

Biosafety Levels for Animal Agriculture Pathogens

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Abstract

Due to the size and importance of agriculture to many countries, it is vital to safely contain highly virulent animal pathogens while conducting research, or diagnostics involving those pathogens. Most agricultural pathogens can be studied safely in the laboratory, or vivarium at Biosafety Level (BSL) -2 or -3, or Animal Biosafety Level (ABSL) -2 or -3. However, when animals are infected with certain highly virulent agricultural pathogens, and there is no alternative other than loose-housing in a room, special requirements for containment are needed. This standard has become known as Biosafety Level 3 Agriculture (BSL-3-Ag) in North America and constitutes a special set of facility, operational and personnel requirements. In order to determine which biosafety level is appropriate for the containment of the animal pathogen being studied, an onsite risk assessment must be conducted, which takes into account many different factors. In addition, the final approval to work with a particular agricultural pathogen is often provided by the local and/or national government and may require further assessments.

Introduction

This document intends to provide recommendations for biocontainment levels required to work with agriculture pathogens or agents that specifically infect animal species traditionally used in agriculture. The scope of this paper does not cover other areas of agricultural risk, which includes foreign diseases of crops, biocontrol activities, and work involving arthropods and genetically engineered agricultural products. Information on these subjects can be found elsewhere (Scott, 2005; Delfosse, 2005; Chapman & Burke, 2006; Traynor, et al., 2001). This document is not only relevant to traditional livestock species, which include cattle, pigs, sheep, goats, horses, and a wide variety of avian species, but also to wildlife when appropriate.

Globally there are a large number of pathogens, including parasites, bacteria, viruses, mycoplasma and prions that infect domestic agriculture species and wildlife. There is a wide range of geographic presence of these pathogens that determines a country's animal health status, at least in part. Many nations have worked diligently for decades to eradicate the most economically significant animal diseases from their national herds. This has provided those countries with better economic animal production and favored trade status. Many of these coun-

tries have a continued interest in studying these now non-endemic, highly infectious agents both *in vivo* and *in vitro*. One of the goals of such research is to ensure adequate diagnostics, vaccines and other tools that could be utilized during a potential outbreak, either naturally occurring, or as a result of agroterrorism. Regardless of how a serious foreign animal disease (FAD) is introduced into a country's animal production system, it is expected that severe losses to the economy would occur. Therefore, this document intends to provide some recommendations for appropriate biocontainment, and practices to conduct studies of significant agricultural pathogens in both laboratory and vivarium settings.

Commercial Agriculture

Agriculture is big business. In the United States, it is estimated that animal agriculture constitutes 13% of the gross national product; 17% of all employment in the U.S. is either directly, or indirectly, working in agriculture. In 2006, there were approximately 88 million beef cattle, 9.2 million dairy cattle and 9.7 billion poultry in the U.S. (National Agriculture Statistics, 2006). When animal health problems occur, and the agriculture economy is affected, it impacts the entire U.S. economy. Since it is possible to start a widespread outbreak from a single source, preventing an accidental release of a highly infectious organism from a biocontainment facility is of the utmost importance. Most animals in the U.S. are not vaccinated against many foreign animal diseases (FADs) and are very susceptible to infection from these agents. If a highly infectious agent is introduced into a modern agricultural system, models have shown that the pathogen can rapidly move from one farm to another via the infected animals. Intensive, modern agricultural practices promote high-density livestock populations, which are bred and reared in close proximity to one another. The outbreak of a contagious FAD at one of these facilities would be very difficult to contain, especially if the disease is airborne and shed in large quantities.

Problems with contagious disease outbreaks are exacerbated by the distant and rapid dissemination of animals from farm-to-farm, or in farm-to-market distribution nodes. In many cases, such as in the cattle industry, dairy and beef animals are born in one location, raised in another location and live their production life in yet another location. For example, a representative survey of U.S. barn auctions revealed that between 20 and 30 percent of cattle were regularly consigned to non-slaughter

