**ILAR J**

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***Balancing Animal Welfare, Human Safety, and Research in Agriculture High Containment***

# **Klages. IACUC and Veterinary Considerations for Review of ABSL3 and ABSL4 Research Protocols, pp. 3-9**

Domain 3: Research; T1. Facilitate or provide research support and T2. Advise and consult with investigators on matters related to their research

SUMMARY: This article is a thoughtful presentation of considerations for research institution review and approval of research that is proposed and planned for investigations of high risk infectious agents (ABSL3 and ABSL4 agents) using animal models. Those studies can only be conducted after the review, approval and under the oversight of a variety of entities within the institution. The institutional processes and oversight are themselves based on regulations and guidelines that come from and are overseen by multiple government and professional organizations. The goals of the design and review of such work include: assuring the protection of the involved people, the welfare of the animal subjects, and the integrity of the science itself. With these broad, interconnected goals, the author emphasizes the need for “professional and collegial interactions (among ‘stakeholders’) across all levels of the proposed project”.

The author presents the institutional goals as revolving around three interconnected concepts: biocontainment, bioexclusion and bioprotection – termed in this article as the “3B’s”. He also describes several types of biocontainment protocol designs: investigation of unknown/new agents associated with health issues; investigations directed at therapy against the infectious agent and the clinical issues that occur during the infection; and investigation of preventive measures (immunization and therapeutics that can prevent infection and disease).

The discussion then touches on key topics to be considered, and related questions, for such work to be appropriately reviewed to assure human safety, animal welfare and scientific integrity prior to approval.

These include the following:

* Basic pre-review questions
* Questions about clinical observation and protocol procedures
* The timing and type of interventions and supportive care to be carried out
* Clearly defined study/scientific endpoints
* Establishment and use of clinical scoring and euthanasia criteria
* Plans for biosampling during the studies, with consideration of numbers and size/volume of necessary samples from individual animals and size of the animal subject species
* documentation/animal health records
* Personnel training and certification
* Emergency response/Contingency Plans
* Security
* Biosecurity related to facility design, operation (biosecurity procedures) and size, as multiple studies, including different agents and animal species are proposed

KEY POINTS

* Key resources/references
  + CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL)
  + FDA Good Laboratory Practice for Nonclinical Laboratory Studies, and the associated Animal Rule
* Institutional entities and stakeholders involved in proposal, review and approval of work involving high containment (ABSL3 and ABSL4) infectious agent animal models
  + Research scientists
  + Institutional veterinarians – Attending Veterinarian
  + IACUC
  + IBC (institutional Biosafety Committee)
  + Environmental Health and Safety department
  + Occupational Medicine Department
  + Facilities Operations
* Three concepts for infectious agent research
  + Biocontainment – “keeping agents in – preventing release of a pathogen or agent.”
  + Bioexclusion – preventing pathogen entry (also referred to as biosecurity)
  + Bioprotection – keeping humans safe as they work in the project/facility
* Three general types of ABSL3 and ABSL4 investigations
  + Investigation of unknown/new agents
    - What is the agent?
    - What happens to infected hosts?
    - How infectious is the agent ?
    - How does it spread from host to host ?
    - What is the agent’s lethality?
    - What is the apparent risk group for this agent?
  + Therapeutic investigations
    - How to stop disease progression
  + Infection prevention measures – immunization and preventive therapeutic measures
    - These generally are conducted with FDA Animal Rule conditions (GLP) as a basis
* For work with infected animals – consider how the clinical observations and biosampling will be accomplished and verified
* Types of interventions related to animal welfare and scientific goals
  + To assure appropriate animal care
  + Intervention that mimics scenarios in human clinical settings
  + Intervention that permits disease manifestation or a particular condition of interest
* Three types of care
  + Standard animal husbandry and preventive health
  + Supportive care (clinical–condition support and care while considering how does this inter-relate with the research goals)
  + Critical care – “saving life, limb, eyesight”

QUESTIONS

1. Which of the following is not one of the infectious agent investigation 3B’s?

a. Biosafety

b. Bioassessment

c. Bioexclusion

d. Biocontainment

2. What federal regulation has to do with the ability to assert that animal subject based work will assure safety of new medical products?

a. US Government Principles

b. Animal Welfare Act

c. Good Laboratory Practices Act – Animal Rule

d. Guide for the Care and Use of Laboratory Animals

3. Consideration of a biological agent’s pathogenesis factors, the research-related laboratory testing/environmental factors, and human research and animal care personnel factors are major parts of which of these terms?

a. Animal welfare

b. Occupational health

c. Risk assessment

d. Contingency/emergency plan

ANSWERS

1. b. Bioassessment

2. c. Good Laboratory Practices Act – Animal Rule

3. c. Risk assessment

# **Harper et al. High-Containment Agriculture Animal Research: An AAALAC International Perspective, pp. 10-17**

Domain 4: Animal Care

Domain 5: Regulatory Responsibility

SUMMARY

Background: AAALAC International is a non-profit organization which is a voluntary accreditation system with more than 1000 animal accredited animal research programs across 49 countries. AAALAC was first established in 1965. The primary focus of AAALAC International is “the conscientious and humane treatment of animals in science and medicine.” It is not a regulatory agency and does not make or enforce regulations.

The performance based assessments rely on 3 primary standards:

* National Research Council’s Guide for the Care and Use of Laboratory Animals
* The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Council of Europe
* The Federation of Animal Science Societies Guide for the Care and Use of Agricultural Animals in Research and Teaching

NOTE: The 4th Edition of The Ag Guide is published by The American Dairy Science Association, American Society of Animal Science, and Poultry Science Association (the 3rd Ed. was published by FASS which is a non-profit service organization that support various non-profit animal science and related organizations).

Institutional Responsibilities for Animal Research Involving Biological Hazards: The IACUC or equivalent Oversight Body (OB) is responsible for ensuring that their animal care and use programs meet or exceed the standards as outlined in the above primary standards. If an AAALAC accredited collaborates with another institution that is not AAALAC accredited the elements utilized at the non-accredited program must be included in the program of the accredited institution as part of the program description and on-site evaluation. An effective oversight system includes: “…strong administrative support, adequate resources, clearly defined hazard identification criteria, a comprehensive risk assessment screening process, sound management and control procedures for work with hazardous materials and agents, and a work-specific training program.”

Unique Aspects of Agricultural Research

* Effective biosafety & biocontainment strategies are essential when working with high-consequence, foreign, and/or regulated pathogens due to the potential economic and environmental impact.
* The “biomedical” definition of Biosecurity – measures that are implemented to protect infectious agents and toxins from loss, theft, escape, or misuse. Focuses on physical barriers and work practices to control access to biohazards.
* WHO’s Terrestrial Animal Health Code defines Biosecurity for agricultural programs as a set of management and physical measures designed to reduce the risk of introduction, establishment, and spread of animal diseases, infections, or infestation to, from, and within an animal population.
* Hazards are managed through the use of controls including:   1) facility design, 2) engineering features, 3) management practices, and 4) personnel training
* A risk assessment should be used to identify the appropriate level of containment required for each hazard
* High containment and maximum containment  refer to work with life-threatening agents that can be transmitted via aerosol
* High containment general is used for Biosafety Level 3 (BSL-3) and involve agents that have a medical counter measure (i.e. vaccine). The term is also used for BSL-4 work.
* Maximum containment is used for BSL-4 and are used with high-consequence agents which do not have a medical counter measure.
* For high containment work with agricultural species, animals are commonly housed in open penning or non-containment cages due to their large size and species specific needs. These facilities are specifically designed so that the animal room is the primary barrier (BSL-3 Ag).
* AAALAC expects site specific risk assessments to be performed and which include waste management, ventilation parameters, sanitation and disinfection, PPE selection, and entry/exit protocols.
* AAALAC considers effective training programs essential to ensure that research activities are conducted safely and with compassion for the animals. This should also include continuing education and training.

