Europe (mainly Scandinavia, the Czech and Slovak Republics, Austria, Switzerland, and Germany), the former Soviet Union, China, and Japan. The bacterium is prevalent at all times of the year in animals. Human infections are highest in winter during rabbit hunting season and during the summer when ticks and deerflies are abundant. Biovar type A strains are found predominantly in North America (reports have also surfaced in Europe recently); biovar type B has a much wider distribution, strains are found mostly in Europe, Siberia, Israel, Japan, and, to a lesser extent, in North America. *Mediasiatica* strains have been isolated in the Central Asian republics of the former Soviet Union (Kazakhstan and Turkmenistan); *novidicida* has been isolated from water samples in the United States. Only 100-200 US cases occur a year (mostly in rural areas in south central and western states); type A and type B isolates are most common, and are recovered equally often.

2) Disease Symptoms (human and animal):

Infection leads to a disease called tularemia. The initial symptoms include fever, chills, joint and muscle pain, severe headache, and weakness. Inhalation leads to pneumonic disease, with chest pain, bloody sputum, and difficulty breathing. Skin exposure results in skin ulcers and swollen, painful glands at the infection site. Drinking or eating contaminated water or food may produce a painful sore throat, diarrhea, nausea, vomiting, abdominal pain, and gastrointestinal bleeding. In animals, infection leads to acute, often lethal hemorrhagic septicemia. Chronic cases are possible in which the animals develop abscesses in the liver and spleen, with cachexia.

3) Strain Information:

There are a large number of strains worldwide, organized into four biovars (subspecies): *F. tularensis* subsp. *tularensis* (*nearctica*, biovar type A) is the most virulent of the four known subspecies (often lethal pulmonary infections); *F. tularensis* subsp. *holoartctica* (biovar type B) is of moderate virulence (rarely fatal disease); *F. tularensis* subsp. *novicida* is of relatively low virulence, as is *F. tularensis* subsp. *mediasiatica*. Biovar type A strains are associated mostly with rabbits and humans; biovar type B strains are associated with a greater variety of animals, but mostly hares and rodents. Subspecies *mediasiatica* has been found in ticks and hares, but not humans.

4) Reservoirs/Vectors:

The bacteria are maintained in nature in a cycle involving vertebrate hosts and arthropod vectors. Over 125 species of animals are susceptible, including hares, wild rabbits, beavers, hamsters, rats, mice, squirrels, foxes, and bears. Domestic animals such as cattle and sheep, as well as dogs and cats can also be infected. Numerous birds such as pheasants and quail may act as hosts. Arthropods such as ticks, deerflies, fleas, lice, and mosquitoes (in Russia) can carry the bacteria. Humans also act as a reservoir.

5) Agent Sources:

Bacterial sources include lesion exudates (for the duration of one month of infection) and pus, blood (during the first two weeks of infection), organs and lymph node biopsies, respiratory and conjunctival secretions, CSF, sputum, urine, and feces of infected animals or humans. The fluids of infected arthropods are also potential sources as ticks carry the bacteria for life. The bacteria could also be present in contaminated water and soil. Bacteria are difficult to recover from field specimens, with recovery rates from carcasses at only 30%.

6) Stability:

F. tularensis is a non-spore-forming organism that survives for long periods in water (~90 days), moist soil, straw (~192 days), animal carcasses and organs (~133 days), and in frozen meat (years). Bacteria are

susceptible to 1% hypochlorite, 70% ethanol, glutaraldehyde, and formaldehyde. Amies medium (with charcoal) commercial transport system can keep the organism viable for seven days at room temperature.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Relatively large volume of research (PubMed)

3) Survey Information:

18/722 (2.49%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *F. tularensis*.

4) Past Weapons Activity:

F. tularensis was one of the principal BW agents studied by Japan in Manchuria. Canada also conducted BW research on the organism. The Soviet Union developed *F. tularensis* weapons, producing the agent in large quantities; the United States also developed it as a BW.

5) Commercial Sources:

Little information is available.

6) General Growth Conditions:

F. tularensis is difficult to recover in culture. Chances improve when the organism is recovered from fresh tissues on nonstandard media. Fresh ulcer and wound specimens, or tissue can be inoculated directly on agar plates using swabs. Blood, aspirates, and urine need to be concentrated before plating. Often sulfhydryl compounds (cysteine, etc.) are required for growth. The bacterium will grow well on cysteine heart blood agar with 9% chocolate sheep blood (CHAB), chocolate agar, buffered charcoal-yeast extract agar, Thayer-Martin agar, and in thioglycolate broth. Bacteria grow slowly with at least a 48 hour incubation time at 37°C (aerobic conditions) is necessary. Antibiotics in media can help selection away from normal flora. Colonies are raised, gray, smooth, moist, and 2-4 mm in diameter. On CHAB, it appears white, with green tint and opalescent sheen. Serology is often used to diagnose the disease. ELISAs and PCR can effectively detect the organism in clinical specimens and in culture. It is difficult to distinguish between subspecies, but PCR methods have been developed for this purpose.

Safety Factors

1) Risk of Disease Contraction:

Bacteria are transmitted to humans by inoculation of compromised skin (even through extremely minute lesions) or mucous membranes with the blood, secretions, tissues or excretions of infected organisms. Transmission also occurs via ingestion of contaminated food or water, inhalation of dust from contaminated soil, hay or grain, and by arthropod bites. Bacteria are most frequently transmitted to humans by direct contact with infected rabbits or other rodents (particularly during the slaughter of infected hares). Tularemia is the third most commonly acquired laboratory infection. Human-to-human transmission is extremely rare. Individuals frequently in close contact with wild game animals are at high risk of infection. Hunters, cooks, and farmers are particularly at risk. The infectious dose is very small: ten to 50 organisms via skin inoculation or inhalation; approximately one million organisms by ingestion.

2) Countermeasures (PPE and Medical):

BSL3 containment and practices should be used for any clinical specimen suspected of containing the bacteria, and any culture manipulation or experimental animal studies. All clothing or linens used in *F. tularensis* work should be disinfected often. Gloves, gowns, and face masks should be worn when handling samples. Waterproof gloves should be used when handling potentially infected animals. In the field, protective clothing and tick-repellent chemicals can prevent arthropod-mediated infection. Numerous

antibiotics are effective. Streptomycin and gentamycin are frequently used, ciprofloxacin is also sometimes used, and tetracyclines are effective as well. Some strains are resistant to streptomycin. Attenuated vaccines are available. A live vaccine is available from the CDC for occupational risk groups only.

Agent: Herpes B virus

Environmental Factors

1) Geographic Distribution:

Little is known about the natural prevalence and geographic distribution of herpes B virus in primate populations. Presumably the virus is found wherever Old World monkeys (particularly macaque species) are found. Rhesus monkeys live mostly in Asia, particularly in India, southern China, and Afghanistan. Human cases are almost entirely associated with laboratory and zoo infection.

2) Disease Symptoms (human and animal):

In humans, infection results in an onset of febrile illness with headache, and vesicular skin lesions at the infection site accompanied by pain, numbness, and itching. Flu-like aches and pains develop with fever, chills, fatigue, vomiting, and cramping. Variable neurological symptoms develop quickly and may include diplopia, altered sensations, shortness of breath, muscle incoordination, paralysis, and seizures. Ascending encephalitis and myelitis occurs in >90% cases; case fatality is approximately 75%. In most monkeys, primary infection is asymptomatic or causes very mild disease; some may develop ulcers in the mouth, on the tongue, and around the face, lips, genitals, and eyes. The virus typically establishes latency in monkeys, and can spontaneously reactivate later. Virus is detectable as early as six hours post-infection, but serological testing can be difficult due to cross-reactivity with other herpes viruses. A competitive ELISA has been developed that works well and an effective PCR strategy has been developed relatively recently.

3) Strain Information:

Herpes B virus (*Herpesvirus simiae*) strains are thought to have co-evolved closely with their host species. Strains from different hosts are very closely related but can be separated into three distinct genotypes related to the macaque species of origin: rhesus and Japanese macaque isolates, cynomolgus monkey isolates; and pigtail macaque isolates. Human interaction with rhesus monkeys (*Macaca mulatta*) accounts for the vast majority of human infections, suggesting that strains associated with rhesus monkeys may be most virulent to people. Indeed, even in cases when cynomolgus monkeys were the potential sources of infection, the infected individuals were also exposed to rhesus monkeys.

4) Reservoirs/Vectors:

Herpes B infects Old World monkeys of the genus *Macaca*, particularly rhesus and cynomolgus monkeys in different parts of Asia. Japanese, Taiwan, and pigtail macaques are also often infected. The virus may possibly be present in other species as well. Little is known about the seroprevalence in wild macaque populations, although it is approximately 22% in animals younger than 2.5 years of age raised in outdoor breeding corrals, and more than 97% in animals 2.5 years of age and older. However only 2 to 3% are believed to shed the infectious virus at any given time; the reactivation of shedding occurs spontaneously but can be induced by stress, crowded conditions, immunosuppression, and pregnancy. Animals may shed the virus in the absence of visible symptoms. There are no known vectors. Rabbits, guinea pigs, and mice can be experimentally infected with the herpes B virus.

5) Agent Sources:

Animals shed the virus in oral secretions. It is also present in thoracic and abdominal viscera, blood (very low levels) and the CNS tissues of infected macaques. Lesion swabs should be collected from multiple sites and placed in viral transport medium (balanced salt solutions with protein-stabilizing agents such as gelatin or BSA, and antibiotics. Salt solutions often used include Hanks' balanced salt solution, Leibovitz-Emory medium, and Stuart's medium). A number of transport mediums are available commercially. Biopsy tissue can also be used to culture the virus. Samples should be kept very cold, on dry ice.

6) Stability:

Herpes B is an enveloped virus that is inactivated rapidly in the environment when not protected in cells. It can remain viable for some time in monkey saliva, CNS tissue, and monkey kidney cell cultures; may

survive for up to seven days at 37°C, weeks at 4°C, and is very stable at -70°C. Lipid solvents, light, heat (50-60°C for 30 min), and acidic pH rapidly inactivate the virus. The virus is susceptible to 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, and formaldehyde.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Low volume of work (PubMed)

3) Survey Information:

No survey information

4) Past Weapons Activity:

No known attempts at weaponization

5) Commercial Sources:

Very few commercial resources such as culture collections are expected to have this virus.

6) General Growth Conditions:

Mucosal swabs or CSF tissue are preferred for virus isolation; blood is less preferred. Herpes B virus can be cultured on established epithelial cell lines from humans, monkeys, rabbits, and hamsters; monkey kidney and chick embryo cells are also often used. The virus can also be amplified in human and nonhuman primary cell lines, including those used for herpesvirus culture. Several days after infection, characteristic cytopathic effects become visible. PCR can be used to detect the virus in clinical samples or in culture. Monoclonal antibodies are available for identification of herpes B in culture.

Safety Factors

1) Risk of Disease Contraction:

The herpes B virus is highly pathogenic to humans but infections are very rare. Transmission occurs from exposure of mucous membranes or broken skin to the saliva, secretions, or tissues of infected monkeys, most often administered through bites, scratches, and splashes. Aerosol infection is a potential risk. A total of approximately 40 human cases have been reported worldwide. While infection risk from macaques is actually very low, most infections have occurred from exposure to monkeys in laboratories or zoos. Person-to-person transmission has been reported once. Primary cell cultures produced from macaques can also be very dangerous. Virus spreads among monkeys via biting, sexual activity, and close contact.

2) Countermeasures (PPE and Medical):

Any work with this virus should occur in BSL4 laboratories. BSL3 laboratories may be used for handling potentially contaminated materials. Appropriate PPE, including laboratory coats, face shields (or mask/goggles), and gloves should be worn. Special care must be taken to prevent bites, scratches, or exposure by spitting when working with potentially infected animals. In the event of a bite or scratch, the wound should be washed extensively and treated promptly with 0.25% sodium hypochlorite. Acyclovir appears to be effective against the virus, although treatment is experimental (15 mg/kg every eight hours may work). Ganciclovir may be more effective, but there has still been little clinical work. Postexposure prophylaxis with valacyclovir can help prevent the development of acute disease.

Agent: Highly pathogenic avian influenza virus (HPAI)

Environmental Factors

1) Geographic Distribution:

Highly pathogenic avian influenza has circulated in wild birds and poultry throughout the world, but appears to have spread particularly rapidly in Asia. Nine Asian countries (Korea, Vietnam, Japan, Thailand, Cambodia, Laos, Indonesia, China, and Malaysia) have reported H5N1 outbreaks in birds. The virus is endemic in several of these countries but has been eliminated in Japan, Korea, and Malaysia. Bird outbreaks have also occurred in Russia, parts of Central Asia, and the Middle East, and Africa. Thus far, the virus has killed millions of birds. There has been 291 human cases (in Azerbaijan, Cambodia, China, Djibouti, Egypt, Indonesia, Iraq, Laos, Nigeria, Thailand, Turkey, and Vietnam). Indonesia has particularly been hit hard in recent years and has experienced the largest number of human cases.

2) Disease Symptoms (human and animal):

The clinical symptoms vary greatly depending on the bird species infected, the virus strain, host immune system, and environmental conditions. The typical clinical signs are nonspecific and include severe lethargy, loss of appetite, and vast decline in egg production. Sudden death may occur in the absence of symptoms. Mortality is close to 100%. In chickens, symptoms also include dehydration, nasal and oral discharge, respiratory congestion, respiratory tract and gizzard hemorrhages, and diarrhea. Diagnosis can be difficult; avian influenza is often misdiagnosed as Newcastle disease or cholera. Some bird species may shed the virus without any clinical signs (e.g. ducks appear unaffected). In humans, the virus causes severe influenza and pulmonary symptoms.

3) Strain Information:

Only H5 and H7 avian influenza A subtypes are highly pathogenic to domestic poultry. H5 and H7 strains are capable of infecting humans; H5N1 appears to be particularly virulent and pathogenic. Influenza viruses mutate very quickly. New strains continually arise, and the prevalence of circulating strains constantly change. Numerous strains have been shown to be resistant to amantadine and rimantadine—drugs that are often used to treat human illness.

4) Reservoirs/Vectors:

All birds are thought to be susceptible to some extent. The H5N1 virus has been detected in hundreds of species thus far. Poultry such as chickens and turkeys are particularly susceptible. Migratory waterfowl, notably wild ducks, appear to be resistant to disease and are widely considered to be significant reservoirs. Numerous mammals can be infected, including pets like cats and dogs as well as mice, ferrets, guinea pigs, macaques, and humans.

5) Agent Sources:

The virus replicates quickly in the GI tract of birds causing systemic infection and severe respiratory symptoms. The virus is present in respiratory fluids and feces (birds may excrete the virus for ten days or more), and can be collected by taking tracheal, oropharyngeal or cloacal swabs from live (or recently deceased) birds. Cloacal swabs are most likely to yield virus. Organs including the trachea, lungs, air sacs, intestine, spleen, kidney, brain, liver, and heart can be processed and may contain high virus concentrations. Equipment, vehicles, feed, cages, or clothing contaminated with infected excretions are potential sources. Cage swabs (swabs of fresh fecal matter) may also provide appropriate material. Cages housing mixed species are more likely to harbor virus.

6) Stability:

HPAI viruses can survive for long periods in the environment, especially at colder temperatures.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Relatively high volume of laboratory/academic research (PubMed)

3) Survey Information:

49/722 (6.79%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain HPAI. More than half—26 of the 49—are in Asia (300 laboratories total).

4) Past Weapons Activity:

No evidence of any BW research.

5) Commercial Sources:

Little is actually known, but a number of sources are expected to have viral samples because of the high prevalence worldwide.

6) General Growth Conditions:

Specimens (tissues, feces, tracheal, and cloacal swabs, etc.) containing virus should be placed in isotonic PBS (pH 7.0–7.4) containing antibiotics (penicillin, streptomycin, gentamycin, mycostatin; higher concentrations for feces samples). Feces and finely minced tissues should be prepared as 10–20% (w/v) suspensions. All samples should be kept cold. The conventional method of growing, isolating, and characterizing avian viruses requires inoculation of embryonated specific pathogen free (SPF) fowl eggs, or specific antibody negative (SAN) eggs, followed by the infection of chickens with isolated virus. These procedures can be slow, labor intensive, and expensive. Feces or tissue homogenate suspensions are clarified by centrifugation, and the supernatants are inoculated into the allantoic sac of at least five 9–11 day old embryonated SPF or SAN fowls eggs. The eggs are then incubated for four to seven days at 35–37°C, chilled to 4°C, and the allantoic fluids tested for hemagglutination activity (indicative of Influenza A) or specific H5 subtype. This process is not trivial. It generally requires a host of antibodies for serology, or RT-PCR). Also, numerous kits have been developed to detect H5N1 in clinical samples, but the specificity and sensitivity are often not well tested. The virus may be concentrated from fluid by ultracentrifugation or precipitation under acidic conditions. Pathogenicity can be determined by sequencing genomic segments; but more commonly, a sample is tested by injection in chickens. HPAI is lethal to at least six of eight 4–8 week-old chickens within ten days following injection with infective allantoic fluid.