destinations at least 30 miles from their original point of purchase, and in many cases had crossed several states within 36 to 48 hours of leaving the sales yard (Chalk, 2004). Because farms are tightly interconnected with other farms and distribution nodes, infectious agents can move extremely rapidly across the United States. Thus, small local outbreaks can rapidly become large national outbreaks. Some estimates have determined that an outbreak of Foot-and-Mouth Disease (FMD) in the U.S. would cost \$33 billion dollars to eradicate (Lautner & Meyer, 2003). However, that may be an underestimate based upon outbreak costs in other countries. FMD in the UK in 2002 cost the country \$15 billion; Classical Swine Fever (CSF) in the Netherlands in 1997 cost \$3.4 billion; highly pathogenic avian influenza (HPAI) in the U.S. in 1983 cost \$349 million; Exotic Newcastle disease, in California in 2003, cost \$360 million to eradicate. Many of these costs are due to the effect a disease outbreak has on export trade. Export of agricultural products is one of the few areas where the U.S. has a positive trade balance. World trade has dramatically increased in the last twenty years (\$2.4 trillion in 1980 to \$8.0 trillion in 2000). U.S. exports have been continuously climbing with \$103 billion dollars in exports and 860,000 jobs related to exports. All of those gains could be jeopardized if a serious disease outbreak involving a FAD, or a disease that has been previously eradicated occurred among livestock. Other economic impacts would include: loss of production; losses to related industries such as transportation; the direct costs of outbreak control; and indirect costs to non-agricultural industries, such as tourism due to restriction of movement in and out of impacted areas.

Animal/Livestock Agents of Agricultural Concern

In the U.S., the United States Department of Agriculture (USDA) generally responds to outbreaks of highly pathogenic organisms with a test and slaughter policy. In order to prepare for these outbreaks, pathogenesis studies of the organism are required to develop better diagnostic tools and establish effective countermeasures. There is a need for laboratories where these pathogens are studied, both *in vitro* and *in vivo*, to employ appropriate biocontainment. These laboratories typically require levels of biocontainment beyond what is required for endemic pathogens to ensure that these FADs stay confined to the research environment.

There are many pathogens (Table 1) that infect agricultural species that could cause economic problems to a region, state or nation. Many of these pathogens are still widely distributed around the world and continue to cause agriculture production losses. It has been difficult to devise a single global ranking for agricultural pathogens due to the factors of economic impact and disease status between countries and regions within countries

(Rusk, 2000). A global risk assessment guideline for diseases of livestock and crops is desperately needed. The U.S. and other developed countries have invested significant resources, both human and financial, to eliminate many of the most economically damaging disease agents from their national herds. For many of these agents, there is no treatment or vaccine; therefore, the most efficient and rapid method of removing the agent, if an outbreak should occur, is to remove the susceptible hosts, thus necessitating effective biocontainment when studies are conducted within a laboratory or vivarium.

Risk Assessment

Risk analysis is a “body of knowledge” (methodology) that evaluates and derives a probability of an adverse effect of an agent (chemical, physical or other), industrial process, technology, or other technology (Molak, 1997). The National Academy of Sciences (1983) identified four common elements in risk analysis:

1. Hazard (agent) identification
2. Dose response
3. Exposure analysis
4. Risk characterization

Risk assessment is “risk analysis applied in a particular situation” (Molak, 1997). Many institutions and organizations throughout the world must consider the risks presented by proposed research with agricultural pathogens, and make decisions regarding the placement of these pathogens into proper biocontainment and biosafety categories. In the U.S., the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) published guidelines that are universally accepted as the basic standard for biosafety (Centers for Disease Control & Prevention and National Institutes of Health, 1999). Although this system is appropriate and accepted for agents of human and zoonotic diseases, it cannot be universally applied to biocontainment for pathogens that infect only livestock (Rusk, 2000). In the development of risk assessment and management guidelines for agriculture, it must be recognized that the rationale for agricultural standards will differ from those for human public health standards and those for worker protection. Risk management for agriculture research is based on the potential economic impact of animal and plant morbidity and mortality, and the trade implications of disease.

In conducting a risk assessment, agricultural regulatory agencies, such as the United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS), will consider a wide variety of factors when determining what level of biocontainment and biosafety is appropriate, and what additional enhancements may be required for research involving livestock pathogens. Just a few of these include the following:

1. Is the agent endemic, or foreign to the region?

Table 1

Agents of greatest concern to the health and productivity of agricultural animals.