Overview of AAALAC Accreditation Process

* The on-site assessment and Program Description (PD) are used to evaluate an institutional member’s animal care program and occurs on a 3 year cycle.
* AALAC’s Rules of Accreditation

1.  Animal care and oversight should be directed by qualified persons

2.  Animal care personnel should be suitably trained and experienced

3.  Physical facilities and methods of animal care should ensure animals’ well-being and comfort

Areas of Special Interest to AAALAC

* High-containment agricultural research programs may receive “added scrutiny during assessment” due to the highly specialized facilities and occupational health and safety concerns.
* Facility management
* Make sure site visitors are aware of any special entry requirements  (e.g. vaccination, respirator fit testing, animal contact restriction) well in advanced of the site visit
* Biological hazard containment areas need to be clearly identified  (name of agent/hazard, special entry requirements or procedures, emergency contact information)
* Animal Housing
* Site visitors will ask about animal transport, types of procedures performed, who provides care, and will review records and documentation associated with animal care
* Most agricultural species are herd animals and social housing is important. AAALAC expects that individually housing of animals is approved the animal use protocol and/or authorized by a veterinarian for medical or behavioral reasons
  + The environmental enrichment program may be utilized to help alleviate the potential stress of single housing of animals
* Animals should have a clean, comfortable resting space and be able to stand and make natural adjustments
* Flooring surfaces should have good traction
* If bedding is used it should be compatible with the drain and waste system
* All surfaces should be free of sharp edges, pinch points, or gaps that can injury animals or personnel
* Validation of the effectiveness of cleaning and sanitation should be conducted and documented
* If arthropod vectors are used for studies with large animals an effective surveillance program should be used to prevent accidental escape of the vectors.
* Facility Environment
* High-containment spaces are kept under negative pressure. There should be systems in place to alert staff of changes in room pressures
* Back power must be available and tested frequently to ensure uninterrupted service and prevent accidental release of hazards
* Pest (rodent, insects, birds) surveillance and management program is essential
* Procedure & Support Areas
* Site visitors will inspect any support areas (imaging, surgical rooms, necropsy) where animals may go
* Site visitors will inspect equipment and methods used to transport animals within and outside of the facility
* Maintenance logs are expected for any equipment that requires routine calibration
* The institutional disaster plan will be reviewed for provision for routine animal care and well-being

Veterinary Care

* + - Most agricultural animals are procured from production herds rather than Class A commercial dealers which may require a more robust preventative health program and acclimation and quarantine process prior to moving animals into high-containment.
    - Recognition of pain and distress in agricultural species can be challenging. Animals tend to hide pain and/or distress, animals may flock together making it difficult for individual assessment, and viewing of the animals may be more difficult if there are in isolation chambers or viewed through windows.
    - Veterinarians should be involved in the establishment of humane endpoints and the training of researchers and animal care staff on how to monitor animals for humane endpoints.

IACUC/OB Oversight: IACUC/OB must ensure that final study design includes clear, human endpoints to minimize the level of pain and distress that animals experience and appropriate safeguards to protect personnel and animals both inside and outside of the containment facility.

Institutional Policies

* + - Personnel should receive species specific training.
    - The occupational health program must be customized to address the specific hazards of high containment work and agricultural species. Site visitors may ask about practices used to help protect workers from traumatic injuries that may result from unpredictable behavior of large animals in containment space or heavy equipment (i.e. chutes)

QUESTIONS

1. AAALAC International uses which of the following as primary guidance documents when conducting program assessments?

a.  AVMA Guidelines on Euthanasia

b. The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Council of Europe

c.  Guide for the Care and Use of Agricultural Animals in Research and Teaching

d.  National Research Council’s Guide for the Care and Use of Laboratory Animals

e.  Occupational Health and Safety in the Care and Use of Research Animals

f.   OIE's Terrestrial Animal Health Code Chapter 7.8

2. T/F:  When an AAALAC accredited institution collaborates with a non-AAALAC accredited institution elements of animal work conducted at the non-accredited institution must be included in the program description of the accredited institution.

3. After program assessment, AAALAC International Council on Accreditation (Council) may grant all of the following except:

a.  Accreditation granted

b.  Accreditation withheld

c.  Accreditation revoked

d.  Conditional Status

e.   Provisional Status

f.   Probationary Status

ANSWERS

1. b, c, d

2. True

3. d

**Higgs et al. The Use of Arthropod-Borne Challenge Models in BSL-3Ag and BSL-4 Biocontainment, pp. 18-31**

Domain 3: Research

Domain 5: Regulatory Responsibility

SUMMARY: It is important to mimic natural transmission when studying arthropod-borne diseases because arthropod salivary proteins have an impact on virus transmission efficiency, subsequent replication dynamics, and vertebrate immune responses. The American Committee of Medical Entomology 2019 contains revised Arthropod Containment Guidelines (ACGs). Arthropods can be sourced either through an existing laboratory colony or can be field collected. Laboratory arthropod colonies can provide arthropods in sufficient number as needed regardless of season. Field collected arthropods may have limited availability due to season, geographic location, and limited population size. Consideration needs to be given to any necessary transportation regulations and permits. Containment requirements of the biological agent must also be considered.

Facilities for arthropod rearing and containment work need to be designed specifically for this purpose. It is essential to work in facilities that are designed and operated to prevent escape of the arthropods. Three key approaches to prevent escape are 1) accurate inventory of arthropods, 2) appropriate containment for the arthropods, and 3) SOP for safe manipulation of the arthropods. If an arthropod escapes in containment the fundamental rule is that you stay until you find it. In the event that the escaped arthropods cannot be found a commercial insecticide spray should be readily available. Maintaining containment should be prioritized over the research being conducted for the safety of personnel, animals and the environment.

Virus infected arthropods need to be kept in both primary and secondary containers. Mosquitos should be worked with utilizing a glove box as the airflow of a biosafety cabinet can suck the mosquito into the plenum space making it impossible to retrieve. Supply and exhaust vents should be covered with mesh. Ceiling and windows should be sealed to prevent escape. Tacky mats should be placed at door thresholds and doorways should have insect screens. When working with ticks the work area should be outlined with sticky tape and ticks should be chilled prior to manipulation. When housing animals with ticks feeding capsules should be verified secured prior to populating with ticks, rodent cages should have a layer of sticky tape along the perimeter at the top of the cage and white bedding such as Alpha-dri to aid in arthropod visualization should be use. Animals with feeding capsules should be housed alone to prevent cage mates from disturbing the capsule.

Infectious pathogens can be transmitted to an arthropod or host during the bloodmeal feeding process. Live animal feedings many not always be practical. Artificial methods of infection include artificial bloodmeals, artificial infection methods, intrathoracic inoculation, and artificial transmission. Using an artificial means of infection allows the researcher to know the viral titer. Ticks require small mammals as a food source and long engorgement time while mosquitoes can be fed using live animals or artificial meals and have short feeding times. There are methods to feed ticks on artificial feeders but they need to be optimized for the specific tick species and may not work the majority of tick species. The use of synchronous tick infection is a way to effectively and safely to generate a large number of infected ticks. This method utilizes a procedure where the ticks are dehydrated prior to exposure and then the dehydrated ticks are submerged in vial containing the 1.5 -2 ml of viral agent. Infection can be confirmed using PCR 2-4 weeks post exposure. Artificial infection via injection can be accomplished injection into the hemocoel which can result in 100% infection rate and there is no reported difference from mosquitos and ticks infected via oral exposure. When using live animals to infect arthropods success is dependent on the viremic level at time of feeding. In addition to the use of rodents the authors also recommend the blue or king quail (*Excalfactoria chinensis*) for difficult to feed ornithophilic species.

Conversely, for an arthropod to be a competent vector it requires dissemination of the virus to their salivary glands. Needle infection of mammals does not accurately mimic natural pathogen delivery. Since mosquitos have shorter feeding times than ticks, rodents can be anesthetized for infected mosquito feedings and large animals that acclimated to handling can be gently restrained and the feeding chamber can be manually held in place. For ticks, feeding capsules should be adhered to the skin of the animal and left in place due to the longer duration of feeding required. Saliva or salivary gland extract (SGE) can be used to mimic natural infection.