Safety Factors

1) Risk of Disease Contraction:

Human contraction is very rare. Humans appear to have an elevated risk of infection if they have close contact with sick birds, typically poultry, and contaminated surfaces. Infection may also occur by ingestion of undercooked contaminated meat. Infection through indirect contact with birds (at wet markets, etc.) may be possible, but unlikely. Human-to-human transmission also appears very unlikely. Virus spreads to birds via contact with contaminated excretions. Increased transmission is expected where many birds of numerous species are forced into close proximity, such as at migratory lakes where birds congregate or at wet markets.

2) Countermeasures (PPE and Medical):

H5N1 should generally be handled in BSL3 enhanced conditions. Clinical specimens can be tested using standard BSL2 practices in a class II biosafety cabinet. Gloves and masks should be worn at all times. No H5N1 vaccines are available for humans currently, however numerous inactivated, live attenuated, recombinant, and DNA vaccines are in various stages of development. Prophylactic antiviral drugs such as

Tamiflu are effective and available for purchase and can typically be used for both prevention and treatment. Some virus strains exhibit resistance to the common drugs amantadine and rimantadine, but oseltamivir and zanamivir appear to remain largely effective.

Agent: Junin virus

Environmental Factors

1) Geographic Distribution:

Argentinean hemorrhagic fever is present in the rural regions of South America, wherever the rodent reservoir lives. The disease is endemic principally in four provinces in north-central Argentina: Buenos Aires, Cordoba, Santa Fe, and La Pampa. The affected area has been expanding and covers around 58,000 square miles. Each year, several hundred to several thousand cases are reported in these regions; farm workers are especially affected, and men contract the disease four times as often as women. Disease occurs year-round, but infections peak during the annual grain harvest from March to June. The agricultural area of the humid “pampas” in the north-west province of Buenos Aires, 200 km west of the capital city, has a high incidence of disease. Incidence cycles with fluctuations in rodent populations.

2) Disease Symptoms (human and animal):

Disease signs are almost identical to those caused by other New World hemorrhagic fever arenaviruses such as Machupo, Guanarito, and Sabia; clinical symptoms are nonspecific and difficult to diagnose. Initial disease symptoms include gradual onset of fever, malaise, headache, back and muscular pains, anorexia; nausea and vomiting occurs in approximately half the cases. In most cases, the face becomes flushed, as does the neck and chest. The patient dehydrates and blood pressure decreases, urination is infrequent and the heart slows. Blood spots on the upper trunk and the oral mucosa may begin to appear; bleeding begins to occur from the nose, gums, and GI tract, oozing from puncture wounds. Severe cases lose enough blood to go into hypotensive shock. Many exhibit neurological signs that range from mild irritability, abnormalities in gait, and tremors of the upper extremities to delirium, convulsions, and coma. The mortality rate ranges between 10-20%. Acute disease may last two to three weeks; clinically asymptomatic infections are rare.

3) Strain Information:

A large number of strains have been isolated from human and rodent populations. A broad spectrum of virulence has been observed in animal models for wild and laboratory strains.

4) Reservoirs/Vectors:

Junin virus causes chronic viremia in *Calomys musculus* and *Calomys laucha* rodents, which are the most important natural disease reservoirs; species also carry the disease. *C. laucha* rodents live in corn fields. High prevalence (between 5-57%) has been reported for populations of *C. musculus*.

5) Agent Sources:

Significant titers of the virus are shed in the saliva, secretions, and excretion of rodents. The virus may be isolated from organs, body fluids, and urine. From humans, blood/serum taken during the febrile stage is the best source of virus; mucosal secretions and throat wash specimens can also be successfully used. Postmortem spleen, kidney, liver, lymph nodes, and clotted blood can also be used.

6) Stability:

Junin is an enveloped virus, but can be rather stable in blood kept at 4°C. Near complete inactivation of the virus occurs after 192 hours at 4°C, 72 hours at 25°C, 26 hours at 37°C, or ten minutes at 56°C. Junin virus is sensitive to lower pH; inactivation is practically complete after six hours at a pH of 4.5. Junin virus is also rapidly inactivated with ultraviolet radiation, drying, 1% sodium hypochlorite, or 2% glutaraldehyde.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Few laboratories with access/research to agent – most research may be expected in South American laboratories.

3) Survey Information:

No survey information.

4) Past Weapons Activity:

The Soviet Union and the United States had conducted BW research on Junin virus.

5) Commercial Sources:

Little is known; few sources are expected to have this virus.

6) General Growth Conditions:

Virus isolation from clinical samples takes time as it is dangerous and often not successful. Clinical secretions, bodily fluids or clarified tissue homogenates (10% [w/v]) are diluted in maintenance media and used to inoculate Vero cell monolayers which develop distinctive intracytoplasmic inclusion bodies. Isolation and identification of the virus typically takes around five days. Vero cells are grown in Eagle's minimal essential medium (MEM) containing 5% inactivated calf serum and 50 mg/ml gentamycin. Maintenance medium, pH 7.5, consists of MEM supplemented with 1.5% calf serum and gentamycin. The virus is not difficult to continue to propagate in tissue culture. The isolation frequency of Junin virus can be increased by co-culturing peripheral blood mononuclear cells (taken from patients) with Vero cell monolayers. Historically, Junin was isolated by intracranial inoculation of newborn hamsters or mice; young adult guinea pigs have also been used. Animals typically die after an incubation of seven to 20 days. Various immunohistochemistry, RT-PCR, and antigen-capture ELISA strategies have been developed that can specifically detect Junin virus in clinical samples (blood and sera) and in tissue culture supernatant.

Safety Factors

1) Risk of Disease Contraction:

Normally transmitted to people through the inhalation of aerosols generated from rodent urine, feces, and other excreta; ingestion of contaminated food; or direct contact of rodent excreta or body fluids with broken skin or mucus membranes. Person-to-person transmission is rare, but can occur from direct contact with infectious blood and bodily fluids. Nosocomial epidemics (and family infections) have also been observed.

2) Countermeasures (PPE and Medical):

BSL4 containment and practices are required for any diagnostic work or research involving live agent. In the field, minimal protection should include the use of gowns, gloves, leg/shoe coverings, goggles/face shields, and N-95 masks (or some other respiratory device). Strict disinfection procedures may prevent the further spread of illness significantly; barrier nursing procedures must be put into effect in outbreak events. Immediate administration of immune plasma from recovered patients and ribavirin after suspected infection greatly lowers mortality. Replacement therapy is used only in severe hemorrhagic cases. An attenuated live vaccine (Candid #1) was developed jointly by the United States and Argentine governments and proven to be highly effective with no negative side-effects observed. The Candid #1 vaccine is produced in both countries.

Agent: Lassa virus

Environmental Factors

1) Geographic Distribution:

Lassa fever is endemic to West Africa with estimates of 300,000 to 500,000 infected each year. The disease is prevalent in the countries of Guinea, Liberia, Sierra Leone, and Nigeria, but it is probably endemic in additional countries. The proportion of all hospital admissions is 10-16% in Sierra Leone, 0%-15% in Guinea, and 14.3% in Liberia. Outbreaks of disease are sporadic and unpredictable; those in rural, crowded areas with poor sanitation are at greatest risk. The rodent reservoir range extends further into sub-Saharan Africa, suggesting that human cases of Lassa fever may be underreported. Prevalence in rodent populations can vary greatly; in one study in Guinea, prevalence ranged from 0-9%, suggesting focal hotspots.

2) Disease Symptoms (human and animal):

Approximately 80% of human infections are asymptomatic; the remaining cases develop a severe disease that lasts for one to four weeks and affects several vital organs including the liver, spleen, and kidneys. Initial symptoms are nonspecific and difficult to diagnose: sore throat, sharp lower back pain, and conjunctivitis. Symptoms progress to include nausea, vomiting, diarrhea, abdominal and chest pain, headache, cough, and dizziness. In severe cases, fever persists. Swelling of the head and neck may occur, as well as low blood pressure; a maculopapular rash may also develop. In 15-20% of cases, hemorrhage with bleeding from the mouth, nose, vaginal, and/or GI tract may occur. The illness may eventually lead to shock, tremors, and coma. The mortality rate is 15-20%. Few infected individuals exhibit neurological symptoms during disease course, but survivors may suffer from blindness or deafness.

3) Strain Information:

A large number of strains have been isolated from Western Africa. These strains comprise four lineages; three are found in Nigeria, the fourth is found in Guinea, Liberia, and Sierra Leone. Overall genetic diversity among strains—even within the same lineage—is very high; genetic distances correlates with geographic distance, suggesting that strains may co-evolve closely with local rodent populations or species and these rodents do not migrate far. Little is known about the relative virulence of the different lineages; but isolates that are indistinguishable antigenically often differ in virulence.

4) Reservoirs/Vectors:

Rodents of the *Mastomys* genus (multimammate rats), particularly *M. natalensis*, are the natural reservoir. It is unknown how many rodent species carry the virus. When infected, these rodents are asymptomatic and shed the virus persistently. Most infected humans (asymptomatic cases) do not shed the virus.

5) Agent Sources:

Lassa virus is present in the saliva, urine, and feces of infected rodents. In humans, the agent can be isolated from body fluids and secretions (blood, urine, pleural fluid, and mucosal secretions). The virus may be detected in urine one month after symptoms subside. From humans, blood/serum (taken up to 14 days post-onset of symptoms, after antibodies begin to form) is the best source of virus; throat wash specimens can also be successfully used. The virus is present in almost all the organs of infected humans and rodents; postmortem spleen, kidney, liver, pancreas, lymph nodes, and clotted blood can also be used for isolation. The placentas of infected pregnant women often yield virus. Lassa virus is recovered successfully from clinical specimens more frequently than most arenaviruses.

6) Stability:

Lassa is an enveloped virus and not very stable in the environment. It is readily inactivated by conditions of acidic pH, 0.5% sodium hypochlorite, 2% glutaraldehyde, and phenolic disinfectants. Soaps and detergents also rapidly inactivate the virus.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Few laboratories with access/research -- low to moderate amount (PubMed)

3) Survey Information:

No survey information

4) Past Weapons Activity:

Both the United States and the Soviet Union had conducted BW research on Lassa virus.

5) Commercial Sources:

Little is known, but few sources are expected to have Lassa virus since it is a dangerous BSL4 agent with a limited geographic distribution to just a handful of Western African countries.

6) General Growth Conditions:

Clinical secretions and bodily fluids or clarified tissue homogenates (10% [w/v]) are diluted in maintenance media and used to inoculate Vero or Vero E6 cell monolayers. Vero E6 monolayers are cultured in Eagle's minimal essential medium supplemented with 2% fetal bovine serum, L-glutamine, and antibiotics. Virus isolation typically takes about a week or more. Cytopathic effects are seen in culture but usually disappear upon further passage. In addition, effective isolation of Lassa virus has been accomplished by co-culturing lymphocytes (taken from infected animals) with Vero cell monolayers, even late during the disease after the neutralizing antibody appeared. Traditionally, mice and guinea pigs had been used to amplify the virus from serum and other clinical specimens. Animals typically die 12-18 days post inoculation. This is not practical, though, because of the inherent danger. Various immunohistochemistry, antigen-capture ELISA, and RT-PCR strategies have been developed that detect virus in clinical samples or in culture supernatant. RT-PCR works well on serum samples but primers must be designed carefully due to genetic variation among strains.

Safety Factors

1) Risk of Disease Contraction:

Normally transmitted to people through the inhalation of aerosols generated from rodent urine, feces, and other excreta; ingestion of contaminated food; or direct contact of rodent excreta or body fluids with broken skin or mucus membranes. Person-to-person transmission is rare, but can occur from direct contact with infectious blood and bodily fluids; nosocomial epidemics (and family infections) have been observed. Infectious dose is low: one to ten organisms.

2) Countermeasures (PPE and Medical):

BSL4 containment and practices are required for any diagnostic work or research involving live agent. In the field, minimal protection should include the use of gowns, gloves, leg/shoe coverings, goggles/face shields, and N-95 masks (or some other respiratory device). Strict disinfection procedures may prevent the further spread of illness significantly. Barrier nursing procedures must be put into effect in outbreak event. There are no licensed vaccines for Lassa fever; however there is a promising experimental vaccine that is not widely available. Ribavirin, if administered early, can significantly reduce the mortality rate.

Agent: Marburg virus

Environmental Factors

1) Geographic Distribution:

Natural outbreaks of Marburg have been rare and sporadic, occurring in mostly small, isolated cases in Africa (Kenya, Zimbabwe, the Democratic Republic of the Congo, and Angola). However, there have been larger outbreaks, with the most recent occurring in Uige, northern Angola (2005) which caused a total of 252 cases and 227 deaths. The distribution, while overlapping somewhat with Ebola virus, appears to include drier savanna zones of southern and western Africa.

2) Disease Symptoms (human and animal):

Onset of illness is sudden, with fever, headache, joint and muscle aches, sore throat, and weakness early during disease course. This is followed by diarrhea, vomiting, and abdominal pain. By the 5th day of illness, a maculopapular rash develops on the arms, legs and trunk. Mucous membrane hemorrhages subsequently appear. Patients may also have red eyes, hiccups, and delirium. Bleeding may develop in the GI tract, and in 40-50% of patients, extensive internal and external hemorrhaging occurs. Patients may have vacant facial expressions, breathe rapidly, and eventually go into shock or coma before death. Ebola virus causes very similar symptoms. Disease progression is similar in primates (nonspecific early on, followed by extensive hemorrhaging).

3) Strain Information:

There is only one known species of Marburg virus (Lake Victoria), but several strains have been isolated and genetically distributed into two distinct lineages. Two often studied strains are the Popp strain (originally isolated in 1967 in Marburg, Germany) and the Musoke strain (isolated in 1980 in Kenya). Most known strains produce very lethal infections, although the rapidity of disease course may vary and the case fatality rates have generally ranged from approximately 23-50%. The Angola strain from the 2005 outbreak has a fatality rate of 90% and preliminary experiments suggest that strain causes more rapid and severe disease than other strains.

4) Reservoirs/Vectors:

The virus reservoir has not been definitively identified. Evidence suggests that African fruit and insectivores bats may harbor the virus asymptomatically based on two observations: 1) two individuals independently contracted Marburg after visiting the bat-infested Kitum cave in Kenya on separate occasions; and 2) serological data has implicated bats as the likely reservoir for Ebola, a very similar filovirus.

5) Agent Sources:

Marburg virus is present in the bodily fluids, blood, urine, secretions, semen, and tissues of infected (or deceased) people or primates. Blood (serum) collected during acute febrile stages of illness usually yields virus in culture. However, various other specimens including throat washings, saliva, urine, tissue effusions, and semen have also yielded the virus, even during later stages of disease. The virus can usually be isolated from the skin, spleen, lymph nodes, liver, and kidneys of infected humans and primates.

6) Stability:

Marburg virus is relatively stable for a lipid-enveloped virus. It can survive for a week or two in blood specimens at room temperature and in corpses. It is susceptible to 2% sodium hypochlorite, 2% glutaraldehyde, and formalin, ultraviolet irradiation and drying and is inactivated by heating to 60°C for an hour.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Few laboratories with access/research; moderate volume of research (PubMed)

3) Survey Information:

Only 4/722 (0.55%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain filoviruses (Ebola or Marburg). It is uncertain how many contain strains nonpathogenic to humans (such as Ebola Reston).

4) Past Weapons Activity:

The Soviet Union weaponized Marburg virus and produced large quantities for BW use.

5) Commercial Sources:

Marburg virus is a very dangerous BSL4 agent with a natural geographical distribution limited to Africa; thus only in a handful of collections in the world will likely have samples.

6) General Growth Conditions:

Isolation in cell culture is the most reliable mode of disease diagnosis, but serology can be used to detect infection in blood. Virus can be isolated from human blood samples and tissues (clarified 10% homogenate) by inoculation of guinea pigs or by passage in cell culture. Marburg will grow vigorously in a variety of cell lines—Vero and Vero E6 cells are used frequently, but PK 15, MDCK, and BHK cells can also be used—upon inoculation with infected serum, or other bodily fluid or tissue extract. Virus propagation is optimal at 37°C in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin, streptomycin, and L-glutamine—following readily available protocols. For virus propagation, DMEM with 2% fetal calf serum should be used. Cytopathic effects are seen about a week later. Electron microscopy can detect filovirus particles in clinical specimens and culture supernatants; indirect immunofluorescence, immunohistochemistry, ELISAs, and RT-PCR can be used to specifically detect Marburg.

Safety Factors

1) Risk of Disease Contraction:

Marburg infection occurs from direct contact of mucous membranes or broken skin with the virus particles present in infected body fluids, tissues, and blood. Natural human infection is rare, overall, and most outbreaks are traced to a single index case of an individual handling an infected animal carcass. This virus is not transmissible during incubation periods or during the onset of symptoms. Disease has often spread during outbreaks among healthcare workers, and in family members who have prepared dead relatives for burial; poor barrier nursing also often accounts for virus spread. The virus is highly infectious in the laboratory and has the potential to spread by small-particle aerosols generated during virus manipulation. A very small number of virions are required for infection via the aerosol route.

2) Countermeasures (PPE and Medical):

BSL4 facilities, equipment, and practices should be used for any handling of potentially infectious material; biosafety cabinets for any procedures that may produce aerosols. In hospitals, infection control practices should be implemented that reduce contact with patients and the production of droplets. Medical staff should wear eye protection or face shields, impermeable gowns, shoe covers and caps, gloves, and N95 masks or powered respirators. Supportive care with monitoring and maintenance of electrolyte balance, intravascular volume, and blood pressure should be done; prevention of hemorrhage and shock is the only treatment at this time. Ribavirin appears effective *in vitro*, but is probably ineffective as a prophylactic drug. There are no licensed Marburg vaccines but new experimental vaccines appear effective in primates—for example, an attenuated vaccine that expresses the Marburg virus glycoprotein from a recombinant vesicular stomatitis virus completely protects cynomolgus monkeys.