African horse sickness virus	Malignant catarrhal fever virus (exotic strains or alcelaphine herpesvirus type 1)
African swine fever virus	Menangle virus
Akabane virus	<i>Mycobacterium bovis</i>
Avian influenza virus (highly pathogenic)	<i>Mycoplasma agalactiae</i>
<i>Bacillus anthracis</i>	<i>Mycoplasma capricolum</i>
<i>Besnoitia besnoiti</i>	<i>Mycoplasma mycoides</i> (var. <i>mycoides</i> , small colony type)
Bluetongue virus (exotic)	Nairobi sheep disease virus (Ganjam virus)
Borna disease virus	Newcastle disease virus (velogenic strains)
Bovine infectious petechial fever agent	Nipah virus
Bovine spongiform encephalopathy	Peste des petits ruminants virus (plague of small ruminants)
<i>Brucella abortus</i>	Rift Valley fever virus
<i>Brucella melitensis</i>	Rinderpest virus
<i>Brucella suis</i>	Sheep pox virus
<i>Burkholderia mallei</i> (<i>Pseudomonas mallei</i> - Glanders)	Spring Viremia of Carp virus
<i>Burkholderia pseudomallei</i>	Swine vesicular disease virus
Camelpox virus	Teschen disease virus
Classical swine fever virus	<i>Theileria annulata</i>
<i>Coccidioides immitis</i>	<i>Theileria bovis</i>
<i>Cochliomyia hominivorax</i> (Screwworm)	<i>Theileria hirci</i>
<i>Cowdria ruminantium</i> (heartwater)	<i>Theileria lawrencei</i>
<i>Coxiella burnetii</i> (Q fever)	<i>Trypanosoma brucei</i>
Ephemeral fever virus fever agent	<i>Trypanosoma congolense</i>
Eastern equine encephalitis virus	<i>Trypanosoma equiperdum</i> (dourine)
Foot and mouth disease virus	<i>Trypanosoma evansi</i>
<i>Francisella tularensis</i>	<i>Trypanosoma vivax</i>
Goat pox virus	Venezuelan equine encephalomyelitis virus
Hendra virus	Vesicular exanthema virus
<i>Histoplasma (Zymonema) farciminosum</i>	Vesicular stomatitis virus (field strains, exotic)
Infectious salmon anemia virus	Viral hemorrhagic disease of rabbits virus
Japanese encephalitis virus	Wesselsbron disease virus
Louping ill virus	
Lumpy skin disease virus	

2. What is known regarding the morbidity and mortality caused by the agent?
3. Are there effective prophylaxes, treatments or vaccines available?
4. What are the shedding patterns of the agent in relevant species?
5. Are there active control or eradication programs for the disease?
6. Knowledge of environmental stability, quantity and concentration of the agent.
7. How will the agents be used in animals (large or small), or in the laboratory?
8. What is the host range of the agent, and is there ongoing surveillance testing?

In addition to the biological factors of the agent, consideration will also be given to the people using the agent such as their level of experience and knowledge, practices used, experimental design and protocols, standard operating procedures, and facility design and management.

Risk management strategies identify and implement methods to reduce risk to an acceptable level. To mitigate

risk, biosafety professionals can use a combination of practices and techniques, along with safety equipment and facilities working in concert to enable agricultural pathogens to be studied safely (Rusk, 2000). A risk assessor, or manager has additional risk management options available to them for controlling an agricultural hazard that do not represent a risk to human researchers. In an agricultural setting, consideration for seasonal separation, climatic and geographic factors, and host and/or vector availability outside the research environment can all play a role in determining the appropriate biocontainment level. For example, a livestock pathogen only transmitted by a specific arthropod vector, would typically require a high biocontainment level (BSL-3-Ag) when viable vectors and hosts were present in the outside environment. However, this pathogen could potentially be studied at a lower containment level in situations where the vector, or potential hosts are absent, (e.g., where there is climatic, seasonal, or geographic separation).

In addition to describing various biocontainment standards appropriate for working with agricultural

pathogens, this document also describes the facility parameters and work practices that have come to be known as BSL-3-Ag. BSL-3-Ag is unique to agriculture, because of the necessity to protect the environment from an economic, high-risk pathogen in a situation where studies are conducted employing large agricultural animals, or other similar situations in which the facility barriers now serve as primary containment. This document also describes some of the enhancements beyond BSL-3 that may be required by the USDA-APHIS when working in the laboratory, or vivarium with veterinary agents of concern. The document provides guidance to researchers working with veterinary agents of concern, and is not regulatory, or meant to describe national or international policy. Conditions for approval to work with specific agricultural agents are provided at the time USDA-APHIS, or the veterinary authority of the country, permits a location to work with an agent.