QUESTIONS

1.  Due to its small size, average weight of 50 grams, which species of bird is useful for transmission studies to infect mosquitos?

a. *Columbia livia domestica*

b.*Coturnix japonica*

c.*Excalfactoria chinesis*

d.*Gallus domesticus*

e.*Taeniopygia guttata*

2.  T/F: To understand the transmission of most arthropod born viruses, it is necessary to work with arthropods and mimic natural infection as much as possible.

ANSWERS

1. c

2. True

# **Henneman et al. Challenges and Solutions With Agricultural Animal High Containment Waste Disposal, pp. 32-39**

Domain 4:T1. Design laboratory animal facilities, K5 to K7

SUMMARY: When planning high-containment large animal research, the species of animal and required care dictates what waste will be generated and subsequently need to be processed. Agricultural animal high-containment waste streams include animal carcasses, feed, bedding, manure and urine, other effluent, dis- posable personal protective equipment (PPE), other consumables and sharps, and contaminated air. Non-waste items such as laundry, reusable PPE, and tools must also be considered in decontamination process planning.

Carcasses: ABSL-3Ag and ABSL-4Ag facilities must have the ability to process out the large waste volume and carcasses of considerable weight and size. Facilities with large auto- claves, adequate material to process validations, and adequate staff to carry out the process may find this a viable option. The same is true if incineration is the only treatment process. When working properly, incineration produces considerably less final product, which lightens the disposal load compared with just autoclaving.

An advantage of the alkaline tissue digestion method is that the high pH enhances breakdown of the carcasses and, in our experience, decreases solid waste volume up to 80%–90% depending on the animal species used. Alkaline tissue digestion also breaks down prions because of the high pH and is an approved method for prion decontamination.

There are many additional support components and special infrastructure needed to support running a tissue digester, including a reliable consistent heat source to supply steam, contained storage for significant quantities of potassium or sodium hydroxide (up to several hundred gallons), a means of neutralizing the alkaline effluent, and a cooling system or domestic cold water to lower the temperature to below 60◦C before release to sanitary sewer. Also, a reliable hoist/crane rail system is essential for moving heavy objects. These units are absolutely necessary for working with large animal carcasses.

Planning for validation and optimization of tissue digester during construction is critical because procuring carcasses for validating tissue digester systems can be difficult and time consuming. It may take weeks or months to procure and store the carcasses needed to perform required validation runs.

Refrigeration of carcasses results in the greatest ease in loading a thermal tissue digester. Frozen carcasses are difficult to handle and load, especially with larger animal species. Cycle times to reach adequate temperature for decontamination may be more than 4 times that for non-frozen carcasses. The drawback to refrigeration as opposed to freezing is that carcasses still decompose at refrigeration temperatures, resulting in extremely unpleasant smells.

Animal Feed: Feed options should include pelletized or ground grains and hay products. Even effluent decontamination systems with macerators and large self-cleaning strainers have difficulty with hay and straw. Cover grates for drains will help prevent excess feed and other foreign items from entering the drains while still allowing adequate flow and wash-down of urine and feces.

Bedding: In agricultural animal high-containment facilities, it is wise to minimize the use of bedding or even eliminate it if feasible. It must be autoclaved or incinerated because it cannot be processed through a tissue digestion system (TDS) with thermal digestion or go down drains to an effluent decontamination system (EDS) without significant cost in additional equipment. Autoclaving is a difficult option due to the large volume and dense nature of the material.

Manure and Urine: Livestock animal holding rooms usually require at least once- if not twice-per-day cleaning. Ensure the EDS can handle the volume and that drain lines are properly sized for the proposed research.

Proper cleaning of large animal holding rooms requires high- pressure hot water, which adds to the waste stream. This is best plumbed in during construction. It is very difficult to add later because ABSL-3Ag and ABSL-4Ag walls are usually poured concrete. Redundant high-pressure water systems are advisable or at least a way to provide temporary connections that do not compromise containment when a backup is needed.

Because of the amount of cleaning required on a daily basis, more than 1 effluent decontamination tank or similar liquid waste-processing system will be required. Three is ideal, because losing the ability to sterilize and manage liquid effluent makes it almost impossible to carry out research. Exit showers create additional waste effluent whether they are plumbed to the effluent decontamination system or sanitary sewer system. At least 2 properly sized effluent decontamination tanks and 2 digesters for every 20000 square feet of facility would be a minimum estimate.

Also, it is critical to confirm, before operations begin, that there is redundancy in the ability to shut off utilities to EDS and TDS should an emergency occur.

When choosing and installing an effluent decontamination or similar system for liquid waste treatment, considerations must also be given to how the equipment will be moved out of the facility if it needs replacement, how parts will be accessed for easy maintenance while minimizing personnel exposures to active research areas, how run conditions will be monitored and documented, and how the decontamination parameters used will be optimized and validated prior to and during routine use.

General Trash and Sharps: ABSL-3Ag and ABSL-4Ag trash will need to be autoclaved and then processed out of the facility through a validated autoclave cycle.

Laundry and Other Non-disposable Items: Laundry is not a true waste stream, but laundry must be decontaminated out of the facility prior to laundering. Containment clothing items (scrubs, socks, underwear) and towels accumulate quickly in an agricultural high-containment facility, which requires many clothing changes and exit showers.

The non-disposable items such as necropsy procedure tools or electronic recording devices may be decontaminated before removal from agricultural high- containment rooms using chemical disinfectants. Disinfectant “dip tanks”  at room exits or pass-through dunk tanks are options for decontaminating these types of items.

Contaminated Air: For ABSL-3Ag, guidance from Biosafety in Microbiological and Biomedical Laboratories (BMBL) suggests that HEPA filtration on both supply and exhaust is required and these filters must be certified annually.

Challenges and Lessons Learned Summary: Here are design limitations for decontamination equipment. When designing a new processing area or retro-fitting an old one, remember that space is required to work on equipment such as effluent decontamination system and thermal tissue digester equipment. Lack of adequate space to work on equipment leads to safety issues and the use of extra staff and materials to ensure the equipment is safe to handle for repairs. It is common for research facility buildings to lack routes to replace this equipment. Wide doors become essential for maintaining or replacing equipment.

There are many combinations of final waste stream treatment that must be considered. Using just a thermal digester may still require the addition of some other process such as a pathological incinerator or a contract with a rendering company. Consider adding additional tanks to hold and mix liquid effluent before release or the ability to have it trucked away for another use such as anaerobic digestion. These details are essential parts of deciding whether to use alkaline hydrolysis or just thermal processing for carcass decontamination.

Validation of all decontamination equipment and systems is essential prior to use with active infectious materials. Planning is critical because proper process validation takes a significant amount of time.

QUESTIONS (True or False)

1. In ABSL-3Ag and ABSL-4Ag facilities, carcasses are most commonly removed from the research area through the process of thermal tissue digestion, which always involves alkaline pH
2. straw, wood shavings, or any other types of bedding material cannot be processed by a thermal digestor, and therefore should be autoclaved
3. A fast-acting bioseal damper can be used in lieu of a supply HEPA filter to prevent airflow reversal

ANSWERS

1. False
2. True
3. True

**DeTolla et al. The Evaluation of the Containment Efficacy of Semi-Rigid Isolators for Housing Cages of Laboratory Animals Infected with BSL-3 Agents, pp. 40-45**

Domain**s** 3 (Research), 4 (Animal Care), 5 (Regulatory Responsibilities)

**SUMMARY:** Research animals infected with BSL-3 agents require specialized biocontainment housing in order to provide a primary barrier to reduce the risk of release of infectious aerosols. Biocontainment cage systems (negative pressure, HEPA filtered ventilation) and BSC can provide sufficient primary barriers when working with small rodents (e.g. mice, rats). Larger mammals (rabbits, ferrets, small NHP) require larger housing that can function as primary containment. These larger biocontainment cages work well but animals usually have to be moved to a BSC for procedures requiring special transport devices. Alternatively standard housing that can fit inside of a containment device such as an isolator can provide primary containment and allow for animal procedures to occur within the isolator reducing the need to transport the animals to another primary containment space such as a BSC. Semi-rigid isolators can provide primary containment for housing animals by relying on negative air pressure and HEPA filtration of exhaust air. Due to the potential for severe consequences of an isolator containing an BSL-3 agent failing it should have engineering controls, such as directional air flow, control of pressure differentials, HEPA filtration of exhaust air, redundant HAVC motors, emergency power, and alarm systems**.**

**Computational Fluid Dynamics** (CFD) is used to study flow of fluid (liquid and gasses)  - CFD utilizes computer software that can verify, calculate, and visually display how small particles (e.g. bacteria, viruses, gases) behave under certain circumstances. CFD subdivides an air space into thousands or millions of cubes (i.e. cells) of air. Each small volume of a cell is mathematically described using equations of the conversion of mass, momentum, and energy. The software solves for the behavior of each cell and its interaction with each surrounding cell in order to simulate the behavior of the total space

The **objective** of this study was to determine the potential aerosol contamination of the housing room should physical barrier of the isolator be breached and/or if a failure of the exhaust motor should occur in an effort to validate the containment efficiency of the semi-rigid isolators prior to using them to house animals infected with BSL-3 agents.

