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Animal Welfare in High Containment or Barrier Facilities: Addressing Unique Challenges

Speaker:

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Slide 1: Animal Welfare in High Containment or Barrier Facilities: Addressing Unique Challenges
>> Dr. Cate Pritchard: Good afternoon. I'm Cate Pritchard, part of the NIH of Office of Laboratory Animal Welfare. Today is Thursday, June 9th, 2022. I'm pleased to welcome you and our speak to our webinar today, titled "Animal Welfare in High Containment or Barrier Facilities: Addressing Unique Challenges."

There are just a few housekeeping details before we get started. If you have questions throughout the webinar, please enter them in the Q & A box. Dr. Tansey will be taking questions throughout the webinar, and if a question is a little bit more nuanced or context-specific, we'll forward the question to her after the webinar, and then we'll append the question and answer to the end of the transcript. We'll monitor the chat the best we can, and we encourage you to use it to interact with us and other participants.

All right, let's get started with an introduction for Dr. Tansey. Cassandra Marie Tansey, DVM, DACLAM, is Deputy Chief of the Comparative Medicine Branch in the Division of Scientific Resources at the Centers for Disease Control and Prevention. Dr. Tansey received a BA in Sociology from Rice University, and her DVM from Texas A & M University before joining CDC to complete a post-doctoral residency in laboratory animal medicine. In her current position, Dr. Tansey oversees the daily operations of CDC's program of animal care, co-manages the Comparative Medicine Branch's Laboratory Animal Residency Program, and provides veterinary support for high containment research, among other responsibilities. Her interest in public health and infectious disease has led to work in event-based surveillance for the International Task Force in the Zika Response, field epidemiology during the outbreak of Seoul Hantavirus in the U.S., and several roles during the COVID-19 response from household transmission study, assessing SARS CoV-2 infections in pets residing in households with laboratory-confirmed human cases, to leading a team investigating a SARS CoV-2 outbreak at a Wisconsin mink farm. Dr. Tansey is currently a member of a number of professional organizations, including the American Veterinary Medical Association, the American Association of Laboratory Animal Science, and the American College of Laboratory Animal Medicine. She can be reached at ctansey@cdc.gov, and we'll be sure to put that in the chat box.

And with that, I will turn it over to Dr. Tansey.

Slide 2: Animal Welfare in High Containment or Barrier Facilities: Addressing Unique Challenges
>> Dr. Cassandra Tansey: Thank you so much, Dr. Pritchard. My name is Cassandra Tansey. I am, as Dr. Pritchard mentioned, the Deputy Chief of the Comparative Medicine branch here at the Centers for Disease Control and Prevention. I was thrilled to receive the invitation to speak on this particular topic, because animal welfare and high containment are two of my favorite topics to talk about. This is going to be a bit of a whirlwind presentation today, because I could probably talk about this topic for about four hours, and I'm going to try to condense it down into 45 minutes. If you have questions, I absolutely encourage you to submit them, and I will try to answer them as we go through the presentation.

Slide 3: Learning Objectives
So, my goals for today are to have each of you understand the unique animal welfare challenges that come with housing animals in high containment or barrier facilities, for you to be able to evaluate those challenges and implement methods to prevent or address them, and to give you a list of resources for future reference.

Slide 4: Let Us Get to Know You…Polling Questions
Just a couple of quick questions before we begin, so I have an idea of the audience. This is going to be -- we're going to be polling you, so please have your mice ready. First up, your role in your current workplace. All right, so we'll close that one and take a look at the results. Some veterinarians, and a lot of others. Okay, I'm interested in the others -- biosafety, all right, IACUC admin, biosafety, training program manager -- great. Project manager -- fantastic.

Okay, so next up, does your workplace utilize animal models in high containment or barrier facilities? And it is okay if you don't know. This is a short question, so we'll go ahead and close it out. All right. Most of you do -- fantastic.

Have you worked in a high containment or a barrier facility? And this is going to be a Yes-No question, because I'm assuming if you've worked in one of these, you're going to know it. Let's see what we have. Okay, all right, about two thirds have worked in one of these facilities -- perfect.

What species are utilized in high containment or barrier facilities at your workplace? Some common ones, and then if there are some others, please list them in the chat. I'm really curious. Ooh, raccoons, deer, rabbits, hamsters, guinea pigs. All right, some more rabbits. All right. Turkeys! All right, so we can close out this one. Lots of rodents, some ferrets, swine, non-human primates, okay. Okay, got some ag -- very cool. Bats -- love it.

Last question. Does your IACUC review protocols that take place in high containment or barrier facilities, or inspect those spaces? This is again Yes-No-I Don't Know -- I think everybody is probably able to answer their questions pretty quickly at this point so we can see what the answers are -- yes. Okay, fantastic. All right.

Well, I think I will present some information that will be useful to all of you. It does look like we have a fairly diverse audience, so I am going to spend the first couple of minutes just talking about biosafety levels, how they relate to high containment and barrier facilities, just to give everyone an idea of how

they are different from conventional facilities before we move on to the unique challenges that they pose.

Slide 5: Overview of Research Laboratories
Biosafety levels are used to identify the protective measures that are needed in a laboratory setting to protect workers, the environment, and the public. Biosafety in Microbiological and Biomedical Laboratories (also known as the BMBL [book]), define different levels of containment. BSL-1 labs are used to study agents that are not known to consistently cause disease in otherwise healthy adults. BSL-2 labs are used to study moderate-risk infectious agents that pose a risk if accidentally inhaled, swallowed, or exposed to the skin.