Containment Standards Appropriate for Working with Agriculture Pathogens

Biosafety Level

A combination of work practices and physical containment requirements (facility and safety equipment) are designed to reduce the risk of laboratory infection when working with biohazardous material. The degree of protection recommended is proportional to the risk associated with an agent and the proposed research operations. When studying agriculture pathogens in the laboratory, there are 4 proposed biosafety levels that can be used. When studying agriculture pathogens in the vivarium, there are 4 proposed biosafety levels that can be used, with the additional criteria of BSL-3-Ag reserved for studying high consequence pathogens in loose-housed animals where the room becomes the primary containment. The biosafety levels described in the BMBL specify many of the containment features that could be utilized as a basis for agricultural research. However, the authors re-emphasize the difference in risk assessment criteria between public health and worker protection and agricultural containment requirements. Others have also described similar biocontainment principles and practices (Barbeito et al., 1995; Best, 1996).

Proposed Laboratory Biosafety Levels

BSL-1. Facility and practices appropriate for work with well-characterized, low-risk agents not known to cause disease in healthy animals that represent no potential economic loss to agricultural industries. No specialized practices, other than good microbiological technique, are utilized. Facilities should be easily cleanable, have a sink for hand washing, and conform to the facility requirements described in the BMBL for BSL-1. These laboratories are typical of undergraduate, or secondary education teaching laboratories.

BSL-2. Facility, safety equipment and practices appropriate for agents of moderate potential hazard to animals, or agriculture that are generally endemic, cause illness of varying degree, and are typically treatable or preventable. Most research and diagnostic laboratories that work with food-borne pathogens and domestic diseases are designed to perform work at this level.

BSL-3. Facility, safety equipment, and practices that are applicable to clinical, diagnostic, research, or production facilities in which work is done with indigenous, or exotic agents with a potential for transmission, and, which may cause serious and potentially lethal infections, or grave economic consequences if released. Laboratory facility and practices include inward directional airflow, separation from non-laboratory areas, special laboratory protective clothing, and decontamination of laboratory waste. For *in vitro* work with some highly infectious agriculture agents, BSL-3 may be modified further with enhancements specifically designed to protect the environment, such as high-efficiency particulate air (HEPA) filtration of supply and exhaust air, laboratory liquid effluent/sewage decontamination, personnel exit showers, and facility integrity testing (pressure decay test), if required by the risk assessment for the agent, and planned manipulations.

BSL-4. Facility, safety equipment and practices appropriate for research on dangerous and exotic agents that pose a high individual risk of human life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or treatment. While there is no BSL-4 requirement solely for agricultural agents, 2 viruses have recently been discovered that are lethal for agricultural species and for humans (Nipah and Hendra viruses) and for which there are no vaccines. These agents can only be manipulated at laboratories having BSL-4 capability. At present, the U.S. does not have this containment capability for large animals. This underscores the need to establish an ABSL4 laboratory in the U.S. that is designed to accommodate large agricultural species. Currently, fewer than 20 viruses are designated for use at BSL-4.

Proposed Vivarium Biosafety Levels

ABSL-1. Facility and practices appropriate for work with well-characterized, low-risk agents not known to cause disease in healthy animals. No specialized practices, other than good microbiological technique are utilized. These facilities are typical of university or industry research farms.

ABSL-2. Facility, safety equipment and practices, appropriate for agents of moderate potential hazard to animals, or agriculture that are generally endemic, cause illness of varying degrees, and are typically treatable or preventable. Most research and diagnostic vivariums that work with food-borne pathogens and domestic diseases are designed to perform work at this level.

ABSL-3. Facility, safety equipment, and practices

applicable to clinical, diagnostic, research, or production facilities in which work is done with indigenous, or exotic agents with a potential for transmission, and which may cause serious and potentially lethal infections, or grave economic consequences if released. Vivarium facility and practices include inward directional airflow, separation from uninfected animal areas, special laboratory protective clothing, and decontamination of laboratory waste. For *in vivo* work with some highly infectious agriculture agents, ABSL-3 may be modified further with enhancements specifically designed to protect the environment, such as placing animals in isolation containers (isolets or flexible film isolators) with HEPA filtration of supply and exhaust air, sewage decontamination, personnel exit showers, and facility integrity testing (pressure decay test).