**Categories Tested**

* Breach – small, large (6 in.), or hatch seals left open
* Exhaust fan – negative pressure, static pressure, or positive
* Positive pressure of an isolator is normally not a risk as the exhaust motors are wired to prevent the motor from producing positive airflow

**Tested 6 Scenarios**

1.  Negative pressure + small breach à No particles escaped into the room

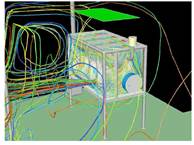
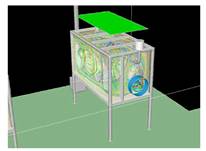
2.  Static pressure + small breach à Majority of particles contained within the isolators, the few tracks that did escaped were quickly scavenged by the room exhaust

3.  Negative pressure + large breach à No particles escaped into the room

4.   Negative pressure + hatch seals open à No particles escaped into the room

5.  Static pressure + hatch seals open  à Suspended droplets in room airspace

6.   Positive pressure + small breach à visualization of mass particle loss from the isolator into the room



#4 - Negative pressure w/ hatch open versus  #6 - Positive pressure w/ small breach

**Conclusion:** CFD testing shows that when the exhaust motor is running properly so that the isolator is at negative pressure to the room, containment can be maintained even with a small breach, large breach, and even with the port hole open. Both a breach of the isolator and failure of the motor need to occur for leakage of particles into the room. Having redundant HVAC motors and power back for the HVAC motors should greatly reduce the risk of a losing negative pressure of the isolator. Semi-rigid isolators with negative air flow can provide safe and effective primary containment for standard animal caging for research animals infected with BSL-3 agents

QUESTIONS

1.   Airflow within an animal room and caging systems for both design purpose and problem solving can be analyzed by using what method?

a.  Air Flow Analysis (AFA)

b.  Air Pressure Analysis (APA)

c.  Computational Flow Dynamics (CFD)

d.  Computational Spacey Dynamics (CSD)

e.   Computational Fluid Dynamics (CFD)

2.   T/F: According to the BMBL, 5th Ed., actively ventilated caging systems must be designed to prevent the escape of microorganisms from the cage and the exhaust air must be HEPA filtered.

ANSWERS

1. e

2. True

**Brake et al. Challenges and Opportunities in the Use of High and Maximum Biocontainment Facilities in Developing and Licensing Risk Group 3 and Risk Group 4 Agent Veterinary Vaccines, pp. 46-61**

SUMMARY: The World Health Organization defines Risk Group 3 (RG-3) and RG-4 pathogens as mainly viruses but also bacteria that serve as the foundation for approximately 60% of emerging infectious diseases that are zoonoses.

The World Health Organization (WHO) and the World Organization for Animal Health (OIE) define Risk Group 3 (RG-3) agents as pathogens that cause serious human or animal disease, are not commonly transmitted from 1 infected individual to another, and cannot be countered effectively with available medical countermeasures (MCMs) such as therapeutics or vaccines.7 RG-4 agents are pathogens that typically cause serious human or animal disease and are readily transmissible between individuals but usually cannot be countered effectively with available MCMs.

A 2020 study identified that an average of 19.3 (min. 5, max. 31) zoonotic viruses are shared between domesticated animals and humans, in contrast to an average of only 0.23 viruses (min. 0, max. 16) shared between wildlife and humans.

Moreover, this same study showed that zoonotic viruses in domesticated animals are also shared with wild animals in the Cetartiodactyla and Carnivora orders.

Considering this information together, it is reasonable to postulate that domesticated and indigenous livestock can serve as an active source of cross-species zoonotic virus transmission and increase the risk of zoonoses spillover to humans. RG-3/-4 veterinary vaccines for these livestock animals can be a useful tool to help mitigate these risks.

There is an escalating consumer demand for meat and specific meat preferences (e.g., higher income switch from poultry to pork and subsequently to beef) to meet the needs of a growing human population with longer projected life expectancies.

Agricultural modernization and intensification practices are driving the creation of new grazing areas for domestic livestock and for growing feed crops, thus clearing and disrupting natural wildlife habitat. In addition, in some countries, novel farming systems for wild carnivores (e.g., mink) and omnivores (e.g., fox and raccoon dog) have created ideal reservoirs and amplifying hosts for some zoonoses. These largely uncontrolled practices have also resulted in the convergence of diverse wildlife (e.g., bush meat) and domesticated and indigenous livestock into the same or overlapping ecosystems and/or the same food supply chains.

There are a number of reasons for the paucity of epizootic/enzootic TAD and zoonotic RG-3/-4 veterinary vaccines. First is the number of knowledge gaps associated with understanding livestock-protective innate and adaptive immune responses in the context of specific bacterial or virus disease pathogenesis. Second, despite tremendous advances in the biological disciplines and the ability to generate and analyze extremely large datasets quickly, the time required to experimentally expose, test, and evaluate vaccine candidates in the target host in high/maximum biocontainment facilities has remained relatively constant. In the Americas, Europe, and Australia, high (United States biosafety level 3 agriculture [BSL-3 Ag; BSL-3-like]) and maximum (US ABSL-4; ABSL-4-like) animal biocontainment facilities provide the essential infrastructure in which to conduct basic and applied research, vaccine regulatory development, and licensing studies using RG-3/-4 pathogens. Challenges in leveraging these high/maximum biocontainment facilities exist mainly due to the total number currently available, with most being owned and operated by government agencies and not controlled directly by the biopharmaceutical private sector. Third, and perhaps most critical, most if not all of the net present value (NPV) models fail to proactively (i.e., in the absence of a major global epizootic or zoonotic disease outbreak) actuate these companies to independently invest in RG-3/-4 livestock vaccine development. Due to the clear and growing market need for such vaccines, smaller regional veterinary companies that are often government-owned and located in LMICs with enzootic and zoonotic TAD diseases have had the financial benefit of developing and licensing RG-3/-4 veterinary vaccines without requiring high biocontainment.

Interestingly, some European countries may need to urgently import some of these LMIC-developed RG-3/-4 veterinary vaccines in response to an outbreak of an OIE-listed disease (i.e., bluetongue and Q fever). Thus, it is acutely apparent that traditional veterinary vaccine R&D paradigms used by the larger global animal health companies located in OIE-listed disease-free countries regularly encounter significant barriers to market entry.

Some mid-size and all of the larger global animal health companies have corporate-level social responsibility programs associated with TAD epizootic/enzootic, EIDs, and zoonotic diseases.

Despite these initiatives, the principal drivers for RG-3/-4 veterinary vaccine development are NPV and return on investment (ROI). More recently, many government funding agencies and non-profit funders are increasingly requiring ROI metrics to justify investments in RG-3/-4 veterinary vaccine R&D. Unfortunately, the vast majority of RG-3/-4 veterinary vaccines have an NPV <0, especially when vaccine-tiered pricing is applied to LMIC markets. Thus, in the absence of a globally active pandemic or significant external funding, most promising RG-3/-4 veterinary vaccine candidates languish in government and academic research laboratory freezers and are never transitioned to veterinary biopharmaceutical industry partners for product development.