Agent: Monkeypox virus

Environmental Factors

1) Geographic Distribution:

Monkeypox was first reported in the Democratic Republic of the Congo (DRC) (formerly Zaire) in 1970 and most cases still occur here. Since then it has also been observed in Liberia, the Ivory Coast, Sierra Leone, Nigeria, Benin, Cameroon, and Gabon. Little is known about the actual geographic distribution and epidemiology of monkeypox virus in nature; it is believed to be endemic only to Central and West Africa. The ecological niches of these regions are similar: both are at low elevations, are forested, and have high rainfall. The prevalence appears to be higher in Central African countries where over 90% of all cases have been reported; only a few cases have been reported in West Africa. Overall, the disease in humans is sporadic and rare—from 1986-1992 there were only 13 reported cases—but it appears to be increasing. In 1997, the largest outbreak of monkeypox (419 cases) was reported in the Kasai Region of the DRC. In 2003, imported Gambian giant rats, carrying the West African strain, infected prairie dogs which subsequently caused 71 human infections in Kansas, Illinois, Indiana, Missouri, and Wisconsin. Little is known about the prevalence in animals—it has only been isolated once from a wild animal (a moribund rope squirrel) in the DRC—or how species may act as a reservoir.

2) Disease Symptoms (human and animal):

The clinical symptoms are similar to smallpox but much milder; lymph nodes are enlarged in monkeypox but not smallpox. Flu-like initial symptoms include fever, headache, backache, chills, sore throat, cough, and lethargy, followed by severe lymphadenopathy. Between one and ten days later, a rash with macules and papules develops, which gradually changes into vesicles and pustules (so-called “pocks”); eventually the pocks scab and fall off. The rash often originates on the face and neck but subsequently spreads centrifugally over the rest of the body. The highest concentration of pocks is on the extremities. Disease course takes two to four weeks; lesions resolve in two to three weeks. Monkeypox in nonhuman primates begins with fever, then a self-limiting rash develops over the entire body; the rash pustulates and eventually scabs. In severe cases, animals may have lymphadenopathy, a cough, discharge from nostrils, anorexia, and oral ulcers. Rodents and rabbits develop fever, conjunctivitis, nasal discharge, cough, lymphadenopathy, and limited lesions.

3) Strain Information:

Strains are divided into two clades: Central African and West African. Strains can vary significantly in virulence to human and nonhuman primates. Central African strains appear to be more transmissible between people (approximately 73% transmission rate), cause more severe disease, and substantially higher case fatality rates. This may be due to genetic differences in the virulence of orthologous gene families located at the terminal regions of the genome (West African strains lack three genes.) The 1997 DRC outbreak involved a strain with more efficient human-to-human transmission that caused more severe disease.

4) Reservoirs/Vectors:

The natural reservoirs and full host range of monkeypox virus are unknown. The virus has only been isolated once from a wild animal—a moribund rope squirrel. Monkeypox virus can infect Old and New World monkeys (rhesus macaques, cynomolgus monkeys, African green monkeys, gibbons, baboons, squirrel monkeys), apes (chimpanzees, orangutans, gorillas), various rodents (rats, mice, squirrels, prairie dogs), and rabbits. Rodents are proven vectors to humans.

5) Agent Sources:

The virus can be isolated by collecting rash pustule and scar swabs, throat and nasopharyngeal swabs, or collecting blood, respiratory fluids, or scabs. Contaminated bedding and clothing may also be a good source of virus. The livers and spleens of infected animals have high virus titers; also from the blood and oropharyngeal swabs a few days post-infection. Thus far, virus strains have been isolated from over 30 human clinical specimens.

6) Stability:

The monkeypox virus is relatively resilient to environmental conditions; it can withstand dry conditions at high and low temps. The virus will remain active after 20 minutes at 40°C; freeze-thawing has little effect on infectivity. Treatment with SDS, chloroform, methanol, and phenol inactivates the virus.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Relatively low volume of laboratory work (PubMed)

3) Survey Information:

5/722 (0.69%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain monkeypox.

4) Past Weapons Activity:

The Soviet Union conducted BW research on the virus and considered weaponizing it.

5) Commercial Sources:

Little is known. Collections and clinics in Africa, where monkeypox is endemic, may have virus samples.

6) General Growth Conditions:

Lesion fluids (and overlying skin) and scabs (two to four) are suitable for virus isolation. Suspensions of pustule samples and scab extracts can be used to inoculate a variety of established cell culture lines: Vero, BS-C-1, CV-1, LLCMK-2, monkey kidney cells, human embryonic lung fibroblast cells, and MRC-5 cells. Traditionally, the virus was grown and isolated on the chorioallantoic membranes (CAM) of 12-day-old chicken embryos; protocols are readily available. The virus is easy to identify, as it produces pocks on the CAMs. Electron microscopy can detect the characteristic virus particles in clinical specimens. PCR, ELISAs, and immunohistochemistry can also be used to detect virus in clinical specimens, in cell culture, and chicken embryos.

Safety Factors

1) Risk of Disease Contraction:

Monkeypox transmission to humans occurs principally (almost all cases) from direct contact of skin abrasions or mucus membranes with the lesions, blood, and bodily fluids of infected animals. Animal bites and aerosols are also possible. Transmission between people is not very efficient generally; it occurs from skin-to-skin contact or in aerosols. Transmission rates between people have been estimated to be 3.3-30%, as compared to around 70% for smallpox. However, recent outbreaks have exhibited higher rates of human-to-human transmission and more severe symptoms: an example is the 1997 DRC outbreak with a 73% transmission rate. Very little is known about disease spread between wild animals.

2) Countermeasures (PPE and Medical):

BSL3 containment and practices should be used for any laboratory work with this virus. PPE should include laboratory coats, gloves, and goggles. Treatment is normally supportive; cidofovir (a systemic antiviral that targets most DNA viruses) is effective *in vitro* in animals, although some monkeypox strains are somewhat resistant. Vaccinia vaccination can significantly prevent infection and reduce symptoms (85% protective efficacy); laboratory researchers in the United States are routinely vaccinated. Post-exposure vaccination should also be beneficial although it is largely untested.

Agent: *Mycobacterium tuberculosis*

Environmental Factors

1) Geographic Distribution:

Mycobacterium tuberculosis is an aerobic, nonmotile and non-spore-forming rod-like bacillus. The *Mycobacterium tuberculosis* complex that causes tuberculosis (TB) in humans includes *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti*. There is currently a global pandemic of *M. tuberculosis*; estimates suggest that one-third, or two billion people are infected. Ninety-five percent of new cases and 98% of deaths occur in developing nations. Particularly susceptible populations include the poor, elderly, and overcrowded populations. Prisoners, alcoholics, intravenous drug users, and immuno-compromised subpopulations, especially those infected with HIV/AIDs, are at extreme risk. Just 22 developing countries, mostly in Southeast Asia and sub-Saharan Africa, carry 80% of the world's TB burden. In 2006, 13,767 TB cases were reported within the United States, a decline from 2005.

2) Disease Symptoms (human and animal):

The clinical features of pulmonary tuberculosis include a prolonged cough with thick, often bloody sputum, fatigue, weight loss, night sweats, low-grade fever, dyspnea, and chest pain. Tuberculosis can also affect the central nervous system, the lymphatic system, the circulatory system, the genitourinary system, bones, joints, and skin.

3) Strain Information:

There is growing evidence that there are differences between tuberculosis clinical strains from distinct geographic regions. The Beijing family of *Mycobacterium tuberculosis* strains has been associated with epidemic spread and an increased likelihood of developing drug resistance. This family is prevalent throughout Asia but has been reported on all continents. There are many strains that appear to be more virulent in the macrophage and animal models such as the common laboratory strains, H37Rv and H37Ra.

4) Reservoirs/Vectors:

Humans are the normal reservoir.

5) Agent Sources:

Tubercle bacilli may be present in sputum, gastric lavage fluids, cerebro-spinal fluid, urine, and a variety of tissues.

6) Stability:

Mycobacteria are able to survive, without active replication, for weeks to months on inanimate objects if protected from sunlight. They are easily killed by heat (>65°C for at least 30 min) and by ultraviolet rays. The organism has a thick, lipid-rich cell wall that renders resistance to harsh treatments (including alkali and detergents) making hospital and laboratory facility decontamination difficult. *M. tuberculosis* is also not affected by freezing or desiccation. *M. tuberculosis* is incapable of replication in the inanimate environment.

Laboratory Factors

1) Security Classification:

Not a US Select Agent

2) General Research Volume:

Large volume of scientific research (PubMed)

3) Survey Information:

93/722 (12.88%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *M. tuberculosis*.

4) Past Weapons Activity:

There is no evidence that *M. tuberculosis* has been used to produce a biological weapon.

5) Commercial Sources:

Many commercial sources and laboratories—research and clinical—have samples.

6) General Growth Conditions:

Compared to other bacteria, the growth of most mycobacterial species is slow, with generation times of up to approximately 20 hours on commonly used media. Most species adapt readily to growth on relatively simple substrates. The optimum incubation temperature is 35-37 °C in 5-10% CO₂ or ambient atmosphere. Depending on the species, visible colonies may appear as soon as a few days to up to six weeks after incubation under optimum conditions.

Safety Factors

1) Risk of Disease Contraction:

M. tuberculosis is carried by airborne droplet nuclei of approximately 1-5 microns in diameter. The bacteria are spread when patients with pulmonary tuberculosis cough, sneeze or talk. Particles are suspended in the air infecting susceptible persons via inhalation and establishing infection preferentially in the lungs. Thus, close contact with actively infected people is the most common source of disease contraction. Immuno-compromised people, especially those with HIV/AIDS infection and drug users, are at extreme risk.

2) Countermeasures (PPE and Medical):

Because of the low infectious dose (i.e., ID₅₀ <10 bacilli) and the potential for aerosolization of *M. tuberculosis*, clinical and laboratory samples must be considered potentially infectious and handled with appropriate precautions. BSL2 practices and procedures, containment equipment, and facilities should be used. All aerosol-generating activities must be conducted in a biosafety cabinet. BSL3 practices, containment equipment, and facilities should be used for the propagation and manipulation of cultures. Medical countermeasures include treatment with first-line antibiotics for several months.

Agent: MDR *Mycobacterium tuberculosis*

Environmental Factors

1) Geographic Distribution:

During the 1990s, multi-drug resistant (MDR) *Mycobacterium tuberculosis* emerged as a global threat. MDR strains are defined as being resistant to at least two first-line antibiotics, isoniazid and rifampin. MDR-TB treatment usually requires the extensive use of costlier and more toxic second-line drugs. While the true global burden of MDR *M. tuberculosis* is largely unknown, strains are most likely present all over the world. Regions of the world with high MDR-TB burden include Eastern Europe, South and Southeast Asia, sub-Saharan Africa, and the Western Pacific region. In 2000, three countries—China, India, and Russia—reportedly accounted for 62% of a total of 424, 000 cases worldwide.

2) Disease Symptoms (human and animal):

The symptoms of MDR-TB are the same as wild-type *Mycobacterium tuberculosis*.

3) Strain Information:

Little information is available on the characteristics of different MDR-TB strains.

4) Reservoirs/Vectors:

Humans are the normal reservoir.

5) Agent Sources:

Tubercle bacilli may be present in sputum, gastric lavage fluids, cerebro-spinal fluid, urine, and in a variety of tissues. *M. tuberculosis* is incapable of replication in the inanimate environment.

6) Stability:

Mycobacteria are able to survive, without active replication, for weeks to months on inanimate objects if protected from sunlight. Bacteria are easily killed by heat (>65°C for at least 30 min) and by ultraviolet rays. The organism has a thick, lipid-rich cell wall that renders resistance to harsh treatments (including alkali and detergents) making hospital and laboratory facility decontamination difficult. *M. tuberculosis* is also not affected by freezing or desiccation. *M. tuberculosis* is incapable of replication in the inanimate environment.

Laboratory Factors

1) Security Classification:

Not a US Select Agent

2) General Research Volume:

Moderate volume of scientific research (PubMed)

3) Survey Information:

93/722 (12.88%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *M. tuberculosis*. Some of these strains are likely MDR.

4) Past Weapons Activity:

There is no evidence that MDR-TB strains have been used to produce biological weapons.

5) Commercial Sources:

Little is known

6) General Growth Conditions:

Compared to other bacteria, the growth of most mycobacterial species is slow, with generation times of up to approximately 20 hours on commonly used media. Most species adapt readily to growth on relatively simple substrates. Optimum incubation temperature is 35-37 °C in 5-10% CO₂ or ambient atmosphere. Depending on the species, visible colonies may appear after a few days to six weeks of incubation under optimum conditions.

Safety Factors

1) Risk of Disease Contraction:

Close contact with infected individuals is the most common route of disease contraction. Immuno-compromised people, especially those with HIV/AIDS infection and drug users, are at extreme risk.

2) Countermeasures (PPE and Medical):

Because of the low infectious dose (i.e., ID₅₀ <10 bacilli) and the potential for aerosolization of *M. tuberculosis*, clinical and laboratory samples must be considered potentially infectious and handled with appropriate precautions. BSL2 practices and procedures, containment equipment, and facilities should be used. All aerosol-generating activities must be conducted in a biosafety cabinet. BSL3 practices, containment equipment, and facilities should be used for the propagation and manipulation of cultures. Medical countermeasures include treatment with second-line antibiotics for several months.

Agent: XDR *Mycobacterium tuberculosis*

Environmental Factors

1) Geographic Distribution:

Extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis* are relatively rare. In addition to isoniazid and rifampin resistance, XDR strains are resistant to any fluoroquinolone and at least one of the three following injectable drugs: capreomycin, kanamycin, and amikacin. The emergence of XDR-TB was first documented in 2005 and while it is still rather rare, the prevalence appears to be increasing; however, its distribution worldwide remains unknown.

2) Disease Symptoms (human and animal):

Symptoms of XDR-TB are the same as wild-type *Mycobacterium tuberculosis*.

3) Strain Information:

Little information is available on the characteristics of different XDR-TB strains.

4) Reservoirs/Vectors:

Humans are the normal reservoir.

5) Agent Sources:

Tubercle bacilli may be present in sputum, gastric lavage fluids, cerebro-spinal fluid, urine, and in a variety of tissues.

6) Stability:

Mycobacteria are able to survive, without active replication, for weeks to months on inanimate objects if protected from sunlight. They are easily killed by heat (>65°C for at least 30 min) and by ultraviolet rays. The organism has a thick, lipid-rich cell wall that renders resistance to harsh treatments (including alkali and detergents) making hospital and laboratory facility decontamination difficult. *M. tuberculosis* is also not affected by freezing or desiccation. *M. tuberculosis* is incapable of replication in the inanimate environment.

Laboratory Factors

1) Security Classification:

Not a US Select Agent

2) General Research Volume:

Small volume of scientific research (PubMed)

3) Survey Information:

93/722 (12.88%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *M. tuberculosis*. A very small percentage of these may be XDR strains.

4) Past Weapons Activity:

No evidence that XDR strains of *M. tuberculosis* have been used to produce biological weapons.

5) Commercial Sources:

Little is known

6) General Growth Conditions:

Compared to other bacteria, the growth of most mycobacterial species is slow, with generation times of up to approximately 20 hours on commonly used media. Most species adapt readily to growth on relatively simple substrates. Optimum incubation temperature is 35-37°C in 5-10% CO₂ or ambient atmosphere.

Depending on the species, visible colonies may appear after a few days to six weeks of incubation under optimum conditions.

Safety Factors

1) Risk of Disease Contraction:

Close contact with infected individuals is the most common route of disease contraction. Immuno-compromised people, especially those with HIV/AIDS infection and drug users, are at extreme risk.

2) Countermeasures (PPE and Medical):

Because of the low infectious dose (ID₅₀ <10 bacilli) and the potential for aerosolization of *M. tuberculosis*, clinical and laboratory samples must be considered potentially infectious and handled with appropriate precautions. BSL2 practices and procedures, containment equipment and facilities should be used. All aerosol-generating activities must be conducted in a biosafety cabinet. BSL3 practices, containment equipment and facilities should be used for the propagation and manipulation of cultures. Medical countermeasures include treatment with second-line antibiotics for several months. XDR-TB strains are resistant to first- and second-line drugs; therefore treatment options are seriously limited. However, good TB control programs and drug compliance have demonstrated successful treatment in approximately 30% of affected people.

Agent: Nipah virus

Environmental Factors

1) Geographic Distribution:

The Nipah virus is a newly emergent pathogen so the actual geographical distribution remains to be determined. The virus was first observed in Malaysia in 1998-1999, where it spread among swine in various pig-farming communities throughout the country and led to 265 suspected cases of human infection, mostly in abattoir workers. During this outbreak, abattoir workers in Singapore caught the disease from infected swine imported from Malaysia. Outbreaks have occurred in Bangladesh (predominantly central and northwest districts) during the winters of 2001, 2003, and 2004; India retrospectively reported cases from 2001. Certain species of fruit and insectivorous bats (*Pteropus* genus) that inhabit a range that spans from Madagascar and Mauritius to Australia, encompassing most of Southeast Asia and parts of South Asia, are the probable virus reservoir.