BSL-3-Ag. The USDA Agricultural Research Service (ARS) has defined enhanced ABSL-3 facilities, safety equipment and practices particular to agriculture research where the facility barriers, usually considered secondary barriers, now act as primary barriers. This standard is used when large animals, such as cows, pigs, bison and deer, are infected with high consequence agricultural pathogens and cannot be placed inside any other animal isolation device. BSL-3-Ag facilities utilize the containment features of the standard ABSL-3 facility (as defined in the BMBL) as a starting point, with a number of enhancements specifically designed to protect the environment, such as HEPA filtration of supply and exhaust air, sewage decontamination, exit personnel showers, and facility integrity testing (pressure decay test). FMD, CSF, and HPAI are representative of agricultural agents assigned to this biosafety level.

ABSL-4. Facility, safety equipment and practices appropriate for research on dangerous and exotic zoonotic agents that pose a high individual risk of human life-threatening disease, which may be transmitted via the aerosol route, and for which there is no available vaccine, or treatment. This standard would have all of the features of a BSL-3-Ag facility with added worker protection. While there is no BSL-4 requirement solely for agricultural agents, recently two viruses have been discovered that are lethal for agricultural species and for humans (Nipah and Hendra viruses) for which there are no vaccines. These can only be manipulated in vivaria having ABSL-4 capability.

Facility Design and Standard Operating Procedure Enhancements for Laboratories and Small Animal Facilities Working with High Consequence Foreign Animal Diseases

There are circumstances where certain agents that typically would be required to utilize a BSL-3-Ag facility for research involving large animals, may be studied in an

enhanced BSL-3 laboratory, or an enhanced ABSL-3 vivaria, for work with small animals in which primary containment devices can be utilized. In these situations, the facility no longer serves as the primary barrier as it does with the large animal rooms. The design and testing requirements for BSL-3-Ag laboratory areas should reflect the difference between the two situations without compromising the environmental protection that is required when working with these agents. Therefore, when manipulating high consequence livestock pathogens in the laboratory, or small animal facility, facility design and work procedures must meet the requirements of ABSL-3 with additional enhancements unique to agriculture. In addition to meeting the basic ABSL-3 requirements, the facility should have personnel enter and exit through a clothing change and shower room, have a double-door autoclave and/or fumigation chamber, HEPA-filtered supply and exhaust air, and a liquid effluent decontamination system. Surfaces must be smooth to support surface wipe-down decontamination, all penetrations should be sealed, and the room capable of sealing a fumigant in instances where gaseous decontamination is required. Since all work with infectious material is conducted within primary containment, there is no requirement for pressure decay testing.

Laboratory Facilities and Small Animal Vivaria

Potential enhancements beyond BSL-3 for containment of agriculture pathogens in the laboratory are agent and site dependent and may include the following:

1. Personnel change and shower rooms that provide for the separation of street clothing from laboratory clothing and that control access to the containment spaces.
2. Access doors to these facilities that are self closing and lockable.
3. Supplies, materials and equipment enter the BSL-3 enhanced space only through an airlock, fumigation chamber, or an interlocked and double-door autoclave.
4. Double-door autoclaves engineered with bioseals should be provided to decontaminate laboratory waste passing out of the containment area.
5. Dedicated, single pass, directional, and pressure gradient ventilation systems must be used.
6. Supply and exhaust air, to and from the containment space, is HEPA filtered, with special electrical interlocks to prevent positive pressurization during electrical, or mechanical breakdowns. Alternatively, the supply air may not be HEPA filtered, but equipped with a fast-acting bioseal damper, which is interlocked with the exhaust fan to ensure that potentially contaminated air is not released through the air intake during an HVAC failure.
7. Liquid effluents (sinks, toilets, floor drains, showers) from BSL-3 enhanced areas may be collected and decontaminated by a method validated to inactivate the agent being used (a central liquid waste sterilization system is recommended) before disposal into the sanitary sewer

system. The treatment requirement will be determined by a site-specific, agent-specific risk assessment.

8. Each BSL-3 enhanced containment space shall have its interior surfaces (walls, floors, and ceilings) and penetrations sealed to create a functional area capable of being validated as airtight.

9. All ductwork within containment serving BSL-3 enhanced spaces shall be airtight (pressure tested—consult your facility engineer for testing and certification details).

10. If Biological Safety Cabinets are installed, they must be installed where their operations are not adversely affected by air circulation and personnel traffic.