However, newly emergent market drivers are an opportunity to re-evaluate traditional NPV RG-3/-4 veterinary vaccine development models. First, veterinary vaccines for some zoonotic diseases offer intrinsic One Health value by functioning as a strategic barrier to human infection. Second, RG-3/-4 veterinary vaccines for Q fever and brucellosis zoonoses provide critical protection to large and small ruminant stocks with high reproductive breeding values based on genetic traits of interest (e.g., milk yield, fat yield, and feed intake) or to those that possess rare genetic traits. Third, enzootic and epizootic TADs for which no differentiating infected from vaccinated animals (DIVA) marker vaccines are currently available for use in companion serology diagnostic testing inevitably result in mass livestock depopulation and disposal campaigns that have significant ethical, ecological, and economic impacts.

Effective, prophylactic DIVA vaccination against OIE-listed diseases can be an important economic tool to help mitigate the need for domestic livestock mass culling and disposal as a result of a sudden outbreak in an OIE disease-free country. However, even in these cases, challenges remain due to strong non-vaccination policies driven by federal government agency decision-makers. Fourth, the One Health concept includes a transcendent, integrative notion of animal health, human health, and environmental healthas exemplified by the increased coordination of transnational organizations, including FAO, OIE, WHO, and the World Bank.

Over the past 70 years, veterinary vaccine product licensure in the food and agriculture sector has largely focused on endemic bacterial and viral diseases. Excluding poultry vaccines (outside the scope of this paper), the vast majority of licensed vaccines are approved for use in a limited subset of domestic livestock with specific product claims for domesticated cattle (*Bos taurus*) or domestic pigs (*Sus scrofa domesticus*). Importantly, in the context of RG-3 endemic zoonoses, there are numerous other indigenous livestock particularly small ruminants that support owners and farmers in Africa and Asia—for which veterinary vaccines have not been specifically developed and licensed for use but may be occasionally used “off-label”

One of the principal factors for the very low penetration rate of veterinary vaccines for small ruminants and indigenous animals found in many parts of Africa and Asia is the relatively high cost of vaccination that includes the omnipresent cold chain supply issue. It is relatively apparent that most of these indigenous livestock pose significant challenges with respect to RG-3/-4 veterinary vaccine development in high-level biocontainment facilities, especially with respect to unique animal husbandry, handling, and disposal challenges.

The global market for veterinary vaccines is usually analyzed via geographical regions, namely North America, Europe, Asia Pacific, and the rest of the world. North America has a high income economy and maintains the largest market with the highest profit, followed by the EU, which has a range of upper middle- to high-income economies. Thus, the top global animal health companies have historically focused on new vaccine products for North American and European markets for the livestock. Private regional and smaller veterinary biopharmaceutical companies, as well as government-owned companies in Africa, Asia, and Russia, have historically served as the principal R&D engine for RG-3/-4 veterinary vaccines in LMIC markets. Unfortunately, these companies do not typically possess the financial strength to develop RG-3/-4 veterinary vaccines using highly innovative or state-of-the-art vaccine methodologies. Over the next decade, the Asia-Pacific region and parts of Africa with growing economies are forecast to have the highest growth in the livestock vaccine global market, fueled by increases in meat/milk product demand and consumption.

One method to prioritize RG-3 and RG-4 veterinary livestock vaccine development in high/maximum level biocontainment facilities is to divide the target pathogens into 2 distinct groups. The first group includes RG-3/-4 agents associated with OIE listed (reportable) livestock epizootic and enzootic TADs that are connected to human well-being). The second group includes RG-3/4 agents with a direct or indirect link to human transmission and zoonotic disease outbreaks.

It is very difficult to predict with any reasonable certainty which RG-3 or RG-4 TAD pathogens are most likely to cause the next wave of major global epizootic outbreaks over the next decade and when and where this may happen. The same holds true for attempting to forecast with relative accuracy the next currently known or perhaps completely new RG-3 or RG-4 zoonosis that will spill over into livestock and/or humans and cause the next major epidemic or global pandemic. Therefore, expanding current veterinary vaccine repositories and vaccine banks needs to be discussed, as has long been the case for FMD (inactivated antigen storage) and more recently for classical swine fever (ready-to-use vaccines). A closer examination of the origins of zoonotic diseases may offer some predictive clues. Of the top 10 mammals associated with the highest number of zoonotic viruses, 6 are domesticated: pigs, cattle, and horses (n=31 each), red sheep (n=30), goats (n=22),and one-humped camels (n=15).13This is a salient finding with respect to prioritizing future investments in livestock immunology, zoonotic disease pathogenesis, and transmission in these specific animals for RG-3/-4 veterinary vaccine development.

Once past the initial NPV hurdle, animal health biologic companies (including the largest and most profitable) clearly understand the remaining significant obstacles to develop and license RG-3/-4 veterinary vaccines, particularly for livestock. High BSL3Ag and maximum ABSL-4 biocontainment facilities to support preclinical and clinical R&D require highly sophisticated, complex infrastructures that are very expensive to operate and logistically challenging to maintain. Proper high/maximum biocontainment facilities are required for target host pathogen exposure, evaluation of rationally gene-deleted live attenuated vaccine candidates for genetic stability/reversion to virulence, and, in some cases, vaccine manufacturing. A general rule of thumb is that the annual operating cost of a US BSL-3 Ag or ABSL-4 biocontainment facility is approximately 10% of the construction cost and can account for roughly 75% of the total annual facility budget.

It is precisely for this reason that the majority of currently registered US BSL-3 Ag and ABSL-4 facilities for livestock vaccine R&D are either government owned and operated or government subsidized. These BSL-3 Ag and ABSL4 facilities and their personnel are highly regulated from both Occupational Safety and Health policy and biosurety. Another barrier to investment in the development of TAD and zoonotic veterinary vaccines is that all licensed high/maximum biocontainment facilities have sophisticated engineering and procedural controls for staff safety and facility security that require validation and continuous maintenance by a large group of highly trained engineers and facility technicians.

High biocontainment facilities for BSL-3 Ag and maximum biocontainment facilities for ABSL-4 require the development of a highly qualified, diverse, sustainable workforce that is properly and continuously trained. All of these individuals must work under the biosafety demands of strict laboratory environments (including specialized personal protective equipment, possibly mandatory vaccinations, quarantine regulations, or special medical and/or personnel reliability requirements). In addition, as it specifically pertains to ABSL-4 vivarium spaces that house large animals in relatively confined areas, challenges include compliance with the myriad of regulations and rigid safety measures to minimize risk for physical injury to animal care staff and R&D. Livestock studies in ABSL-4 maximum biocontainment facilities pose significant challenges with respect to animal welfare compliance, physical space (e.g., extra-wide hallways and doors and additional protective gating), wet animal husbandry conditions associated with daily room cleaning, special necropsy rooms (large carcass movement and cold storage), and large carcass disposal (e.g., alkaline hydrolysis digesters, specially designed incinerators, environmental permits).

Another major limitation is the general absence of veterinary species-specific immune reagents and assays to close knowledge gaps in disease pathogenesis and host protective immune responses. A further challenge is the indispensable requirement for diverse livestock target hosts for disease model development to enable vaccine safety and efficacy clinical studies. This need is particularly problematic for many of the indigenous animals

Additionally, supporting model development in the intermediate (amplifying) host and/or natural reservoir—which ideally includes bats, passeriform birds, and rodents—is possible but can be complicated, time consuming, and very expensive to establish and perform in high/maximum biocontainment. Moreover, high/maximum biocontainment facility use of arthropod vectors (e.g., mosquitoes, ticks, and various types of flies) must meet defined guidelines.

Due to the One Health concept and approach to human and animal vaccine development, another contemporary challenge is the competing practical and moral priorities of human/public health vs veterinary health. In addition, from a funding priority perspective, government and private sector funding for human vaccine development for RG-3/-4 zoonoses far outweighs funding for veterinary vaccine TAD development. Thus, utilization of high/maximum biocontainment facility space is prone to the same disparity. The One Health approach emphasizes the integration of animal and human health but does not provide guidance on best implementation.