2) Disease Symptoms (human and animal):

In humans, encephalitis is the predominant clinical symptom but symptoms range from asymptomatic to very severe. Acute illness is initially flu-like and includes fever, headache, myalgia, drowsiness, sore throat, and disorientation. A quick onset of coma occurs occasionally. Asymptomatic infection appears to be common in pigs; acute illness and sudden death occurs occasionally (more common in young pigs). The disease is very contagious, and is similar to other porcine illnesses. The clinical signs are acute fever with respiratory problems; neurological symptoms are less common.

3) Strain Information:

There appears to be very little genetic diversity among most virus strains in Southeast Asia—isolates taken from humans, fruit bats, and pigs show greater than 99% nucleotide homology; in fact they generally differ by no more than one to three amino acids. However, there is more divergence between Southeast Asian and South Asian strains—an isolate from the outbreaks in Bangladesh shares around 91.8% sequence homology with the original Malaysian strain. Little is known regarding any differences in pathogenicity among strains.

4) Reservoirs/Vectors:

Numerous fruit-eating and insectivore bats are believed to be the disease reservoirs. Virus antibodies were found in bat species living in the Malaysian and Bangladeshi outbreak areas. Neutralizing antibodies to Nipah virus have been detected in five bat species in Malaysia: *Pteropus hypomelanus* (31% of animals), *Pteropus vampyrus* (17%), *Eonycteris spelaeca* (5%), *Cynopterus brachyotis* (4%), and *Scotophilus kuhli* (3%). In Thailand, *P. hypomelanus*, *P. vampyrus*, *P. lylei*, and *H. larvatus* bats have exhibited serological evidence as well as *P. lylei* bats in Cambodia, and *P. giganteus* bats in Bangladesh. Bats are asymptomatic carriers. The virus appears to infect a wide range of hosts. It replicates explosively in pigs and is also seen in humans, horses, dogs, and cats, which all are believed to be 'dead end' hosts.

5) Agent Sources:

The disease is systemic, affecting multiple organ systems including the kidneys, lungs, the nervous system (large amounts in brain), and spleen. Virus is shed by the oral-nasal route and in excretions. It can be isolated from nasal (pigs, humans) and throat (pigs, humans, cats) swabs, urine (humans, cats), and the serum or blood (pigs, cats). Serum, heparinized plasma, whole blood, or cerebrospinal fluid should be collected during early and febrile stages of disease and frozen; nasal or oral swabs, bronchial washes, and urine should be collected in buffer and frozen. Nipah virus has been isolated from swabs of fresh urine (collected under roosting trees) and swabs of partially eaten fruit (collected under mango trees—*M. indica* and *E. aquea*) dropped by a colony of *P. hypomelanus* (flying foxes) bats along the beach of Tioman island (25 km off the eastern coast of Malaysia). However, only 24/588 urine samples and 1/27 fruit swabs produced cytopathic effects in cell culture; of these, only three cultures actually yielded the Nipah virus (two from urine, one from fruit).

6) Stability:

Nipah virus is generally unstable and does not retain infectivity for long periods in the environment. The agent is inactivated at temperatures $>55^{\circ}\text{C}$ (stability at lower temperatures is unknown). In addition, the virus is inactivated rapidly by sunlight, oxidizing agents (such as sodium hypochlorite or calcium hypochlorite), and chlorinated lime.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Low volume of academic research (in PubMed)

3) Survey Information:

7/722 (0.97%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain Nipah or Hendra complex viruses.

4) Past Weapons Activity:

There has been no known attempt to weaponize Nipah virus.

5) Commercial Sources:

Little is known, but few are expected since Nipah is a newly emergent virus. The CDC in Atlanta, The Commonwealth Scientific and Industrial Research Organisation (CSIRO) Australian Animal Health Laboratory, and Laboratoire P4 Jean Merieux in Lyon, France are known to conduct work with live virus.

6) General Growth Conditions:

Nipah virus can be grown in a variety of tissue culture cells—Vero and RK-13 cells produce high virus titers. Infected tissue samples should be homogenized in cell culture media to create a 10% (w/v) suspension, clarified by centrifugation, and inoculated directly onto cell monolayers. Oropharyngeal swabs, urine, and serum can be placed in a small amount of culture media and used directly to inoculate cells. Cytopathic effects are generally seen within three days and exhibit a characteristic formation of multinucleate syncytia. Immunofluorescence can be used to detect the virus in culture, although antibodies cross-react with the Hendra virus. RT-PCR can be used to specifically detect Nipah.

Safety Factors

1) Risk of Disease Contraction:

Humans primarily contract the disease by coming into close contact with the contaminated tissues, body fluids, excretions, and secretions of infected swine. Disease contraction from swine is well-documented; contraction from bats is only suspected. The risk of human-to-human transmission is extremely low, but has been confirmed in the Bangladeshi outbreaks. Aerosol transmission is not regarded as a significant risk.

2) Countermeasures (PPE and Medical):

A BSL4 facility should be used for any work with this pathogen. In dealing with sick animals in the field, one should wear long sleeve overalls, rubber boots, gloves (preferably two sets taped to the sleeves of overalls), a plastic apron that can be disinfected or discarded, a face shield, and a mask (with a HEPA filter) or positive air pressure respirator (PAPR). Treatment is generally supportive, with ventilator support for those in coma. No vaccines have been developed; ribavirin may be effective but is largely untested.

Agent: Norovirus

Environmental Factors

1) Geographic Distribution:

Viruses of the *Norovirus* genus (*Calciviridae* family) are the most important cause of nonbacterial acute gastroenteritis (for all ages) in both developed and developing countries. The virus is estimated to cause approximately 50% of all infectious human gastroenteritis globally. Improved sanitation in the developed world has reduced bacterially-induced gastroenteritis with little effect on virally-induced illness. Global seroprevalence studies have shown that 70-100% of individuals (over the age of five) have antibodies to noroviruses and sapoviruses. There has been a large increase in the number of norovirus epidemics in 2006; only the common cold is a more frequently reported illness in the United States. Large outbreaks occur sporadically, but an increased incidence appears to occur on cruise ships; several outbreaks, where thousands have become ill, have occurred between 2002 and 2007. Hospitals, educational settings, and nursing homes are also often affected.

2) Disease Symptoms (human and animal):

Norovirus infection causes an acute gastroenteritis with nonspecific clinical signs: watery diarrhea, vomiting, anorexia, abdominal pain and fever, lethargy, weakness, and muscle aches. Headache and low-grade fever may occur. The disease usually resolves within a few days and severe illness is rare.

3) Strain Information:

Norovirus strains exhibit a vast amount of genetic and antigenic diversity. There are a huge number of strains with many more discovered all the time. Strains of the *Norovirus* genus are grouped into five genogroups (GG): GG I, II, and IV contain human disease strains, GG III contains bovine strains, and GGV contains the murine norovirus. The genogroups are further subdivided into 20 genetic clusters. Very little is known about the comparative virulence of strains. In 2006, two emerging strains (2006a and 2006b) caused the majority of infections.

4) Reservoirs/Vectors:

Norovirus infection appears to only make humans ill, and it is widely believed that a zoonotic reservoir does not exist. Recent studies have detected closely related enteric calciviruses in animals though, suggesting the possibility of animal reservoirs. However, this has not been resolved scientifically.

5) Agent Sources:

Stool samples are the principle source of virus. While some individuals may shed virus in stool for two weeks or more after onset of symptoms, it is best to collect specimens within the first 48 hours of illness from sick individuals. Stool samples can be stored at 4°C for weeks but should be frozen if stored for prolonged periods. Vomitus can also be used.

6) Stability:

Noroviruses are non-enveloped and very stable outside of hosts in the environment. Virions can survive freezing, temps as high as 60°C, and moderate-to-high levels of chlorine. Sodium hypochlorite is an effective disinfectant.

Laboratory Factors

1) Security Classification:

Not a Select Agent

2) General Research Volume:

Low volume of research overall (PubMed); may be understudied because of an absence of suitable animal models and effective culture strategies have only recently been developed.

3) Survey Information:

No survey information

4) Past Weapons Activity:

There are no known attempts to weaponize norovirus.

5) Commercial Sources:

Little is known

6) General Growth Conditions:

Stool cultures are used as sources of virus for tissue culture infection; a 10-20% stool suspension (one gram stool in 0.01 M PBS) is clarified and filtered (0.22 micron filter) to remove bacteria. The supernatant is then inoculated on cells. Suitable tissue culture systems and animal models for the amplification of noroviruses have not been available for some time. Numerous attempts to infect and grow virus in a wide variety of monolayer cell cultures have failed. Only within the last few years have tissue culture systems been developed that are susceptible to norovirus infection and can amplify virus over numerous passages. The virus can infect and grow in a cell culture model that mimics the human small intestinal epithelium morphologically and physiologically. The culture system is composed of INT-407 cells grown on porous collagen-I coated microcarrier beads under conditions of physiological fluid shear in rotating wall vessel bioreactors. Thus far, GG I and II viruses have been grown in the culture system; however, it is a nontraditional culture system and may be relatively difficult to set up and maintain. Diagnostic assays do not cover all norovirus strains. As expected, antigenic cross-reactivity is greatest among strains within the same genogroup. Several ELISA and PCR assays have been developed for the detection of norovirus in clinical samples (particularly stool samples) and cell culture. A small number of commercially available, rapid detection tests are also available. PCR primers can be designed (e.g. to the RNA-dependent RNA polymerase) to detect almost all norovirus genetic clusters; primers can also be designed to type the viruses.

Safety Factors

1) Risk of Disease Contraction:

Norovirus is transmitted by the fecal-oral route. The transmission mostly occurs by ingestion of contaminated water and foods (often shellfish) but also by indirect oral contact with contaminated environmental surfaces. Person-to-person transmission is considered a common phenomenon and has often been observed on cruise ships. Close contact with infected individuals (and their aerosols generated during speaking, vomiting, etc) can also spread illness. The virus is highly contagious, only around ten virions may be sufficient.

2) Countermeasures (PPE and Medical):

BSL2 containment and practices are recommended for any work with potentially infected samples or norovirus cultures. Laboratory staff should wear masks and gloves. Improved personal hygiene with frequent and thorough hand washing, along with the disinfection of environmental surfaces can help prevent infection significantly. Surgical masks and gloves should also be worn when in the presence of sick individuals and when cleaning areas heavily contaminated with vomitus or feces. Gastroenteritis is self-limiting and antiviral drugs are not effective and seldom used. Treatment is supportive in nature, patient hydration should be maintained.

Agent: Rabies virus

Environmental Factors

1) Geographic Distribution:

Rabies is a common disease globally and is endemic to five continents. Third-world countries are particularly affected, and Asia and Africa have especially high disease incidences. There are an estimated 25,000 to 50,000 human cases of rabies a year; only approximately 0.1% of these occur in the Americas and Europe. Asia has, by far, the most cases: in 1997, there were 33,000 Asian cases, 30,000 of these occurred in India alone. In contrast, there were only 32 human cases documented in the United States from 1980 to 2003. Rabies is much more common in wild animal populations. In 2004, approximately 7,000 animal cases were reported in the United States; 92% were wildlife cases. Rabies epidemiology is complicated; it differs from continent to continent depending on the fauna and state of developmental progress. Urban rabies occurs mostly in developing countries (in Asia and Africa); stray dogs transmit the disease to people and are reservoirs. The sylvan form is the primary type seen in developed countries. Domestic animals come into contact with sick wild reservoir animals and then transmit the disease to humans. Island countries may be free of canine rabies but bats act as reservoirs.

2) Disease Symptoms (human and animal):

The rabies virus causes an acute and progressive encephalomyelitis. The prodromal period symptoms are nonspecific and flu-like. Symptoms include nausea, vomiting, headaches, and pain/numbness at the bite location. The acute neurological phase then sets in. Most cases present as the encephalitic or furious forms, less than 20% manifest as the paralytic form. Encephalitic symptoms include restlessness, hyper-excitability, excess saliva, difficulty swallowing, cold sweat, confusion, hallucinations, aggressive behavior, fear of death and water, insomnia, convulsions, spasms, and muscle twitching. Eventually individuals go into coma and cardiac arrest. Paralytic syndromes include general muscle weakness, head muscle paralysis, bilateral deafness (sometimes), coma, and respiratory and heart failure. In animals, the disease can vary greatly; generally animals are restless, excitable, aggressive, in a daze, and undergo paralysis. Animals may also go into hiding.

3) Strain Information:

Rabies strains vary greatly genetically and antigenically. Strains are grouped into seven genetic variants. A panel of seven monoclonal antibodies is commercially available to type the variants. Incubation periods may vary with strains, but all natural viruses appear to be very deadly to humans. Strain variants generally circulate within a particular host species in one geographic area.

4) Reservoirs/Vectors:

Nearly all animals are susceptible. The primary reservoirs are wild animals such as raccoons, bats, foxes, jackals, skunks, mongooses, and wolves. Domestic animals and pets such as dogs, cats, cattle, sheep, goats, and pigs can also act as reservoirs. Dogs and cats (pets and strays) are major reservoir species in developing countries and are key human vectors. In the United States, raccoons along the eastern coast are the predominant host species, followed by skunks and foxes. Canine rabies has largely been eliminated in developed countries due to effective vaccination programs.

5) Agent Sources:

In infected animals, all tissues and secretions may harbor virus, but the highest titers are in the saliva, salivary glands, and the CNS.

6) Stability:

The rabies virus is enveloped and very unstable outside of a host. It is viable for only a few hours in dried secretions or blood under normal environmental conditions, and is rapidly inactivated by sunlight and drying. In cold conditions (winter temperatures or refrigeration), cadavers may harbor live virus from one to several months. The virus is susceptible to heat (50°C for one hour), 1% sodium hypochlorite, 2%

glutaraldehyde, 70% ethanol, and formaldehyde. One hour acetone treatment does not inactivate rabies virus fully, so special care must be taken during laboratory work.

Laboratory Factors

1) Security Classification:

Not a Select Agent

2) General Research Volume:

Relatively large volume of laboratory work (in PubMed); new vaccine work

3) Survey Information:

30/722 (4.16%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain Rabies.

4) Past Weapons Activity:

No known attempts at weaponization.

5) Commercial Sources:

Many commercial sources and culture collections will have the rabies virus.

6) General Growth Conditions:

Rabies virus can be isolated in cell culture or by intracerebral inoculation of mice (classical method) using postmortem CNS tissue—a 20% brain (brain stem and cerebellum) homogenate is best. Alternatively, it can be taken from saliva samples from living infected animals. Several successful tissue culture systems have been developed and are commonly grown in BHK-21 cells, CCL 131 cells, and mouse neuroblastoma cells (available commercially). Virus can be detected in clinical samples (CNS tissue) and tissue culture by microscopic examination of histopathological stains, a direct fluorescent-antibody test (standard, highly specific, and fast), immunohistochemical assays, or RT-PCR. RT-PCR is especially useful for the detection of virus in saliva, as well as skin, cornea impressions, tears, eye swabs, and throat swabs—materials on which other tests are not very effective at viral detection.

Safety Factors

1) Risk of Disease Contraction:

Virus is transmitted into open wounds from infected saliva, usually by the bite of an infected animal. The virus cannot penetrate intact skin. In the absence of a bite, the virus may penetrate existing scratches, other small lesions, and mucous membranes when infectious material (saliva, contaminated hair or fur, CNS tissue, etc.) comes in contact. Aerosol inhalation is another infection route but rare under natural conditions (documented in bat caves). Infection by non-bite exposure is extremely rare. Human-to-human transmission is possible. Only 20% of exposed persons develop rabies—it depends on the location of wound and the amount of virus present.

2) Countermeasures (PPE and Medical):

BSL2 facilities can be used for diagnostic purposes; BSL3 should be used for any work involving virus amplification. Minimum PPE should include a laboratory coat, double gloves, and safety glasses; a biosafety cabinet should be used for handling infectious materials. A face shield and heavy gloves should be worn when dissecting infected carcasses and handling brain matter. In the event of suspected infection, simultaneous post-exposure vaccination (active and passive) must be applied within 24 hours after exposure to increase the chance of disease prevention. Bites and scratches should be washed immediately with soap, water, and disinfectants. Human rabies serum or immunoglobulin should be inoculated on wounds promptly. If symptoms appear, the person will die. Numerous human and animal vaccines have been developed from whole virions, purified rabies proteins, and recombinant viral vaccines encoding rabies proteins; these are generally quite effective at inducing protective immunity. In the United States, a

licensed vaccine is administered to individuals (with no prior vaccination) in five doses over the course of a month. There are only three documented cases of individuals surviving in the absence of vaccination.

Agent: *Ricinus communis* plant (source of ricin toxin)

Environmental Factors

1) Geographic Distribution:

Ricinus communis plants are native to tropical Africa, especially in and around Ethiopia, but have since been introduced to many warm temperate and tropical regions worldwide. The plant grows as an erect tropical shrub or tree; plants may reach 30 feet tall in the tropics and 15 feet or more in cooler zones. The plants are hardy, resistant to drought, and can grow rapidly under the right conditions— up to eight or more feet during the summer. Several varieties are commonly grown in large numbers in order to harvest castor oil; many varieties are also often grown in ornamental gardens because of their distinctive foliage. Plants have bold foliage, leaves are composed of eight radiating, pointed, and serrated leaflets that are green in many varieties but reddish-brown to purple in some. Plants produce very distinctive soft-spined fruits that contain mottled seeds. The fruit browns when ripe.