Facility Design and Standard Operating Procedure Enhancements for BSL-3 Agriculture

The BSL-3-Ag facility can be a separate building, but, more often, it is an isolated zone contained within a facility operating at a lower biosafety level, usually BSL-3. This isolated zone has strictly controlled access, special physical security measures, and functions with the “box within a box” principle. All BSL-3-Ag facilities employing animals that cannot be readily housed in primary containment devices require the same features as for an animal ABSL-3 facility with the following enhancements typical of BSL-4 facilities (Heckert & Kozlovac, 2006):

1. Personnel change and shower rooms that provide for the separation of laboratory clothing from animal facility clothing, and that control access to the containment spaces.

2. Access doors to these facilities are self closing and lockable.

3. Supplies, materials and equipment enter the BSL-3-Ag space only through an airlock, fumigation chamber, an interlocked and double-door autoclave, or shower.

4. Double-door autoclaves, engineered with bioseals, are provided to decontaminate laboratory waste passing out of the containment area.

5. Dedicated, single pass, directional, and pressure gradient ventilation systems must be used.

6. Supply and exhaust air, to and from the containment space, is HEPA-filtered, with special electrical interlocks to prevent positive pressurization during electrical, or mechanical breakdowns. Alternatively, the supply air may not be HEPA filtered, but must be equipped with a fast-acting, bioseal damper, which is interlocked with the exhaust fan to ensure that potentially contaminated air is not released through the air intake during an HVAC failure.

7. Liquid effluents from BSL-3-Ag areas must be collected and decontaminated in a central liquid waste sterilization system before disposal into the sanitary sewers system.

8. Each BSL-3-Ag containment space shall have its interior surfaces (walls, floors, ceilings) and penetrations

sealed to create a functional area capable of being validated as tightly sealed (airtight). It is recommended that the validation process includes a pressure decay test (for new construction only). Information on how to conduct a pressure decay test may be found within Appendix 9B of the ARS Facilities Design Standard (Policy and Procedure 242.1M-ARS).

9. All ductwork within containment serving BSL-3-Ag spaces should be airtight (pressure tested—consult your facility engineer for testing and validation details).

10. The hinges and latch, or knob areas of all passage doors shall be sealed to meet pressure decay validation requirements (pressure decay test).

11. All airlock doors shall have air inflated, or compressible gaskets.

12. Animal restraining devices shall be provided in large animal rooms.

13. Necropsy rooms shall be sized and equipped to accommodate large farm animals.

14. Pathological incinerators, or other approved means, must be provided for the safe disposal of the large carcasses of infected animals.

15. HEPA filters, or the equivalent, must be installed on all atmospheric vents serving plumbing traps as near as possible to the point of use, or the service cock, of central, or local vacuum systems, and on the return lines of compressed air systems.

Conclusions

Many of the practices and policies described in the U.S. CDC/NIH publication entitled *Biosafety in Microbiological and Biomedical Laboratories* can be applied to work with agricultural pathogens. However, it must be noted that the risk assessment criteria for agriculture are different than those for public health and worker safety. Risk management strategies for work involving agriculture pathogens must focus on biocontainment and environmental protection, in addition to worker protection, since the primary concern is the potential economic impact of the morbidity and mortality on agricultural species, and the international trade implications of a disease outbreak. Those pathogens that have the highest economic consequence to the animal health status of the country in which the work is being done require the highest level of biocontainment. For most agriculture pathogens, BSL-2 or BSL-3 and ABSL-2 or ABSL-3 standards are acceptable and achieve an appropriate level of biocontainment. However, for some agriculture pathogens, there is a special concern for reducing the risk of environmental exposure, or escape from the facility. Therefore, ABSL-3 facility standards with enhancements for safety equipment and practices particular to agriculture research (i.e., infectious disease work with large agricultural animals) where the facility barriers (usually considered secondary barriers) now act as primary barriers have been

established (USDA Agriculture Research Service Manual 242.1, CDC/NIH BMBL 5th edition; Heckert & Kozlovac, 2006; Best, 1996). The U.S. standard, referred to as BSL-3-Ag, utilizes the containment features of the standard ABSL-3 facility (as defined in the BMBL) as a starting point, and includes the majority of enhancements typically assigned to ABSL-4. These enhancements are provided specifically to protect the environment, and include HEPA filtration of supply and exhaust air, decontamination of liquid effluent, decontamination of solid wastes, including carcass disposal, personnel exit showers, and facility integrity testing (pressure decay test). This type of laboratory is appropriate for non-endemic pathogens causing serious livestock, or poultry disease that are readily transmitted to agricultural species and wildlife.

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