BSL4ZNet, an international consortium established in 2016 and funded by the Canadian Safety and Security Program,is actively addressing knowledge and operational gaps in RG-3/-4 zoonotic disease preparedness and response. BSL4ZNET is comprised of working groups of laboratory-based experts associated with 11 high and/or maximum biocontainment animal and public health laboratories, spanning 5 countries: Australia (CSIRO); Canada (Canadian Food Inspection Agency, Public Health Agency of Canada, Department of National Defense, and Global Affairs Canada); Germany (FLI); United Kingdom (Animal and Plant Health Agency, The Pirbright Institute, and Public Health England); and the United States (Department of Agriculture [USDA], Department of Homeland Security, Centers for Disease Control and Prevention). BSL4ZNET has 4 working groups encompassing strategic goals associated with international disease response, knowledge sharing and institutional cooperation, training, and scientific excellence. BSL4ZNet will continue to play an important future role in strengthening operational capabilities at the One Health and science-biosafety interfaces.

Some knowledge gaps remain on how to effectively design, implement, and execute successful RG-3/-4 veterinary vaccine regulatory development plans in high/maximum containment, particularly for newer, next-generation, first-in-class vaccines. This is due in part to the relatively small number of RG-3/-4 veterinary vaccines licensed, the majority of which are based on older, first-generation vaccine technology. Improvements are needed in the identification of pathogen-agnostic, rapid response vaccine platforms for target pathogens within each major epizootic, enzootic, and zoonotic virus family and within most species of a livestock family or subfamily. There also is a need for a more robust decision-tree analysis flow to determine whether high or maximum biocontainment is more appropriate for certain pathogens and disease models. (Note: globally, risk group pathogen classification does not translate 1-to-1 to biocontainment level requirements.) In addition, there are numerous operational gaps in understanding the types of nontraditional indigenous livestock.

There is rarely significant financial incentive for any single animal health biopharmaceutical company to bring a new RG-3/-4 veterinary vaccine to the market. The involvement of public-private partnerships (PPPs) in the TAD and zoonotic disease health sectors is more than 5 years old and was primarily started based on the recognition that the myriad of RG-3 and RG-4 human disease threats could not be tackled by government agencies alone. PPPs promote sustainable business models that allow for innovation in bringing new products to the market.

The current landscape of RG-3/-4 large animal licensed vaccines is relatively limited with respect to both pathogen type and animal host. A few notable examples are detailed below.

WNV (RG-3): Several WNV equine vaccines have been successfully developed in the United States over the past 15 years. The first inactivated WNV vaccine received a USDA license in 2003.64A recombinant live canarypox-vectored vaccine received USDA approval a year later and confers protection following a single dose. A live chimeric vaccine, based on a yellow fever virus backbone, was also developed and licensed in 2006. In the United States, the WNV fraction is often included as part of a larger multivalent equine vaccine that includes other zoonotic viruses, such as eastern equine encephalitis virus and western equine encephalitis virus. These WNV vaccines illustrate that RG-3 innovative veterinary vaccines can be developed under the One Health banner.

### Coxiella burnetii (RG-3): Many of the vaccines have a marginal safety profile and fail to prevent bacterial shedding through vaginal discharge, placenta (a major route in zoonotic transmission), and milk. A consortium named Q-VaxCelerate was established to develop and produce a new vaccine During the 2007–2009 human outbreak of Q fever in the Netherlands, a policy decision was made to use a livestock vaccination with the goal of reducing the number of human cases.These authors concluded that vaccination under field conditions contributed to a reduction in *C. burnetii*shedding in dairy goat and red sheep and a reduction risk of human exposure.

### Mycoplasma mycoides subsp. mycoides (RG-3): A notable recent achievement among the RG-3 TAD diseases listed in the article is the development of a recombinant subunit CBPP vaccine through a highly successful PPP.

### HeV (RG-4): The relatively rapid development and launch of the Equivac®HeV vaccine in late 2012 was a remarkable achievement, propelled by initial positive results from the US Uniformed Services University of the Health Sciences. The subsequent formation of a PPP comprised of US Uniformed Services University of the Health Sciences, the Henry M. Jackson Foundation for the Advancement of Military Medicine, CSIRO’s Australian Centre for Disease Preparedness, and Zoetis accelerated the program through regulatory approval by the Australian Pesticides and Veterinary Medicines Authority.

QUESTIONS

1. Over the past few years, there has been a coalescence of new market drivers for RG-3 and RG-4 veterinary vaccines for TADs and zoonotic diseases, due to:
   1. Emerging rapidly growing product markets, specifically in Southeastern Asia and subSaharan Africa.
   2. An increased frequency and magnitude of EIDs and human zoonoses
   3. Favorable changes in NPV paradigms, principally due to One Health and PPP supported programs.
   4. None of the above
   5. a, b, and c
2. T/F: There has been a disruption in NPV paradigms for RG-3/-4 vaccine development traditionally associated with the driving need for commercial product sales in the private sector to generate ROI as part of a coordinated One Health benefit
3. T/F: There is an opportunity to directly test and evaluate EID and zoonotic human vaccine candidates for safety and efficacy in veterinary livestock disease models, but it does not change international collaborations for RG-3 and RG-4 agent prioritization at the high/maximum biocontainment global facilities

ANSWERS

1. e

2. True

3. False

# **Schiffman et al. The Ferret as a Model for Filovirus Pathogenesis and Countermeasure Evaluation, pp. 62-71**

Domain 1; Task 3 (Diagnose disease or condition as appropriate) and Task 4 (Treat disease or condition as appropriate)

Domain 3; Task 2 (Advise and consult with investigators on matters related to their research)

Secondary Species: Ferret (Mustela putorius furo)

SUMMARY: This article presents an overview of the ferret as a model for filovirus infection and evaluation of countermeasures. Characteristics of two of the five genera of family Filoviridae are outlined as well as of the current animal models (mouse, ferret, NHP). Based on its disease characteristics, the ferret represents a good “intermediate” model between the mouse and NHP, although some limitations do exist.

Key Points: **Filoviruses** are single-stranded, non-segmented, negative-sense **RNA** viruses. While there are five genera within family Filoviridae, **only two (**Ebolavirus **and** Marburgvirus**) produce disease in humans**. Ebolavirus contains six species, each of which contains a single virus (Zaire, Sudan, Bundibugyo, Reston, Tai Forest, and Bombali). Marburgvirus has a single species containing two viruses (Marburg and Ravn). With three exceptions (Tai Forest, Reston, and Bombali), filoviruses are **highly lethal**.

Human infection is acquired through **direct contact with infectious bodily fluids**. The disease course in humans starts by infecting **macrophages, monocytes, and dendritic cells** to suppress the immune response and facilitate virus dissemination. Clinical disease follows an **incubation period of 7-10 days** and is characterized by an early and peak phase. The **early** phase consists of non-specific clinical signs of illness (fever, fatigue, anorexia, myalgia, headache, GI symptoms) and lasts anywhere from 0-7 days. Clinical signs become more severe as the patient enters the **peak** phase (around day 7), which is characterized by viral replication and severe immune dysregulation. Clinical signs include rash, renal failure, respiratory failure, cardiac dysfunction, hemorrhage, and in severe cases organ failure and hypovolemic shock leading to death anywhere from 7-14 days or as late as 21-28 days. **Recovery** can and does occur, requiring both cellular and humoral immune responses, but survivors often have lifelong complications.

The nonhuman primate (typically **rhesus** or **cynomolgus** macaques) is considered the **gold-standard animal model** as they are susceptible to the wild-type virus and exhibit disease very similar to that observed in humans. In addition to pathogenesis studies, they are often a **confirmatory model of medical countermeasures**.

**Mice** are used for **primary evaluation of medical countermeasures** due to their small size, ease of handling, availability of reagents, and general suitability for high-throughput studies especially in maximum biocontainment (ABSL-4). However, they are **not susceptible to the wild-type virus**, which must undergo serial passages to become mouse-adapted. In addition, these passages can and do induce multiple mutations to the viral genome, the impact of which on viral pathogenesis is unknown. **STAT1 and IFNAR knock-out mice experience lethal disease with some wild-type viruses**, although the lack of an intact immune system may confound some of these countermeasures studies. **“Humanized mice” with reconstituted immune systems are also susceptible to lethal Ebola virus disease**, but their high cost and ethical considerations limits the use of this model. **Guinea pigs** have disease **similar to that seen in humans** but require host adaptation of the virus for it to be lethal. **Hamster** models with host-adapted viruses have been used for Ebola and Marburg, but a lack of reagents limits their use as well.