2) Disease Symptoms (human and animal):

The symptoms of ricin poisoning are identical to those of abrin poisoning. Initial post-ingestion symptoms include excess salivation, vomiting, abdominal pain, dehydration, and diarrhea (may become bloody). A fever may also develop, as well as low blood pressure. In severe cases, hallucinations and seizures may develop, followed by death. If ricin is inhaled, likely symptoms include respiratory distress, fever, cough, nausea, and tightness in the chest. Heavy sweating may follow and fluid may accumulate in lungs making breathing difficult. The skin might turn blue, and low blood pressure and respiratory failure may occur, leading to death. Exposure of skin and eyes to ricin mist or powder results in redness and pain.

3) Strain Information:

Numerous varieties of *Ricinus communis* are grown for castor oil production and for gardening purposes. There is little information available on the relative toxicity of the different varieties, but all natural varieties produce dangerous toxins. Some researchers are trying to develop plant strains with lower concentrations of ricin toxin to make castor oil production safer.

4) Reservoirs/Vectors:

None

5) Agent Sources:

The entire *Ricinus communis* plant is poisonous but seeds contain the highest concentration of toxin. Ricin toxin is water soluble so it resides in the seed-pulp after the seeds are smashed during castor oil production; seeds are 5% toxin by weight. Seeds can be purchased from gardening and agricultural suppliers.

6) Stability:

Ricinus communis seeds are typically viable for only a short period of time. They are susceptible to desiccation and do not withstand low temperatures well. Ricin toxin is soluble in water, but solutions are less stable than dry product. In the dry state, the toxin is normally stable at room temperature but denatures at elevated temperatures; the stability decreases with increasing moisture content. Toxin is inactivated by 20 minutes of 140°C heat.

Laboratory Factors

1) Security Classification:

US Select Agent [laboratories with over 100 mg of toxin must abide by regulatory requirements (registration, etc.)]. Plant material is unregulated.

2) General Research Volume:

Generally large volume of research (PubMed).

3) Survey Information:

12/722 (4.29%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain ricin toxin, but quantities are unknown.

4) Past Weapons Activity:

Canada, France, South Africa, and the United States are known to have conducted research to weaponize ricin. Syria is suspected to be conducting ricin research. Iraq is known to have produced biological weapons from ricin. Some reports indicate that traces of ricin were found in Al Qaeda caves in Afghanistan.

5) Commercial Sources:

The seeds of numerous plant varieties are available for purchase online from a number of commercial sources. One online source sells 18+ seeds (labeled as poisonous) for US\$2.50, or 500 seeds for US\$32.50. Another online source sells the seeds of numerous varieties and 20 seeds cost US\$4.60. In addition, small amounts of purified, powdered toxin can be bought for research purposes, but sale of these materials is subject to strict regulation.

6) General Growth Conditions:

Directions are available online for growing plants. The plant is easy to grow and thrives under a variety of conditions, especially in full sun. Toxin is most effectively extracted from the seeds; methods for isolating and purifying toxin are widely available in open literature searches, however many of the popular recipes (e.g. Poisoner's Handbook; Silent Death; Al-Qaeda's manual, etc.) are inadequate. Numerous past terrorist attempts to isolate ricin have largely been unsuccessful.

Safety Factors

1) Risk of Disease Contraction:

Ricin poisoning is rare. The toxin typically enters the body by ingestion (plant material, contaminated food or water). Castor bean seeds are only toxic if the hard outer shell is chewed, releasing the toxins before swallowing. Other parts of the plant are also poisonous, but the highest concentration of toxin is in the seeds. The toxin can also be inhaled when in droplet, mist, or powdered form. Depending on the route of exposure and dose received, death by ricin poisoning can occur within 36 to 72 hours of exposure. Very small amounts—approximately 0.5 to 1 mg—can kill an adult (~75x higher dose is needed relative to abrin toxin).

2) Countermeasures (PPE and Medical):

BSL2 containment and practices are recommended for work with ricin toxin or potentially contaminated materials. PPE should include a laboratory coat, gloves, and a protective mask. Ricin should generally be handled as a nonvolatile toxic chemical in the laboratory. Special care should be taken if handling potentially contaminated clinical, diagnostic, and post-mortem samples because ricin may retain toxicity in fluids, respiratory secretions, and unfixed tissues. A biosafety cabinet or chemical fume hood equipped with an exhaust HEPA filter and charcoal filter should be employed for any likely aerosol-generating activities. In the event of suspected exposure, it is very important to get the toxin off or out of the body as quickly as possible. Poisoning is normally treated by administering supportive medical care. An antidote to the toxin does not exist.

Agent: *Rickettsia prowazekii*

Environmental Factors

1) Geographic Distribution:

R. prowazekii is an obligate intracellular bacterium that causes typhus worldwide, particularly in poorly hygienic or louse-infested areas. The disease (caused by the human subtype) is regularly encountered in North and Central Africa (Ethiopia, Sudan, Somalia, Kenya, Uganda, Burundi, and Rwanda), the mountainous regions of Central and South America (Mexico, Colombia, Peru, and Bolivia), the countries of the Soviet Union, and other parts of Asia. Recently large epidemics have been observed in Russia and Burundi; yearly outbreaks occur in Andean communities. Outbreaks can be explosive and epidemic typhus is most commonly associated with wars and disasters. In the United States, the organism (zoonotic subtype) is now limited to small foci associated with flying squirrels; a louse-borne outbreak has not occurred since 1921.

2) Disease Symptoms (human and animal):

The onset of typhus varies somewhat, but the prodromic signs begin nonspecifically; febrile illness later develops with high fever, conjunctivitis, headache, chills, general pains, dry cough, nausea, anorexia, vomiting, and diarrhea. At around the fifth day, a pink spotted rash develops that becomes red and maculopapular; the rash erupts first on the upper trunk and spreads to the rest of the body except for the face, palms, and soles of the feet. Encephalitis may develop with confusion, restlessness, alterations in speech and movement, and hypotension; kidney and liver function may become impaired. The disease usually resolves around two weeks after the onset of fever.

3) Strain Information:

R. prowazekii is a species in the typhus group of the genus *Rickettsia*. There are two genetically-distinct subtypes: 1) the epidemic form infects humans only, and 2) the zoonotic form that is often associated with flying squirrel populations. The zoonotic strains sporadically cause human disease, particularly in winter months and symptoms are normally mild.

4) Reservoirs/Vectors:

Bacteria reside in arthropod hosts for part of their life cycle; arthropod bites lead to horizontal transmission to mammalian hosts. Humans are the main reservoir in most of regions; the bacteria cycles between the *Pediculus humanus corporis* body louse and people. Lice remain infected for life. Flying squirrels (*Glaucomys volans*) are the primary reservoir for the zoonotic subtype (in United States mostly); bacteria cycle between squirrels and fleas and/or lice.

5) Agent Sources:

Blood should be collected as early as possible during the course of illness and treated with heparin. Blood needs to be processed immediately or stored temporarily at 4°C; for delays over 24 hours, blood should be frozen rapidly and stored at -70°C. Arthropod and vertebrate tissues (spleen, lung, lesion biopsy tissue) are also potential sources of bacteria. Lesion biopsy specimens (punch specimens 3 mm in diameter) should be collected from maculopapules. Bacterial isolation is most effective if clinical samples are collected before antibiotic treatment begins.

6) Stability:

R. prowazekii is an obligate bacterium dependent on the host cell for survival. While the organism is generally sensitive to environmental stimuli such as sunlight, bacteria may remain viable in louse fecal material and dead lice for weeks. Bacteria are susceptible to 1% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, as well as moist and dry heat.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Moderate amount of research (PubMed)

3) Survey Information:

15/722 (2.08%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *R. prowazekii*.

4) Past Weapons Activity:

Germany, Japan, the Soviet Union, and the United States had conducted BW research on *R. prowazekii*; North Korea is believed to have conducted research on and perhaps produced typhus BW weapons.

5) Commercial Sources:

Little is known but the clinical isolation of *R. prowazekii* is relatively rare. Before 1999, the last clinical strain was isolated 30 years beforehand. Thus, few legitimate culture collections can be expected to have *R. prowazekii*.

6) General Growth Conditions:

The organism can be isolated from heparinized blood (buffy coat), biopsy samples, and infected ticks. Specimens are suspended in tissue culture medium before inoculation. Historically, *R. prowazekii* was typically amplified in and isolated from adult male guinea pigs, mice, and the yolk sacs of embryonated chicken eggs. These methods have been supplanted by cell culture; bacteria are now grown in Vero, L-929, HEL, or MRC5 cells growing in antibiotic-free media. The best results are obtained when the bacterial specimen is inoculated on confluent layer cells growing on round coverslips (12-mm-diameter) in 3.7-ml shell vials. Centrifugation ($700 \times g$ for one hour) enhances the attachment and entry of bacteria into cells. After inoculation, the shell vials are washed with PBS; then incubated in minimal essential medium containing 10% fetal calf serum at 34°C with 5% CO₂ for 48-72 hours. Cell cultures typically produce a small quantity of bacteria. Coverslips can then be examined after Giemsa or Gimenez stain, or by immunofluorescence after treatment with antibodies specific to *R. prowazekii*. Group-, species-, and strain-specific monoclonal antibodies are available. PCR can also be used to identify bacteria in acute-stage blood, lesion biopsy specimens, and cell culture. Serology is often used to diagnose disease.

Safety Factors

1) Risk of Disease Contraction:

Most infections occur when louse feces is rubbed into broken skin after a bite. Infection may also occur when mucous membranes come into contact with infected materials such as dead lice and their excreta and dried feces, and through inhalation of airborne particles carrying contaminated materials. Person-to-person transmission does not occur. Bacteria are very infectious, a very low dose—ten bacteria or less—can lead to infection; consequently laboratory work is very hazardous.

2) Countermeasures (PPE and Medical):

BSL3 containment and practices should be used for any manipulation of infectious materials. PPE should include laboratory coats or gowns, and gloves. When collecting potentially infectious materials, treatment of clothing with insecticides (such as 1% permethrin powder) can help prevent infection resulting from lice bites. In the event of infection, treatment with doxycycline or tetracycline (drugs of choice) should be administered early after exposure to prevent organ damage. Chloramphenicol is also effective. There are no vaccines available commercially, but live attenuated vaccine and formaldehyde-inactivated vaccines have been effectively used in the past. Doxycycline can be taken prophylactically in endemic areas to prevent infection.

Agent: Rift Valley fever virus

Environmental Factors

1) Geographic Distribution:

Rift Valley Fever (RVF) is an epizootic disease that has been observed in more than 30 countries. The disease is endemic in parts of sub-Saharan Africa (eastern and southern Africa), and has also, to a lesser extent, been observed in Northern Africa. The disease is believed to be endemic in Kenya, Uganda, Namibia, Angola and Nigeria, and perhaps in Mauritania, Madagascar, Egypt, Somalia, and Tanzania as well. The incidence is particularly high in areas (or during periods) of high rainfall and flooding—conditions that are friendly to mosquito breeding. Human epidemics occur periodically and can be quite large: an outbreak in East Africa (centered in Kenya) in late 1997 affected 89,000 people and caused over 500 deaths. A recent outbreak in Kenya (2006-2007) affected hundreds of people. The only non-African epizootic outbreaks have occurred in Saudi Arabia (the southern Tehama coastal plain) and in Yemen (adjoining the Tehama region in the west); an outbreak in 2000-2001 affected thousands of people.

2) Disease Symptoms (human and animal):

In humans, Rift Valley Fever is rarely fatal and infection is often asymptomatic. In acute cases, a largely nonspecific febrile illness develops with a sudden onset of moderate-to-severe flu-like clinical signs. Symptoms usually include fever, malaise, headache, back pain, retro-orbital pain, and photophobia; a maculopapular rash may also develop. Frequently, the illness resolves within a week. In a very small percentage (1-3%) of patients, delayed symptoms such as ocular lesions, encephalitis, or severe hepatic disease with hemorrhage may develop weeks after the initial illness and multi-organ failure can occur. Jaundice is also common. In domestic animals, the disease usually results in high rates of abortion and neonatal mortality. RVF causes hepatitis and encephalitis in young livestock. The disease is difficult to detect in older animals since they often present asymptomatic or nonspecific symptoms. Mortality is 70% in calves as compared to 15% for adult cattle. Outbreaks are usually preceded by heavy rains, followed by many unexplained livestock abortions.

3) Strain Information:

RVF strains are phylogenetically separated into three broad groups: North African, West African, and East/Central African isolates. Arabian Peninsula isolates have been very similar to the East African strains. There is very little information available on the relative virulence and pathogenicity of the different isolates.

4) Reservoirs/Vectors:

Aedes mosquitoes are the usual natural reservoir; mosquitoes lay infected eggs that are resistant to desiccation. Over 40 species of mosquito from many genera (including *Aedes*, *Culex*, *Anopheles*, and *Mansonia*;) can act as vectors; geographically distinct disease regions often have distinct vectors. Many animals are susceptible including wild and domestic ruminants (cattle, sheep, buffalo, camels, and goats serve as major hosts), and several rodents and carnivores. Human outbreaks are invariably connected to outbreaks in livestock.

5) Agent Sources:

During the acute disease stage, humans and animals have viremia—blood and serum have high virus titers. Viremia ceases later when encephalitis and retinitis may appear. The organs of dead animals and aborted fetuses (such as the liver, spleen, and brain), nasal discharge, and vaginal secretions after abortion harbor large amounts of virus. Infected meat and milk may also be a potential source; the virus has been isolated from pooled mosquitoes during epidemics.

6) Stability:

RVF is an enveloped virus that is unstable in the environment. The offspring of infected mosquitoes often harbor the virus. This provides a durable mechanism for maintaining the virus in nature, as mosquito eggs may survive for periods of up to several years in dry conditions.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Moderate amount of laboratory based work (PubMed)

3) Survey Information:

Only 2/722 (0.28%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain RVF.

4) Past Weapons Activity:

The US offensive biological weapons program conducted weapons research on RVF.

5) Commercial Sources:

Little is known.

6) General Growth Conditions:

The virus is typically isolated from 5 ml of blood or serum, or 5 grams of liver, brain or spleen. Blood/serum can be diluted in culture media (with antibiotics), then used for inoculation; one gram of tissue can be homogenized in cell culture media (10% suspension), clarified by centrifugation, and then the supernatant may used for inoculation. Mosquitoes can also be pooled, homogenized in culture media, clarified, and then the supernatant used for inoculation. Virus isolation and growth is typically performed by injecting samples into hamsters or mice, or by inoculation of various cell lines. Vero, BHK and CER cell monolayers are commonly used, but animals are good for testing virus virulence. Numerous protocols are available online. Virus inoculation should be for one hour at 37°C; then the culture media should be changed and the cells incubated in Eagle's media supplemented with 5% fetal calf serum and antibiotics at 37°C with 5% CO₂. Within a week, cytopathic effects typically appear. The addition of stabilizing compounds (bovine albumin and protamine sulfate) at inoculation time results in increased titers. Virus neutralization tests, immunofluorescence (and immunohistochemistry) microscopy, antigen-capture ELISAs and RT-PCR are often used to identify the virus.

Safety Factors

1) Risk of Disease Contraction:

Transmission may occur through the bite of infected mosquitoes or through direct contact with the blood, body fluids or the organs of infected animals during slaughter. Infection is also possible by ingesting raw milk, or through inhalation of aerosols generated by infected animals or contaminated material. There is a low risk of person-to-person transmission and nosocomial infections are also rare.

2) Countermeasures (PPE and Medical):

BSL4 containment and practices should be employed for any work with known infectious materials. When in the field, mosquito repellents and bednets should be used to prevent mosquito bites. Individuals should wear long sleeves, gloves and a mask or respirator. Long heavy rubber gloves, respirators, and a face shield should be worn if infected animal blood or tissues are handled. There is no established course of treatment for patients infected with RVF virus. Studies suggest that interferon, hyperimmune globulin, and convalescent-phase plasma may be effective if administered early. Vaccines have been developed to prevent animal disease; vaccines have also been used in humans, although in limited capacity (laboratory staff, during outbreaks). Ribavirin may be an effective prophylaxis.

Agent: SARS virus

Environmental Factors

1) Geographic Distribution:

The severe acute respiratory syndrome coronavirus (SARS-CoV) is a newly emergent pathogen that has caused several outbreaks. First, in November 2002, SARS infected people in China's Guangdong province. The virus spread to Hong Kong, and within a matter of months, expanded to 29 countries. Asia was especially affected, but there were also imported cases in numerous European countries, the United States, and Canada. By the end of the epidemic, over 8,000 cases had been reported (774 deaths) worldwide; the vast majority occurred in China (mainland and Taiwan), but Canada and Singapore were also hit hard with 251 and 238 cases reported, respectively. In late 2003-2004, new independent outbreaks occurred in China but were quickly contained. The virus does not appear to be currently circulating in human populations.

2) Disease Symptoms (human and animal):

SARS-CoV causes an infectious atypical pneumonia-like disease in humans. A range of mild, moderate, or severe respiratory disease may occur. Asymptomatic infection is rare but isolated cases may escape detection. The initial symptoms are nonspecific and look like an upper respiratory tract viral infection. A low-grade fever develops with malaise and coughing for a few days, then rapid progression into full-blown pneumonia. Patients may have one or more of the following: cough (75% of cases), myalgia (45%), shortness of breath (40%), nausea, vomiting, anorexia, watery diarrhea (30%), and/or headache (20%). Atypical pneumonia is apparent with a chest X-ray. Symptoms can last for weeks; about 25% of patients require intensive care and some may require mechanical ventilation.

3) Strain Information:

There appears to be a fair amount of genetic variance among SARS-CoV isolates. The SARS virus is believed to undergo a very high rate of mutation and rapid evolution in an intermediate host (civets, etc.) is required before the virus can infect humans. The epidemiology from the 2002 outbreak suggests that initial human-to-human transmission was poor and inefficient; virulence improved only after some adaptation within humans. Supporting this, human virus isolates taken from early 2002 were more genetically similar to animal isolates than were mid-2003 human isolates. The 2003-2004 outbreak human isolates were also more similar to animal isolates than to the 2002-2003 human isolates, suggesting that they did not adapt enough to acquire very efficient transmission capability.

4) Reservoirs/Vectors:

SARS-CoV is a zoonotic agent believed to have first horizontally transferred into humans from animals kept in the wild-game markets of Guangdong, China. Masked palm civets, raccoon dogs, and Chinese ferret badgers sold for human consumption are suspected to have yielded virus; however, these animals are unlikely reservoirs since they become very ill when infected. Serologic testing has repeatedly been positive in market civets, but repeatedly negative in farmed and wild civets. SARS-like viruses have been detected in several species of Chinese horseshoe bats (*Rhinolophus* genus; around Hong Kong, 39% of anal samples taken from bats had virus, 80% of serum samples were seropositive); bats are asymptomatic, distributed over large parts of China, and believed to be the reservoir host.

5) Agent Sources:

Viral loads are highest in human lower respiratory tract specimens (sputum). However, upper respiratory specimens are most suitable and practical for virus isolation (and RNA detection); naso- and oropharyngeal swabs and aspirates can yield high titers of organism when collected during the second week of symptoms (often negative during the first week of illness). The virus is also shed in urine and stool, although at lower titers; these sources can be cultured during the first two to three weeks of illness. The specimen type and timing of collection are very important for virus detection: viral loads are low in the upper respiratory tract and in the feces during the first four days of illness, but peak at around day ten. Virus has also been detected in the blood and serum of patients, but the viremia is believed to be short lived.

6) Stability:

SARS is an enveloped virus and therefore quite unstable in the environment. The virus is stable in feces and urine at room temp for one to two days but is more stable in stool from diarrhea patients because of the higher pH. Common disinfectants and fixatives can neutralize the virus rapidly.

Laboratory Factors

1) Security Classification:

Not a Select Agent

2) General Research Volume:

Relatively high volume of laboratory based work (PubMed)

3) Survey Information:

High volume of academic research; 34/722 (4.71%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain SARS.

4) Past Weapons Activity:

No known weaponization attempts

5) Commercial Sources:

Little is known, but few sources are likely to ship samples upon request.

6) General Growth Conditions:

Isolation of coronaviruses in cell culture is not always reliable; some isolates will grow in cell culture only after some time spent adapting in humans. Numerous attempts (in half a dozen studies) to culture SARS-like virus (in numerous cell lines) from fecal swab samples taken from bats have failed. Clinical human samples such as nasopharyngeal (or throat swabs), and feces should be suspended in cell culture media containing antibiotics, clarified (if necessary), and used to inoculate monolayers of Vero-E6 cells. If infection is successful, cytopathic effects and cell destruction will be visible within a few days. Numerous RT-PCR assays, ELISAs, and immunofluorescence antibody methods have been developed to detect virus in cell culture or clinical specimens. No antibody-based assays are available commercially, but RT-PCR detection kits have appeared recently for sale.

Safety Factors

1) Risk of Disease Contraction:

Transmission to humans is primarily by the respiratory route (inhalation of infected droplets and aerosols). It is unknown whether infection can occur via the oral-fecal route. Human-to-human spread has been extremely efficient in past outbreaks; the risk of nosocomial infection is high. However, the disease does not appear to spread as efficiently in humans as influenza.

2) Countermeasures (PPE and Medical):

BSL3 containment should be used for any viral manipulation or culture work; WHO accepts BSL2 facilities (with biosafety cabinet) for routine diagnostic work. It is critical that PPE such as gowns, gloves, and goggles/face shield be worn; use of N95 masks or respirators are also very important. There is no specific antiviral therapy yet; current treatment is supportive in nature. Oxygen ventilation may be needed for >50% of hospitalized patients. No vaccine is available currently, however a number are under development.

Agent: *Salmonella* serotype Typhi

Environmental Factors

1) Geographic Distribution:

Salmonella serotype Typhi is endemic to Central America, South America, Africa, the Middle East, East Asia, and Southeast Asia; several million cases are reported annually. Typhoid fever—the disease caused by this bacterium—is most prominent in developing nations with poor sanitation and increasingly rare in developed countries. Papua New Guinea, Indonesia, and the Philippines have the highest annual incidence of typhoid fever. Typhoid fever is rare in the United States (and other developed nations) where approximately 800 cases occur each year; >70% of cases are related to international travel.

2) Disease Symptoms (human and animal):

Typhoid fever is much more severe than the disease syndromes caused by other *Salmonella* serotypes. Symptoms are systemic and typically present as a debilitating high fever and headache. Anorexia, fatigue, chills, stomach pains, and a flat rash (rose spots) in approximately 25% of patients are also possible symptoms. Severe cases may result in hemorrhage, intestinal perforation, blood in feces, rapid rise in pulse rate, hypotension, abdominal tenderness, and subsequent rigidity, shock, and altered mental state. Extremely severe cases may exhibit delirium. Greater than 10% of cases may be fatal. Children usually display mild illness with nonspecific fever. Accurate diagnosis requires laboratory examination of stool, bone marrow aspirates, or blood.

3) Strain Information:

Salmonella serotype Typhi is a set of strains grouped according to the immunologic composition of surface antigens; these strains are members of the *S. enterica* species (*S. enterica* subspecies *enterica*). There appear to be two major groups of this serotype: a worldwide set of strains and an African set of strains. Outbreaks seen in South America and parts of Southeast Asia, typically due to the worldwide type, are usually mild (low fatality; low incidence of neurological symptoms). African-type outbreaks in Africa and Indonesia often result in severe illness (high mortality; neurological complications). Great genomic discrepancies, varying virulence and antibiotic resistance levels are often observed among isolates. MDR resistant strains have appeared in several regions worldwide.

4) Reservoirs/Vectors:

Humans are the sole reservoir for *Salmonella* serotype Typhi, carriers may not exhibit symptoms. Insects may act as vectors.

5) Agent Sources:

S. serotype Typhi is more frequently isolated from blood cultures than the fecal specimens of infected individuals. Eighty percent of blood cultures taken from typhoid patients during the first week of fever yield bacteria; positive results decrease gradually thereafter. Bacteria may also be isolated from rectal swabs, urine, gall bladder bile, rose spot lesions, or bone marrow aspirates taken from infected individuals. Acquisition of *S. typhi* from a fly vector is unrealistic. The bacteria may also be isolated from contaminated food or water but this is more difficult. Specimens should be placed in a chilled transport medium (buffered glycerol saline or commercial media) and processed quickly.

6) Stability:

Bacteria are rather resilient and capable of surviving for long periods, perhaps years, in the environment. Virulence is often uncompromised regardless of environmental conditions. Bacteria are known to be able to survive for 17 days in rabbit carcasses, 62 days in feces, ten to 20 minutes on skin, 240 days in ice, up to 30 days in dust, and 130 days in ashes. Survival in sea water is possible up to nine days and in sewage for weeks. *S. typhi* is sensitive to moist heat (121°C for 15 min), dry heat (160°C for one hour), and many disinfectants including 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, and formaldehyde.

Laboratory Factors

1) Security Classification:

Not a Select Agent

2) General Research Volume:

Relatively high volume of academic research (PubMed)

3) Survey Information:

189/722 (26.18%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *Salmonella* serotype Typhi.

4) Past Weapons Activity:

Canada and the United Kingdom have conducted BW research on *Salmonella* serotype Typhi. Japan had weaponized and used *Salmonella* serotype Typhi on Chinese military units and civilians. North Korea is believed to have a BW program.

5) Commercial Sources:

Salmonella serotype Typhi commonly causes outbreaks, some of epidemic proportion, in developing countries. Consequently, it is many commercial sources and research and diagnostic laboratories will have specimens.

6) General Growth Conditions:

Numerous protocols are readily available for bacterial isolation. Fecal specimens from acutely ill individuals can often be directly plated; *Salmonella* serotype Typhi can also be enriched from fecal samples in Selenite broth before plating. Numerous plating media of varying selectivity can be used for isolation of *Salmonella* (including MacConkey's, eosine methylene blue agar, *Salmonella-Shigella* agar, xylose lysine desoxycholate agar, Hektoen Enteric Agar, and desoxycholate citrate agar) but the preferred medium for *Salmonella* serotype Typhi is bismuth sulfite agar because it is highly selective and can be used to detect the lactose-positive *Salmonella* colonies. Most of these media can be purchased readily from commercial sources, but are also easy to make. *Salmonella* serotype Typhi can grow in the presence or absence of air but is typically grown under aerobic conditions. PCR is usually used to distinguish *Salmonella* serotype Typhi from closely related bacteria such as *S. typhimurium*. Commercially available rapid serodiagnostic tests, biochemical tests, and latex agglutination tests can be used to type strains. However, the reliability of these kits varies.

Safety Factors

1) Risk of Disease Contraction:

Poor hygiene is a large contributor to outbreak severity. Human-to-human transmission is by the fecal-oral route; people often contract the disease by ingesting contaminated food or water. Sewage contamination of drinking water (with inadequate chlorination) often leads to outbreaks. The infectious dose is typically low—around 10^3 bacteria—and varies according to gastric acidity. Death is typically communicable through the first week of illness; 10% of patients discharge bacteria for three months after infection; 2-5% of patients are chronic carriers, shedding bacteria for years in the absence of symptoms. Although probably an extremely rare phenomenon, flies may transmit bacteria.

2) Countermeasures (PPE and Medical):

Any potentially contaminated materials and cultures should be handled in BSL2 facilities. Gloves and laboratory coats should be worn at all times. Thorough hand washing can diminish infection risk significantly. Resistance to one or more drugs (especially ciprofloxacin) is increasingly common and several multidrug resistant strains have been isolated. Chloramphenicol is often used for treatment; however the emergence of resistant strains has led to an increased use of ampicillin, amoxicillin, and trimethoprim; quinolone derivatives like cephalosporins are also used. Several typhoid fever vaccines are

in use worldwide (oral and injectable), with efficacy typically ranging from around 60% to 75%. These vaccines typically need a booster every three years.

Agent: Saxitoxin

Environmental Factors

1) Geographic Distribution:

Certain planktonic algae (mostly Gonyaulacoid dinoflagellates), various photosynthetic cyanobacteria, and diatoms can produce saxitoxin. These microorganisms can typically produce numerous analogues (~20) as well; saxitoxin is only one component of all toxins produced. Typically, the proportion of toxin types varies and is likely affected by environmental stimuli. Among these microorganisms, the cyanobacterium *Anabaena circinalis* appears to produce the highest levels of toxin. This species is found worldwide, but there may be a geographical segregation of saxitoxin-producing strains—Australian freshwater isolates often produce saxitoxin and it has also been detected in several lakes in Denmark. Other *Anabaena* species may also produce saxitoxin. The plankton sometimes expands into algal blooms throughout the world; it is exceedingly difficult to predict the location of these blooms. Filter-feeding bivalves feed on the toxin-producing plankton, accumulating the toxin in tissue; various shellfish present along the Pacific coast of the Americas have been shown to contain toxin. Human cases of parasitic shellfish poisoning (PSP)—caused by ingestion of saxitoxin-like toxins—are reported worldwide but occur more often in outbreaks along coastal areas. There is little quality statistical data available on the incidence of shellfish poisoning. Algal blooms associated with fish or bird kills are indicative of high toxin levels, however PSP can occur in the absence of a red tide; shellfish are not necessarily poisonous during red tides. The incidence of PSP has been in decline in developed countries due to careful monitoring, beach closures, and improved public awareness. A 1987 outbreak on the Pacific coast of Guatemala affected 187 people.

2) Disease Symptoms (human and animal):

The symptoms of PSP are the most severe of all shellfish poisonings. Saxitoxin ingestion produces the most severe symptoms. The timing and severity of symptoms varies according to the type of toxin ingested, the concentration of toxin in the shellfish, and the amounts of contaminated shellfish consumed. Saxitoxin ingestion produces the most severe symptoms. The clinical symptoms of PSP are principally neurological, and include tingling and burning sensations, numbness, drowsiness, and nausea. In moderate cases, the numbness spreads to the face and neck; in severe cases, there may be difficulty swallowing, incoherent speech, and respiratory paralysis. Symptoms develop rapidly after the initial ingestion of toxin (from within several minutes to a few hours). Progression to respiratory paralysis can occur within two to 12 hours. Patients may recover gradually, with few residual symptoms within a few days. Diagnosis is based primarily on clinical observation combined with knowledge of recent dietary history.

3) Strain Information:

Numerous cyanobacteria produce saxitoxin-like toxins; the freshwater *A. circinalis* appears to produce the most saxitoxin. Marine dinoflagellates and diatoms also produce saxitoxin.

4) Reservoirs/Vectors:

Numerous types of plankton produce saxitoxin. All filter-feeding mollusks (bivalves) can potentially be poisonous, but PSP is generally most often associated with mussels, clams, cockles, oysters, and scallops. The Alaskan butterclam can accumulate very high concentrations of toxin. Humans, birds, and fish can be affected by saxitoxin.

5) Agent Sources:

The concentration of toxin in the sea during algal blooms is too low for significant isolation. Shellfish digestive organs and soft tissues can accumulate saxitoxin in rather high concentrations. The toxicity of shellfish tissue is normally examined using a mouse bioassay: mice are injected with different dilutions of tissue suspensions, and then observed for signs of poisoning. An indirect ELISA has also been developed for the detection of saxitoxin in shellfish tissue.

6) Stability:

Toxin-producing plankton is generally heat and acid stable. The saxitoxin itself is soluble in water, heat stable (it can maintain its activity in water heated to 120°C), and very stable in acidic solutions (several months). The toxin is inactivated by strong alkalis.

Laboratory Factors

1) Security Classification:

US Select Agent [laboratories with over 100 mg of toxin must abide by regulatory requirements (registration, etc.)]

2) General Research Volume:

Moderate amount of laboratory work (PubMed)

3) Survey Information:

15/722 (2.08%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain this toxin.

4) Past Weapons Activity:

The United States military had isolated saxitoxin; stocks were destroyed in the 1970s.

5) Commercial Sources:

Small amounts of toxin can be purchased from commercial entities however strict regulations are in place for any significant amounts.

6) General Growth Conditions:

It is difficult to grow plankton in the laboratory and induce them to produce significant amounts of saxitoxin. Glycerol-peptone medium in a controlled environment consisting of a 12/12 light/dark cycle and appropriate light conditions is typically required to grow saxitoxin-producing cyanobacteria. One study indicated that growth in 5% CO₂ with the addition of Tris buffer, bicarbonate buffer, and trace amounts of the minerals molybdenum, selenium, and strontium may stimulate growth of some saxitoxin-producing algal species. Addition of ammonium and high levels of nitrate inhibits growth of *A. circinalis* in the laboratory but increases toxin release. Cultures may require one or more months to produce a substantial quantity of the organism. There is little information on the growth of shellfish under conditions that favor the accumulation of saxitoxin in tissues.

Safety Factors

1) Risk of Disease Contraction:

PSP is a foodborne illness contracted by consuming raw or cooked shellfish, or the broth in which shellfish were cooked. The toxin is rapidly absorbed through the GI tract and excreted in the urine. The risk of poisoning is increased along coastal areas with poor food monitoring and control programs. Contraction of PSP by ingestion of contaminated fish or birds is extremely rare. A purified toxin (usually a powder) can be inhaled in laboratory settings; parenteral injection is another potential laboratory hazard. In mice, the LD50 for saxitoxin is 3-10 µg/kg body weight parentally; 263 µg/kg body weight orally. Humans are more sensitive than mice: the LD50 oral dose is 1-4 mg.

2) Countermeasures (PPE and Medical):

BSL2 facilities are required for saxitoxin work. PPE should include a laboratory coat, safety glasses, and disposable gloves. Respirators should be worn and a biosafety cabinet should be used for any manipulation of high concentrations of toxin (such as powders), as well as for any procedures that may generate aerosols. No specific antidotes exist and treatment is primarily supportive. If ingestion of contaminated material was recent, gut decontamination with activated charcoal or dilute isotonic bicarbonate solution may help. The

toxin is normally cleared rapidly from the body via the urine thus, diuretics may help. If support is provided within 12 hours of exposure, recovery is usually seen. Ventilation is required for 3-6% of cases.