The **ferret (**Mustela putorius furo**)** is a small carnivore that shares multiple anatomical, metabolic, and physiological features with humans. It is most well known as the preferred model for **influenza research** due to its **susceptibility to wild-type virus**, its **efficient transmission of the virus**, and its recapitulation of many aspects of human disease. It is also a model for multiple other human respiratory viruses, including **human respiratory syncytial virus, SARS-CoV, and SARS-CoV-2**. It is also a natural host for **canine distemper virus** – infection with CDV causes a disease similar to **measles** in humans, making the ferret a model for **measles pathogenesis and medical countermeasures**.

Overall, the ferret provides a desirable intermediate model for evaluating Ebola medical countermeasures between the mouse and the NHP. The ferret **does not require viral adaptations** to become infected with Ebola, Sudan, Bundibugyo (currently the only small animal model of BDBV), and Reston, and these viruses are **100% lethal** using intranasal and intramuscular routes of infection. The ferret also shares a lot of the same hallmarks of disease with humans, including **high viremia, coagulopathy, and a pro-inflammatory immune response**. Disease course is also similar, with peak viremia occurring from 5 to 10 days post-infection and time to death between 5 and 11 days (both virus dependent).

Limitations of the ferret model are few, but significant. Interestingly, the ferret is not susceptible to infection with Marburg viruses despite these viruses being lethal in humans and NHPs. There is a lack of reagents and other tools needed to study host-response pathways in the ferret, although active work here is ongoing to rectify that. Finally, there are few studies published on vaccine or therapeutic efficacy in the ferret, which raises the question as to whether results in this model can be translated to efficacy in humans.

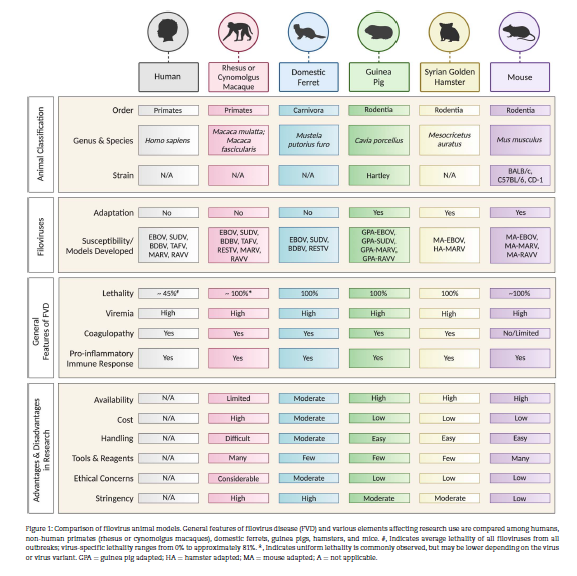
QUESTIONS

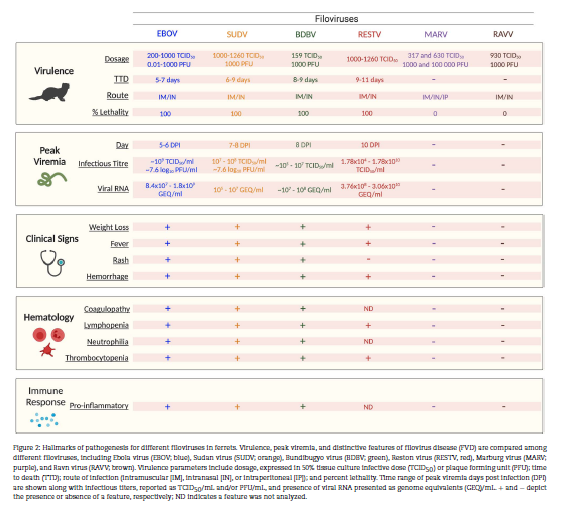
1. Describe the immune dysfunction in STAT1 mice.
2. Describe the immune dysfunction in IFNAR mice.
3. Describe the process of viral passage to create host adaptive strains.

ANSWERS

1. Signal Transducer and Activator of Transcription 1 (STAT1); homozygous knockout mice do not respond to IFN-alpha or IFN-gamma and are highly sensitive to infection by viruses and other microbial pathogens. IFN-alpha is mainly involved in innate immunity, and is secreted by both immune (lymphocytes, NK cells, B-cells and T-cells, macrophages) and non-immune cells (fibroblasts, endothelial cells, osteoblasts) in response to a viral infection. IFN-alpha detects abnormal double-stranded DNA and inhibits viral multiplication. IFN-gamma is critical to both innate and adaptive immunity; its primary function is to activate macrophages as well as stimulating NK cells and neutrophils.
2. Type 1 interferon receptors (IFNAR); homozygous knockout mice cannot generate a complete immune response.
3. Virus is inoculated into the desired host species. After a specified incubation period, the host is sacrificed, and desired tissues collected and homogenized. Virus is detected in the homogenate via PCR (or other appropriate method) and inoculated (“passaged”) into subsequent hosts. This process is repeated multiple times. Adaptation of the virus to the desired host species is tracked by recording the clinical signs and symptoms displayed.

Helpful charts in the article:





# **Falendysz et al. Outside the Box: Working With Wildlife in Biocontainment, pp. 72-85**

# **Lewis and Pickering. Livestock and Risk Group 4 Pathogens: Researching Zoonotic Threats to Public Health and Agriculture in Maximum Containment, pp. 86-102**

Domain 3: Research

Domain 4: Animal Care

SUMMARY: This article highlights the importance of high consequence pathogen research utilizing large (agricultural) animal models in both zoonotic and agricultural disease studies. It begins with a succinct summary of the challenges facing the use of agricultural animals in maximum biocontainment (A/BSL-4, BSL-4Ag), especially how to manage large, potentially destructive animals outside of traditional primary containment caging (i.e., in open floors or pens) while wearing positive-pressure suits. Attention is paid to increased regulatory oversight, both due to the nature of the research as well as the species utilized, and on the increased monetary costs of running a maximum containment lab. This section ends with a discussion on important questions to consider when designing a study utilizing livestock in maximum containment, including the safety of personnel, the challenge agent dose and route, and the number of animals needed to balance scientific integrity with humane care and use. The second half of the article highlights several historical examples of the use of livestock in risk group 4 research, including the emergence and classification of Hendra and Nipah viruses utilizing horses, Zaire and Reston Ebolaviruses utilizing NHPs and pigs, and filovirus studies utilizing NHPs and pigs. The article concludes with a restatement of the importance of high consequence pathogen research using agricultural models and makes a case for transparency in this type of research to the general public. Multiple figures summarizing the different biosafety and risk group levels, as well as differences between ABSL-4 and BSL-4Ag research are included in the paper and are reproduced here.

QUESTIONS

1. True or False: Risk Group always corresponds to the Biosafety Level.
2. Define the following terms: Spillover host, intermediate host, amplifying host, reservoir host
3. What is a “target-host model” and how is this research utilized in policy decisions?
4. Describe why “over-challenge” can be an issue in infectious disease research.
5. What is the number one resource for infectious disease research at all levels of biocontainment?