Agent: *Shigella*

Environmental Factors

1) Geographic Distribution:

Shigellosis occurs worldwide and may be the most common cause of human dysentery. Shigellosis is endemic in developing countries, especially in places with poor sanitation (no running water or plumbing). According to the CDC, "in the developing world, shigellosis is far more common and is present in most communities most of the time." While Shigellosis is endemic in both tropical and temperate climates, the incidence is higher in warmer regions and during the summer months. Four *Shigella* subgroups cause disease. *S. dysenteriae* 1 (Sd1) commonly causes epidemic dysentery in the developing world but is rather rare in developed countries. In the United States (and other developed countries), *S. sonnei* and *S. flexneri* are most common; an estimated 450,000 cases occur each year, but around 20% are related to international travel. Shigellosis is most common at institutions with poor hygienic practices such as child care centers and nursing homes.

2) Disease Symptoms (human and animal):

The symptoms of shigellosis are watery diarrhea (bloody or non-bloody), fever, abdominal cramps, and rectal pain. The symptoms often progress to classic dysentery in which stools contain blood, mucus, and pus. In young children and the elderly (mostly), diarrhea can be so severe that the patient needs to be hospitalized. Severe infection with high fever may lead to seizures (particularly in children less than two years of age). However, some of those infected do not develop noticeable symptoms. All four *Shigella* subgroups can cause dysentery, but Sd1 causes especially severe disease with profuse bloody diarrhea; approximately 5-15% of Sd1 cases are fatal.

3) Strain Information:

The *Shigella* genus is divided into four subgroups: *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C), and *S. sonnei* (subgroup D). There are hundreds to thousands of *Shigella* strains, and most do not produce enterotoxin. *S. dysenteriae* and *S. flexneri* strains are the most virulent generally. *S. dysenteriae* serotype 1 is most virulent of the four subgroups, is the only cause of epidemic dysentery, and the strains are often capable of releasing Shiga toxin. *S. sonnei* strains often cause asymptomatic infection.

4) Reservoirs/Vectors:

The only natural reservoirs of *Shigella* are humans and other large primates. Humans are the primary reservoir. Humans shed bacteria in large numbers when they exhibit symptoms during the acute stage of disease and for one to four weeks afterwards. Flies may act as vectors.

5) Agent Sources:

Shigella bacteria are mostly isolated from the intestines of infected humans. Dysentery can be caused by numerous organisms, so shigella must first be detected in bloody stools before isolation. Positive cultures are most often obtained from blood-tinged plugs of mucus in freshly passed stool specimens obtained during the acute phase of disease. Whole stools and rectal swabs may also be used to culture *Shigella*. The specimen should be transported and processed rapidly or deposited in a chilled buffered glycerol saline holding solution (or commercial transport media); it should be frozen after three days. Bacteria may also be isolated from food or water contaminated with feces, but the organism probably does not live freely in the environment.

6) Stability:

The bacteria may survive in feces for 11 days, flies for up to 12 days, water for two to three days and on clothing for eight days. *Shigella* bacteria are sensitive to moist heat (121°C for 15 min) and dry heat (160°C for 60 min). Disinfectants such as 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, and formaldehyde kill the bacterium.

Laboratory Factors

1) Security Classification:

Not a Select Agent

2) General Research Volume:

Relatively high volume of academic research (PubMed)

3) Survey Information:

110/722 (15.24%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *S. dysenteriae*.

4) Past Weapons Activity:

Japan had weaponized *Shigella*, Canada conducted BW research on *Shigella*.

5) Commercial Sources:

Little information, but many sources are expected to have samples due to the high prevalence of disease worldwide

6) General Growth Conditions:

Scientists and clinicians have been growing *Shigella* for a long time and many effective methods have been developed. A variety of basic, easy to make media can be used to grow bacteria; most of this media can be purchased pre-made commercially. *Shigella* isolation typically involves initial streaking of sample on a more selective media with aerobic incubation to inhibit the growth of typical anaerobic flora, followed by streaking on a less selective media such as MacConkey. An enrichment step is sometime used beforehand [growth in "*Shigella* broth" (very basic; recipe online) with novobiocin] to select against other bacteria present in the sample, however no enrichment media is suitable for all isolates. A liquid enrichment medium (Hajna Gram-negative broth) may also be inoculated with the stool specimen and subcultured onto the selective/differential agarose media after a short growth period. Commonly used primary isolation media include xylose lysine desoxycholate agar (XLD), Hektoen Enteric Agar (HE), desoxycholate citrate agar (DCA), and *Salmonella-Shigella* agar (SS). SS media may inhibit the growth of some Sd1 strains. These media contain bile salts to inhibit the growth of other Gram-negative bacteria and pH indicators to differentiate lactose fermenters (coliforms) from non-lactose fermenters such as *Shigella*. Following overnight incubation of primary isolation media at 37°C, colonies are very small, slightly pink, translucent, and non-lactose-fermenting. Colonies can be further evaluated using PCR and/or a variety of other selective media. *Shigella* bacteria can be difficult to distinguish from *E. coli* antigenically. Plasmid profiling and RFLP analysis can be used for subtyping.

Safety Factors

1) Risk of Disease Contraction:

Human-to-human transmission is possible via direct or indirect fecal-oral route. As few as ten to 200 bacteria may be enough to cause infection. The organism is communicable particularly during acute stage of illness. Ingestion of contaminated food can lead to infection. The disease can also be acquired by drinking or swimming in contaminated water. Water may become contaminated if sewage flows into it or if someone with shigellosis swims in it. Children are particularly at risk: 2/3 of all cases are children.

2) Countermeasures (PPE and Medical):

Contaminated material should be handled in BSL2 facilities. Gloves should be worn whenever infected materials are handled. The spread of *Shigella* is best prevented by frequent and careful handwashing with soap. The illness usually resolves in five to seven days in the absence of any treatment; severe cases can be treated with antibiotics such as ampicillin, trimethoprim/sulfamethoxazole, nalidixic acid, or ciprofloxacin. However, antimicrobial resistant strains (many MDR strains in developing world) are thought to be common. Anti-diarrheal agents should not be used. No vaccine exists to prevent shigellosis.

Agent: *Staphylococcus aureus* (enterotoxin B)

Environmental Factors

1) Geographic Distribution:

S. aureus is highly prevalent throughout the world as it grows on the skin and mucous membranes of mammals and birds. The organism is particularly prevalent in areas with suboptimal hygienic practices. The bacterium is a major source of nosocomial infection, often entering skin abrasions and causing localized infections. Certain strains produce and secrete enterotoxins and are a major cause of food poisoning, and, much less frequently, non-menstrual toxic shock syndrome (TSS). Enterotoxin B-producing strains (SEB) are believed to be a common cause of classic food poisoning, but the actual incidence is unknown.

2) Disease Symptoms (human and animal):

Clinical disease manifestations vary greatly depending on the bacterial strain and route of infection. Toxin-producing strains may cause food poisoning, scalded skin syndrome, and toxic shock syndrome. Strains that produce enterotoxin B can cause food poisoning and toxic shock syndrome. Ingestion of materials (normally foodstuffs) contaminated with preformed enterotoxins can cause severe gastrointestinal symptoms, with clinical signs that include abrupt onset of nausea, vomiting, abdominal cramps, and non-bloody diarrhea as few as six hours later in humans. Most food poisoning cases resolve within a few days. Inhalation of the toxin can cause TSS with severe inflammatory reactions stimulating the host's immune system to release excessive amounts of cytokines. However, around 75% of all TSS cases are caused by bacteria that produce the TSS-1 toxin; the remainder is caused by strains that produce enterotoxin B or C. Clinical signs of TSS include sudden (within a few hours) onset of fever, headache, chills, myalgia, chest pain, and a nonproductive cough. The symptoms are incapacitating and may last up to two weeks, a cough may last four weeks. High doses of toxin can cause widespread systemic failure with septic shock and even death, although fatality is rare. Clinical diagnosis is typical, although ELISA can detect the toxin in tissue and body fluids. Animals exhibit similar symptoms upon ingestion or inhalation of toxin.

3) Strain Information:

Most *S. aureus* strains are often a normal component of a host's flora, and usually act only as opportunistic pathogens. However, many strains can produce one or more exotoxins, including enterotoxins (A, B, C, D, E), toxic shock syndrome toxin (TSST-1), and exfoliative toxins (A, B), that may cause more severe illness. Certain coagulase-positive *S. aureus* strains secrete enterotoxin B. High, medium, and low-toxin producing SEB strains have been identified. It is unknown what percentage of all *S. aureus* strains produce enterotoxin B.

4) Reservoirs/Vectors:

S. aureus bacteria may produce resident and/or transient populations on humans and other primates. Primates are major hosts for the bacteria; specific biotypes can occasionally be found on domestic animals and birds. The bacteria prefer to colonize and grow in the anterior nasal passages. Most humans are colonized only intermittently; 30-40% of people are colonized persistently. *S. aureus* normally have a benign or symbiotic relationship with hosts.

5) Agent Sources:

S. aureus bacteria may be found on the skin, and in the mouth, mammary glands, intestinal, genitourinary, and upper respiratory tracts of hosts. Toxin-producing strains thrive and produce toxins in un-refrigerated meats, dairy, and bakery products.

6) Stability:

S. aureus can survive in carcasses or organs for up to 42 days, floors for less than seven days, meat products for 60 days, skin for 30 minutes to 38 days, and in sunlight for up to 17 hours. The bacteria are relatively resistant to drying but are destroyed by heat (121°C for 15 minutes under moist conditions), and are susceptible to numerous disinfectants including 1% sodium hypochlorite, ethanol, glutaraldehyde, and formaldehyde. Enterotoxin B is relatively stable and soluble in water. It is resistant to high temperatures

and can withstand boiling for several minutes. The toxin can be destroyed with treatment of 0.5% hypochlorite for ten to 15 minutes.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Relatively high volume of academic research (PubMed)

3) Survey Information:

74/722 (10.25%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain this toxin.

4) Past Weapons Activity:

The United States conducted research on enterotoxin B as a potential incapacitant in the 1960s.

5) Commercial Sources:

Little is known.

6) General Growth Conditions:

S. aureus specimens should be plated onto blood agar (preferably sheep blood agar). Colonies are detectable after 24 to 36 hours of growth at 37°C. Specimens that are expected to be heavily contaminated with other bacteria should be streaked onto more selective media such as mannitol-salt agar or lipase-salt-mannitol agar. Two commercial chromogenic agars (CHROMagar staph aureus and *S. aureus* ID) have been developed for the isolation of *S. aureus* bacteria from clinical specimens. Colonies are typically pigmented (cream yellow to orange), smooth, slightly raised, and hemolytic on blood agar; they are 6-8 mm in diameter after three days growth. The bacteria can also be grown in flasks containing dialyzable beef-heart medium or brain heart infusion broth at 37°C with aeration. PCR can be used to detect *S. aureus* in clinical specimens such as nasal swabs and blood cultures, sometimes after enrichment on selective media. In a routine laboratory test, pathogenic strains are frequently detected by their ability to clot plasma (due to coagulase production). A large number of other laboratory tests can also be used to differentiate strains. ELISA, reverse passage latex agglutination, and PCR can detect the potential for strains of *S. aureus* to produce enterotoxin. Finally, numerous easy to use commercial kits can identify *S. aureus* strains with good accuracy.

Safety Factors

1) Risk of Disease Contraction:

Enterotoxin B-producing strains can grow vigorously on food, secreting toxin. The ingestion of food contaminated with *S. aureus* is a fairly common cause of food poisoning. The toxin can also be inhaled in an aerosol, although it is unclear how this would happen naturally. The bacteria may spread between people through contact with the nasal discharge of carriers. *S. aureus* is a leading nosocomial pathogen in hospitals, often infecting postoperative wounds or small abrasions caused by intravenous devices.

2) Countermeasures (PPE and Medical):

Materials contaminated with exotoxin B-producing strains should be handled in BSL2 facilities. Manipulation of high concentrations of SEB, or powders should occur in a biosafety cabinet, as should any procedures that may result in aerosol generation. Respirators should be worn to protect against potential aerosols. Laboratory staff should also wear laboratory coats and gloves, and should wash their hands frequently and thoroughly. Treatment of SEB intoxication is supportive and may include rehydration therapy, cough suppressants, and antihistamines. Assisted ventilation may be required for severe respiratory symptoms. Many strains of *S. aureus* are resistant to multiple antibiotics. No vaccine is available.

Agent: Variola major virus

Environmental Factors

1) Geographic Distribution:

The WHO announced the global eradication of smallpox in 1979; this declaration was subsequently sanctioned by the World Health Assembly in 1980. The last reported naturally-occurring case occurred in 1977 in Somalia. Some sources suggest that surviving specimens of virus may yet be present in the environment from victims that had succumbed to smallpox and were buried in cold, permafrost areas such as Siberia. However, there is no evidence to support or refute this scenario currently.

2) Disease Symptoms (human and animal):

There were four clinical forms of smallpox caused by *Variola major*: ordinary smallpox (responsible for ~90% of cases), modified smallpox (responsible for 5% of cases), flat smallpox (responsible for 5% of cases), and hemorrhagic smallpox (responsible for <1% of cases). The first clinical signs of ordinary smallpox include fever, malaise, prostration, head and back aches, and often vomiting. The fever is usually high, in the range of 101 to 104°F. Within a day or two, a rash emerges first as small red spots on the tongue and in the mouth. These spots develop into sores that break open and spread large amounts of the virus into the mouth and throat. The rash then begins to appear on the skin in a centrifugal distribution, starting on the face and spreading to the arms and legs and then to the hands and feet; concentration is greatest on the oral mucosa, face, and extremities. The rash then develops to be macular, then papular; papules enlarge and become vesicles by days four or five; pustules develop by day seven. The pustules become encrusted and then scab by day 14, sloughing off later in the illness, leaving scars. Skin lesions develop together through the various stages. Toxemia and shock can produce mortality rates close to 30% for ordinary smallpox. Modified smallpox produces a mild prodrome and few skin lesions; flat smallpox produces slow developing lesions and generalized infection with a higher mortality rate (~50%). Hemorrhagic smallpox induces bleeding in the skin and mucous membranes and is invariably fatal.

3) Strain Information:

There are two variations of smallpox: *Variola major* and *Variola minor*. *V. major* was by far the more dangerous type. Thousands of strains were estimated to have circulated in nature. Studies had shown considerable variability in virulence and pathogenicity among isolated, naturally occurring strains. The Soviet Union weaponized an extremely virulent strain—India-1—which was isolated during a natural outbreak.

4) Reservoirs/Vectors:

Humans are the only natural hosts of *Variola* virus. Smallpox is not known to be transmitted by insects or animals.

5) Agent Sources:

Infected body fluids (collected from oral secretions or skin lesions) contain large amounts of virus. Scab material harbors the virus for long periods; objects such as bedding or clothing that may contain remnant scab material are also infectious. Tissues such as spleen, lymph nodes, liver, bone marrow, kidneys, and other viscera may also contain large quantities of virus. Venous blood and mouth washings collected during the prodromal febrile phase or early during the rash phase, and vesicular/pustular fluid swabs (with overlying skin) have often been used in the past to isolate the organism.

6) Stability:

Virions are rather stable; smallpox can persist for up to two weeks on clothing and certain surfaces indoors. The virus is believed to be able to survive for long periods (up to a year) in scab material at room temperature. However, in the environment, smallpox virus may be inactivated within two days by sunlight, elevated heat and humidity.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

There is very little research with whole, active virus today. Only two WHO-sanctioned facilities can legitimately conduct research on live virus; research that includes more than 20% of the virus genome is prohibited at any other locations. The US Centers for Disease Control and Prevention in Atlanta, Georgia or the State Center for Virology and Biotechnology (Vector) in Koltsovo, Russia are the only two facilities that have been sanctioned by the WHO to store smallpox samples legitimately. Shortly after the virus was eradicated in nature, the WHO mandated that all laboratories that had smallpox either destroy their samples or transfer them to one of the two legitimate facilities. However, the extent to which these terms had been met remains contentious.

3) Survey Information:

No survey information

4) Past Weapons Activity:

The Soviet Union had weaponized *Variola* virus, producing large volumes of an extremely virulent strain known as India-1. The United States had conducted research on smallpox virus for BW purposes. North Korea is suspected of retaining smallpox stocks for BW purposes.

5) Commercial Sources:

No commercial sources

6) General Growth Conditions:

Growth requires infection of a living host—often chick embryos or established cell culture lines (including Vero, BS-C-1, CV-1, BHK-21, MDCK, MRC-5). Infected samples are used to directly inoculate (with antibiotics) the chorioallantoic membranes (CAMs) of 12- to 14-day-old chicken embryos. Seventy-two hours after incubation at 36°C, embryos are checked for pox lesions. Orthopoxviruses are the only human poxviruses that produce pocks on CAMs. To purify virus particles from the pox lesions, a 25% emulsion can be made of the portions of the CAMs containing pocks in physiological saline with pestle and mortar. The emulsion should then be centrifuged for ten minutes; supernatant can be stored or used for further inoculations. Alternatively, virus can be grown in monolayers or spinner flasks of mammalian cells; infection causes obvious cytopathic effects (with cells rounding) after a few days. Virions have a characteristic morphology that can be identified in sample smears using electron microscopy. PCR and ELISAs on clinical samples are also effective.

Safety Factors

1) Risk of Disease Contraction:

The virus was most often transmitted between people by inhalation of large-droplet respiratory particles produced by infectious individuals during close, face-to-face contact. However, infection is possible through any direct contact of mucous membranes or broken skin with virus particles present in bodily fluids, scabs, contaminated objects, or aerosols. Person-to-person transmission requires fairly prolonged face-to-face contact. Generally, the virions found in scabs (in the fibrin matrix) are not very infectious. Transmission rates ranged from 30% to 80% among unvaccinated individuals.

2) Countermeasures (PPE and Medical):

Any work with whole, active virus should occur in either of the two WHO-sanctioned BSL4 facilities. PPE appropriate for BSL4 laboratories should be worn at all times when handling specimens. In the event of infection, there is no proven treatment; patients can benefit from supportive therapy which includes the addition of intravenous fluids, medicine to control fever or pain, and antibiotics for any secondary bacterial

infections that may occur. Vaccinia immune globulin may be offered as a prophylaxis. There is a vaccine to prevent smallpox, present in some national stockpiles, although it is not readily attainable. There is a high risk of side effects and potential complications associated with the vaccine.

Agent: *Vibrio cholerae*

Environmental Factors

1) Geographic Distribution:

V. cholera has a worldwide distribution. It is ubiquitous in many water sources—freshwater rivers and lakes, estuarine, and marine environments. Bacteria may be isolated from sediment, the water column, plankton, and bivalves. Generally, bacteria concentration peaks during warmer periods. Most epidemics are associated with hot weather. When conditions (temperature, salinity, and nutrients) are suitable, the bacteria can flourish. Under less optimal conditions, bacteria switch from an active to dormant state allowing them to survive in harsh conditions. Pandemic cholera has been very rare in industrialized nations for the past 100 years, but the disease is still common today in parts of the developing world with inadequate treatment of drinking water and sewage. Refugee settings are frequently at risk. The Indian subcontinent and sub-Saharan Africa are particularly affected and recently there have been large outbreaks in South America. In 1999, 28 countries in Africa reported disease (Mozambique had 44,329 cases; Malawi had 26,508), 11 countries in Central and South America, and 14 countries in Asia. Africa had, by far, the largest number of cases. Recent research shows that epidemic cholera (typically caused by the O1 and O139 subgroups) is endemic in the coastal areas of Bakerganj and Mathbaria, Bangladesh (Bay of Bengal), particularly in the spring and summer months; in 2002, over 30,000 cases were reported.

2) Disease Symptoms (human and animal):

The majority of infections is asymptomatic or have rapid onset of profuse but self-limiting watery diarrhea and leg cramps. Severe cholera results in massive diarrhea with stools composed of clear fluid with flecks of mucus. Rapid loss of body fluids leads to dehydration and shock. In very severe cases, coma and death may occur—even in healthy adults—within a few hours, if untreated. Non-O1 strains may cause mild diarrhea, and can also infect wounds, sometimes leading to septicemia.

3) Strain Information:

V. cholerae is divided into three major subgroups: *V. cholerae* O1, *V. cholerae* O139, and *V. cholerae* non-O1. Only bacteria carrying somatic antigens O1 and O139 can produce CT enterotoxin; both cause cholera with very similar clinical symptoms. *V. cholerae* serogroup O1 has been the cause of most pandemics. The O1 biotypes are serologically indistinguishable from Classical and El Tor strains. O139 emerged recently and is now the primary type in South and Southeast Asia. Non-O1 strains are one of the most commonly isolated vibrios. Strains with multi-drug resistance have also been seen.

4) Reservoirs/Vectors:

The organism lives in aquatic environments, normally attached to various plankton—primarily algae and copepods—and crustacean shells and shellfish. Birds and herbivores may also harbor the bacteria in endemic zones. Humans are also a reservoir.

5) Agent Sources:

The organism may be present in water with algae, copepods, and other zooplankton. The stool of infected humans has large numbers of bacteria and a characteristic appearance: grey, slightly clouded fluid, without blood but with some mucous. The bacteria are present in stool throughout the course of illness, and a few days afterwards. Stool should be collected during the acute stage of disease, before any treatment. Rectal swabs and vomitus from acute cases may also be reliable sources. Specimens should be placed in a transport medium, which may maintain bacteria for four weeks.

6) Stability:

The organism can survive temperatures of up to 117°C. It thrives in salty water or water contaminated with organic matter for up to six weeks, dust for three to 16 days, feces up to 50 days, glass up to 30 days, and soil up to 16 days. Survival depends on the temperature and moisture. Bacteria cannot survive in pure water for long and are very sensitive to cold temperatures. *V. cholerae* is readily killed by desiccation,

steam, dry heat, boiling, common disinfectants such as ethanol, glutaraldehyde, formaldehyde, sodium hypochlorite, and by chlorination or ozonization of water.

Laboratory Factors

1) Security Classification:

Not a Select Agent

2) General Research Volume:

Relatively high volume of academic research (PubMed)

3) Survey Information:

100/722 (13.85%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *V. cholerae*.

4) Past Weapons Activity:

France, North Korea, and South Africa conducted BW research on *V. cholerae*. Japan produced biological weapons and used them on Chinese military units and citizens during the invasion of China.

5) Commercial Sources:

V. cholerae has caused many epidemics throughout human history, killing millions. Presently, cholera appears to be reemerging. It is not a Select Agent and there is a high volume of laboratory research. Consequently, many sources will have the agent.

6) General Growth Conditions:

Cholera is typically diagnosed clinically using bright- or dark-field microscopy on stool samples. Clinical diagnosis takes only a matter of minutes; samples can then be diluted in peptone broth (pH 8.5) and streaked on appropriate media. The organism does not grow well on media often used for growth of enteric organisms (such as MacConkey agar or eosin-methylene blue agar); most media containing peptone or meat extracts are suitable for growth. Numerous selective media are also available, but thiosulfate-citrate-bile salts-sucrose agar (TCBS) is used most frequently. TCBS does not require autoclaving (prepared by boiling) and is available from many commercial sources. The organism should be grown under aerobic conditions. The inclusion of sucrose in media allows preliminary typing of colonies; sucrose-fermenting *V. cholerae* colonies are distinctively flat and yellow on TCBS. Enrichment can be performed in commercially-available alkaline peptone water. Selection of *V. cholerae* colonies is usually not very difficult since the organism grows so rapidly, it typically out-competes commensal microflora. There are numerous biochemical tests for identification, and commercial assays for the detection of bacteria in human clinical samples are available (although the effectiveness of such kits varies). Agglutination tests with O1 or O139 sera or PCR can be used to distinguish between strains.

Safety Factors

1) Risk of Disease Contraction:

Cholera is primarily transmitted to humans through the ingestion of water or food contaminated with feces or vomit. Epidemics associated with raw fish and undercooked shellfish have also been reported, but in most epidemics, the contamination source is usually the feces of an infected person. The disease is communicable throughout the course of the illness to only a few days after symptoms have been resolved. The infectious dose ranges from $\sim 10^6$ to 10^{11} in healthy individuals but varies with gastric acidity.

2) Countermeasures (PPE and Medical):

Cultures and potentially infectious clinical materials should be handled in BSL2 facilities; ABSL2 facilities should be used to house infected animals. Hygiene (frequent hand washing) and sanitation are extremely important in preventing transmission. For treatment, fluids and electrolytes should be continually replaced in patients; intravenous fluid replacement should be used for patients that have lost $\geq 10\%$ body weight. Antibiotics such as ciprofloxacin, doxycycline, co-trimoxazole, and tetracycline may reduce the duration of

disease. Prophylaxis with ciprofloxacin is apparently ineffective, but tetracycline may be effective. Killed cholera vaccines are also ineffective and not recommended by the CDC. A number of new oral vaccines are currently under development.

Agent: *Yersinia pestis*

Environmental Factors

1) Geographic Distribution:

Yersinia pestis is endemic in large numbers of small natural foci (corresponding to rodent populations) present in Africa, the countries of the former Soviet Union, the Americas, Asia, and the Middle East. The foci cover approximately 6-7% of global land area. There is a consistent average of 2,000 to 3,000 human cases per year worldwide, although this is probably an underestimate. The highest incidence is in Africa (accounts for over 90% of cases worldwide). The most active African foci are in the eastern and southern regions of the continent (Ituri province in the Democratic Republic of the Congo is the reportedly the most affected area). The Andean mountain region and parts of Brazil contain the most active South American foci. Ninety percent of US cases occur in the states of New Mexico, Arizona, Colorado, and California.

2) Disease Symptoms (human and animal):

There are three forms of plague: bubonic, septicemic, and pneumonic. Bubonic plague is the most common form, causing nonspecific early flu-like symptoms (chills, headache, fatigue) and very obvious symptoms later in illness (lymph gland swelling and formation of painful buboes). Septicemic plague occurs in a small percentage of cases. Symptoms are similar to the bubonic form minus the buboes; meningitis and pneumonic disease are more likely. In severe cases, necrosis of acral regions (nose, digits) may occur; mortality is very high. Pneumonic plague is rare and resembles an array of pneumonia-like respiratory illnesses (high fever, cough, chest pain). Plague diagnosis can be very difficult early on. Gram-negative staining in sputum or bubo aspirates can be used for presumptive diagnosis.

3) Strain Information:

Y. pestis species are divided into three biovars (Antiqua, Medievalis, and Orientalis) according to genomic data and the ability to ferment glycerol and reduce nitrate. The number of strains is estimated to be very large. Strains vary greatly (genotypically, in virulence, etc.) due to the large number of distinct, isolated foci and large numbers of host/vector species. Variation in plasmid content also affects the virulence and drug resistance. Plasmid variation appears to be larger in Asian strains. Strains of the Orientalis biovar cause most of the modern cases of plague.

4) Reservoirs/Vectors:

Wild rodents (over 200 species) are the natural reservoir; wild rodent fleas (over 80 species), especially the oriental rat flea, are vectors. Rodent infection is asymptomatic. Fleas may be infectious for months. The organism also circulates among animals such as rabbits/hares and various carnivores. Humans occasionally contract bacteria. Large rodent die-offs frequently prelude human outbreaks.

5) Agent Sources:

Bubo aspirates and blood are most appropriate for culture. Sputum, throat swabs, urine, feces, animal carcasses, and infected tissues are also potential sources; infected fleas may also yield bacteria. Bubonic plague patients shed bacteria intermittently, so numerous specimens should be collected in a 24 hour period. Specimens should be collected before antibiotic treatment.

6) Stability:

Bacteria can survive for weeks outside of a host. The organism can remain viable in blood for approximately 100 days, in dried blood for three weeks, and in flea feces for five weeks. The bacteria may remain in infected human bodies for up to 270 days and can survive in soil for extended periods. Bacteria are killed after 15 minutes at 55°C, and by exposure to sunlight for three to four hours. The organism is also susceptible to most disinfectants including 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, and formaldehyde.

Laboratory Factors

1) Security Classification:

US Select Agent

2) General Research Volume:

Relatively large volume of research (PubMed). *Y. pestis* is attracting more research because of its potential as a BW agent. Many laboratories throughout the world conduct research.

3) Survey Information:

36/722 (4.99%) laboratories surveyed in Asia, the Middle East, Latin America, and Eastern Europe contain *Y. pestis*.

4) Past Weapons Activity:

Canada, the United Kingdom, the United States, Germany, South Africa, and Iraq (perhaps) conducted BW research on *Y. pestis*. The Soviet Union had weaponized the bacteria, producing large quantities for incorporation into aerosol weapons. Ken Alibek estimates that more than ten institutes and thousands of researchers worked with plague. North Korea likely has an active *Y. pestis* BW program.

5) Commercial Sources:

Little is known but North Korea likely attained *Y. pestis* from a Japanese culture collection.

6) General Growth Conditions:

Many bacteria species grow faster than *Y. pestis*, so isolation from sputum or carcasses can be difficult. The organism is also not easily culturable from postmortem tissue samples. Buboes should be thoroughly flushed with saline to maximize isolation chances; several blood cultures should be collected. If mixed flora contaminants are present or there is little *Y. pestis* bacteria, passage through mice may increase isolation chances (infected fleas have been pooled and used to infect mice). Numerous culturing protocols are available: the bacterium can grow well on conventional nutrient-rich media at 28°C in 5% CO₂ but selective media may be required for environmental samples. The WHO and CDC recommend sheep blood agar, brain heart infusion agar, MacConkey agar, and eosin methylene blue agar for relatively sterile samples such as blood or lymph node aspirate. A variety of more selective media has been developed for clinical/environmental samples including BIN media (brain heart infusions with Irgasan, cholate salt, crystal violet, and nystatin). Typically 48 hours are required before growth is observable (1-2 mm in diameter colonies). On sheep blood plates, colonies are gray to yellowish, opaque, and exhibit an irregular "fried egg" appearance; on MacConkey, colonies are small, and non-lactose fermenting. No hemolysis occurs on blood agar media. It is difficult to predict the virulence of strains in humans; guinea pig virulence and rhamnose fermentation appear to be best indicators. Stains with Wright, Giemsa or Wayson's dye, fluorescence microscopy (antibody staining of F1 antigen), antigen-capture ELISA, and PCR are more specific and often used to identify the organism. Commercial systems are generally inaccurate in the identification of *Y. pestis*.

Safety Factors

1) Risk of Disease Contraction:

Human plague is transmitted principally through flea bites and much less often by contact with (bites, scratches) with infected animals. Primary septicemic plague may develop after a flea bite, or may arise secondarily from bubonic disease. Direct person-to-person transmission of bubonic and septicemic plague is not seen. Bubonic and septicemic plague may progress to pneumonic plague if bacteria spread to lungs (very rare) or if aerosol droplets or contaminated material is inhaled directly. Person-to-person spread of pneumonic plague is also very rare. Transmission may occur from two meters away; overcrowding facilitates the spread of pneumonic plague. However, the vast majority of human plague cases occur from flea bites; handling of infected carcasses can also be dangerous.

2) Countermeasures (PPE and Medical):

BSL2 facilities, practices, and equipment should be used to handle any clinical material or cultures potentially containing *Y. pestis*. BSL3 should be used when aerosols may be generated (centrifugation, milling, animal use, etc.). Disposable masks and respirators are effective at preventing the transmission if aerosols are present; gloves, gowns, and eye protection should be worn if infected material or animals are handled. There are a host of effective antibiotics—most strains are susceptible to streptomycin, gentamycin, doxycycline, chloramphenicol, ciprofloxacin, and tetracycline—although they must be administered early during illness at the development of fever/cough. Pneumonic plague is almost 100% fatal if not treated within first 24 hours of symptoms. Drug resistant strains are rare, a few isolates have been resistant to streptomycin or tetracycline; only one isolate from Madagascar has been multidrug resistant. Vaccines are sometimes used to protect laboratory personnel, but are not widely available; a booster is often required every six months.

Agent: MDR *Yersinia pestis*

Environmental Factors

1) Geographic Distribution:

Yersinia pestis is a gram negative bacterium that causes plague, a serious disease in humans that is thought to have caused an estimated 200 million deaths historically. Today, the plague is considered a re-emerging disease, regularly causing small epidemics in different regions of the world including Africa, the Americas, the Middle East, Asia, and the former Soviet Union. In 1995, the first multi-drug resistant isolate of *Y. pestis* (strain IP275) was identified in a single patient presenting bubonic plague in Madagascar. The strain was found to contain a self-transmissible plasmid (pIP1202) conferring resistance to at least eight drugs traditionally used for treating plague, including streptomycin, tetracycline, chloramphenicol, and sulfonamides. Furthermore, the plasmid demonstrates a high degree of sequence identity with other MDR plasmids isolated from *Salmonella*, *E. coli* and other enterobacterial pathogens. Experimental studies have shown high transferability of this plasmid to other strains of *Y. pestis in vitro* suggesting a common and mobile resistance mechanism among these pathogens. MDR *Y. pestis* is considered rare with only one documented case; however there has been little surveillance for drug resistance.

2) Disease Symptoms (human and animal):

MDR *Yersinia pestis* has been identified in a single case presenting classic symptoms of bubonic plague. There is no evidence to suggest variability in classical symptoms.

3) Strain Information:

MDR *Yersinia pestis* strain IP275 gains its resistance from the pIP1202 plasmid. This plasmid is genetically similar and highly transferable to other strains of *Y. pestis*, *Salmonella*, *E. coli* and other enterobacterial pathogens.

4) Reservoirs/Vectors:

Reservoirs and vectors are assumed to be the same as drug-susceptible *Y. pestis*.

5) Agent Sources:

Agent sources are assumed to be the same as drug-susceptible strains.

6) Stability:

Stability is assumed to be the same as drug-susceptible *Y. pestis*.

Laboratory Factors

1) Security Classification:

Yersinia pestis is a US Select Agent (includes MDR *Yersinia pestis*). Although rare, this drug resistant strain could constitute a significant international public health and biodefense threat.

2) General Research Volume:

Minimal laboratory based research (PubMed). Only a handful of Western laboratories are actively conducting research on MDR *Y. pestis*.

3) Survey Information:

No survey information available

4) Past Weapons Activity:

Ken Alibek has suggested that the Soviet Union had experimented with MDR *Y. pestis*.

5) Commercial Sources:

Very few sources have this organism.

6) General Growth Conditions:

Growth conditions assumed to be the same as drug-susceptible *Y. pestis*.

Safety Factors

1) Risk of Disease Contraction:

The risk of disease contraction is presumed to be the same as drug-susceptible *Y. pestis*.

2) Countermeasures (PPE and Medical):

The PPE countermeasures should be the same as drug-susceptible *Y. pestis*. However, standard medical countermeasures including antibiotic treatment and prophylaxis are no longer effective with MDR *Y. pestis*.

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