ANSWERS

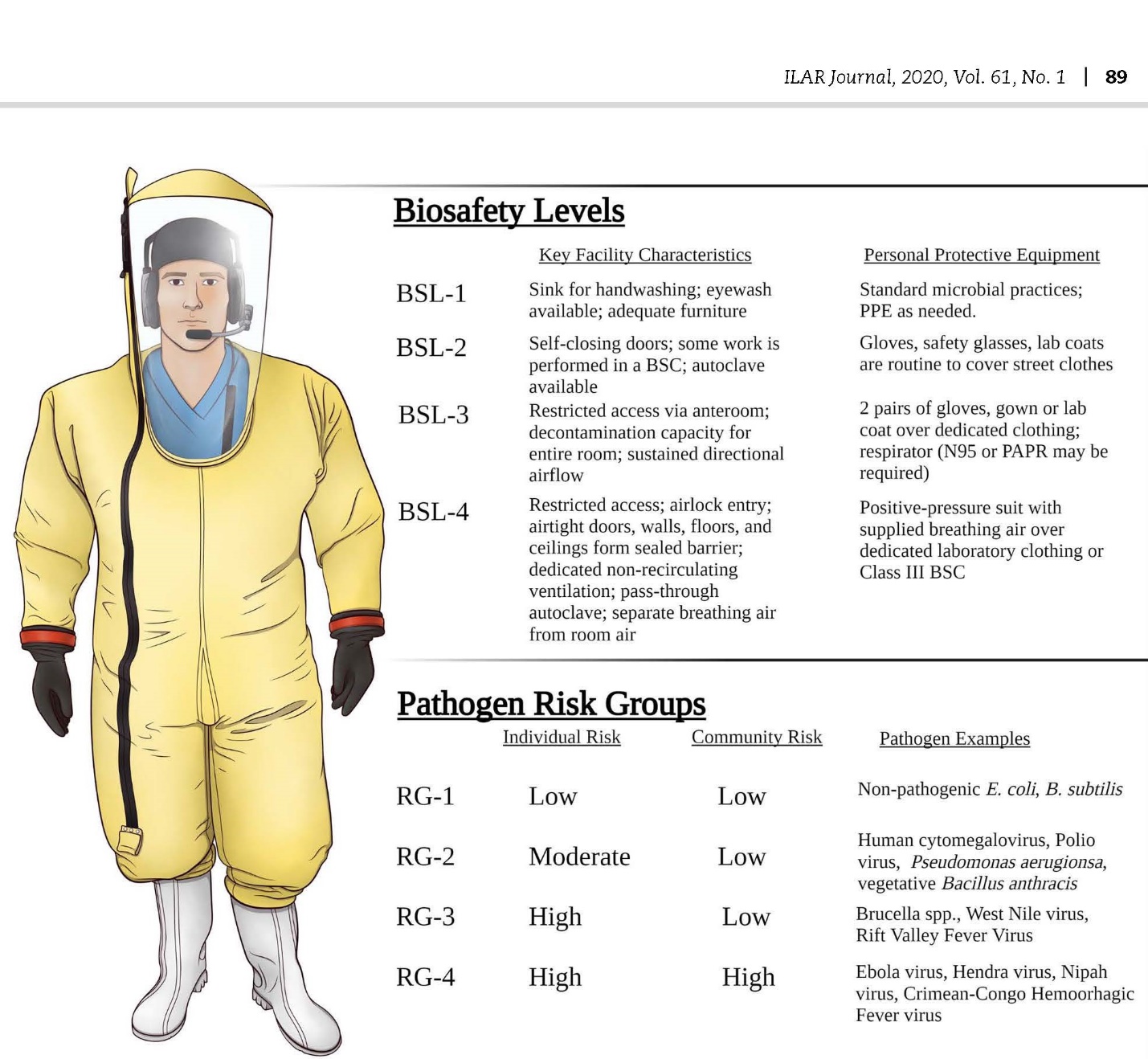
1. False. While they often correspond, they do not always match up.
2. Spillover Host: Also called cross-species transmission; when a virus jumps from one species to a new host species

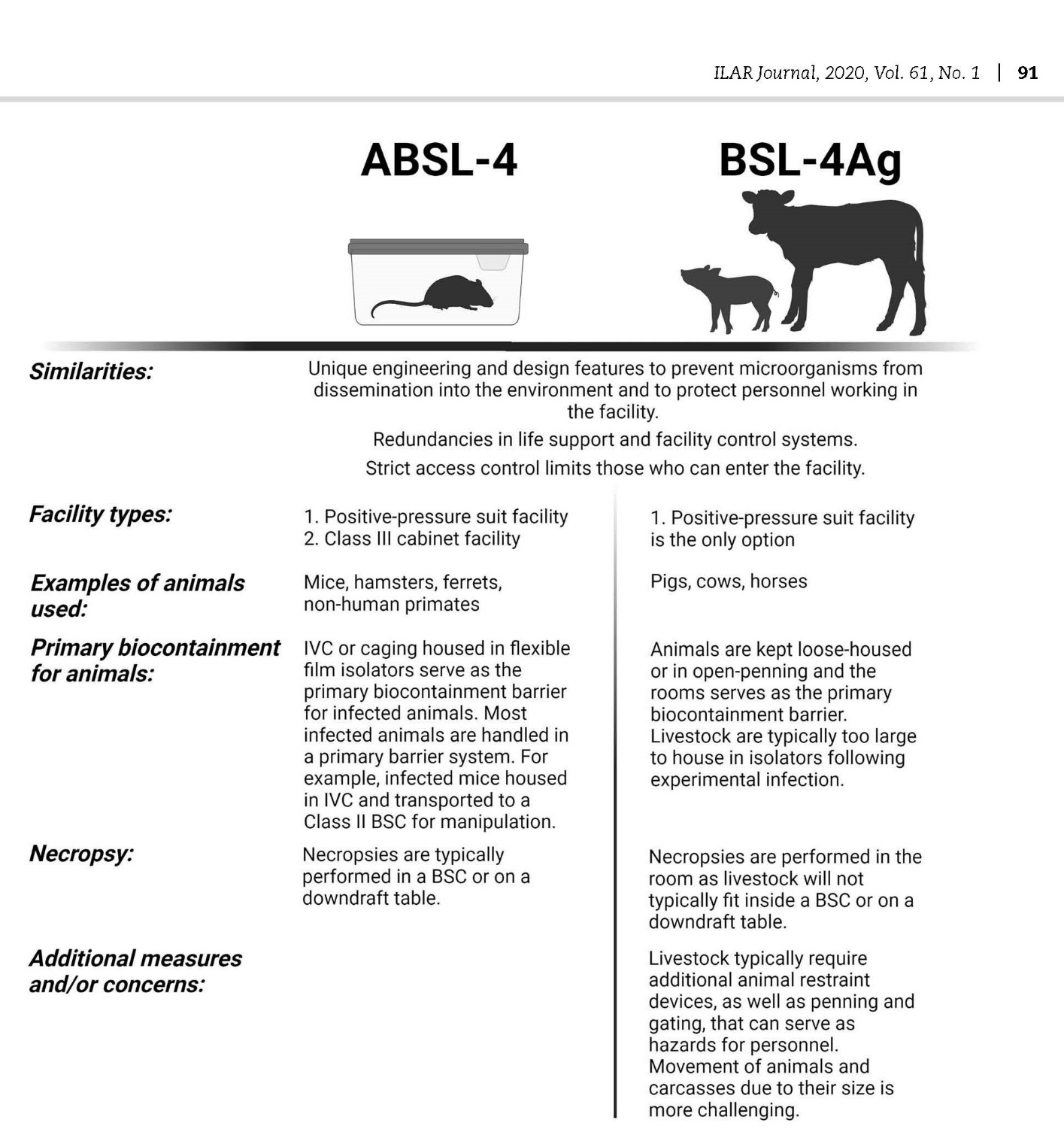
Intermediate Host: In infectious disease, the “bridging” species that links natural origin and susceptible populations and where the disease evolves and replicates

Amplifying Host: An organism in which an infectious agent that is pathogenic for some other species is able to replicate rapidly and to high concentrations

Reservoir Host: Serves as a source of infection and potential reinfection in humans

1. This refers to animals that may serve as a reservoir capable of either sustaining the pathogen in the environment or as a risk for spillover transmission to other susceptible animals, such as humans. These animals are considered models for what may occur during natural infection and serve as surrogates for members of their own species. These “target-host models” are models of species-specific animal disease; research using them involves challenging target hosts to determine susceptibility, understand transmission, and evaluate the potential risk of spillover into the human and/or production animal population. This type of research can serve as a sound basis for risk assessments and policy decisions to determine how to deploy limited resources during a time of need.
2. “Over-challenge” occurs when the amount of pathogen given to an animal is beyond that reasonable for natural exposure. It may mask the natural susceptibility of the animals of interest, as the more robust immune response could prevent disease from occurring.
3. Biosafety in Microbiological and Biomedical Laboratories (BMBL)





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