Slide 6: Table 1. Summary of Laboratory Biosafety Levels (BSLs)
The equipment practices and laboratory design features that are needed to achieve appropriate biosafety and biocontainment are summarized in this table from the BMBL. Biosafety levels 3 and 4, which is what's shown, are what we consider high containment. BSL-3 labs are used to study agents or toxins that can be transmitted through the air and cause potentially lethal infection through inhalation exposure, and BSL-4 labs are used to study agents or toxins that pose a high risk of aerosol-transmitted lab infections and life-threatening disease where we do not have a vaccine or therapy currently available.

Slide 7: HCL Entry and Egress
So, for those who aren't familiar with working in a high containment lab, let me walk you through a typical day in a BSL-4 suit lab. You enter a locker room, you remove all your clothing, you put on dedicated facility scrubs or coveralls, and then you step into the suit room to perform a leak check on your suit, and then you put it on with your other PPE. Here you can see two CDC employees in this picture attaching an air hose to their suit, and that's going to maintain positive air pressure that keeps them safe.

Slide 8: HCL Entry and Egress (continued)
After you suit up, you step through another air-tight door, and you enter the laboratory space. Most of your work is going to be conducted inside a biosafety cabinet, and every task that you do is going to be made more difficult by your limited range of motion and the decreased tactile sensation that you'll have through two layers of very thick gloves.

Slide 9: HCL Entry and Egress (continued)
Once your work is complete, you begin the exit process. You step into a chemical shower, seen here, to scrub your suit down. After the shower, you hang your suit back up, you place your facility scrubs in a bag to be autoclaved, and then laundered, and then you shower yourself out.

Slide 10: High Containment and Barrier Facilities
Many ABSL-3 spaces will have a similar process, but instead of using a suit, they're going to have a powered air purifying respirator and disposable coveralls, as you can see in this picture. High containment facilities are designed to prevent the accidental release of pathogenic organisms like Ebola or Nipah, while providing a safe and secure environment in which to study those organisms, potential treatments, or vaccines for them. Facility design combined with strict safety protocols like air locks,

negative airflow and disinfecting showers help ensure maximum protection for the staff and the community.

In contrast, barrier facilities are designed to protect the health of the animals living within them to prevent inadvertent exposures to microorganisms. So, they're focused on bio-exclusion, and often utilize sterilized, individually ventilated cages, sterilization or disinfection of all supplies and equipment prior to entry and use, sterilized feed and drinking water, air shower or wet shower entry of personnel, and then their personnel change into clean-room processed scrubs and other PPE. All animal facilities have some version of a containment or barrier facility.

Your quarantine suite -- so these areas are focused on both biocontainment and bio-exclusion, where we're trying to keep what's inside the quarantine suite away from the animals in the main vivarium, and vice versa. Whether a high containment or a barrier facility, many of the challenges are going to be similar.

Slide 11: Welfare Challenges
So now that everyone is familiar with what high containment means and the similarities and differences with barrier facilities, let's talk about some of the challenges associated with performing animal research in these spaces.

Slide 12: Working in HCL/Barrier Facilities
Each of these bullet points have differences from conventional animal housing that can potentially impact animal welfare, and we will go through each of these in the next couple of slides.

Slide 13: Entry/Exit Procedures
As you can probably imagine, based on my description, entering and exiting high containment and barrier facilities can be time and resource intensive. It takes me about 20 minutes to get into and out of an ABSL-4 suite, and from a risk management perspective, you want to minimize the number of personnel entrances and exits, because they are very time-consuming, and the more people you have in the suites, the more opportunities you have for an accident or a potential exposure. That can influence how and how often health checks are performed on animals in those spaces. So, at my institution, animals in high containment are only handled with at least two people present, which means that any health check you have that requires actually handling the animal must have two people scheduled.

High containment and barrier facility layouts and space affect the way in which animal experiments are conducted as well. These labs are usually small, and depending on the layout of the facility, animal housing and procedure space may be in the same room. Small housing spaces limit the number of animals that can be housed in the space, particularly non-rodent species. And depending on the nature of the study, animals may need to be singly housed due to transmission concerns, so that further restricts the animal housing numbers, just based on the number of cages that you can fit in.

So, with space for animal housing at a premium in these facilities, really maximizing the informational output from a single animal is incredibly beneficial as you start to plan an animal experiment.

Slide 14: Occupational Health and Safety Considerations
Conducting animal experiments, especially in high containment facilities, involves several logistical challenges that could impact the collection of tissues and samples. Animal infections, monitoring, and tissue collection usually take longer in high containment, so fewer animals can be processed in a single day. The use of sharps is really discouraged in these areas, due to the risk of pathogen exposure posed by an accidental cut or needle stick. And as I mentioned before, many institutions have policies that require that animals in these spaces are only handled under certain circumstances; so, two people must be present, and potentially the animal must be anesthetized due to human safety concerns. It's a completely understandable risk mitigation measure, but anesthesia is not inherently benign. So, each anesthetic episode does have an impact on animal health and welfare.

Slide 15: Study Design
Assessment of animal welfare depends on measurement of a number of parameters, which will vary according to the species, the animal's environment and the study design, all of which are inter-related. So, the authors of this article developed a matrix to assess the combined effects of environment and experimental events on welfare of macaques. Each of the four parameters – physical, psychological, environmental, and procedural, are scored between 1 and 10, where the score of 1 indicates the best possible state, and a score of 10 would be the worst possible state, with the highest impact on welfare. You can see here that as animals change environments from breeding colonies to conventional housing to high containment housing, the score for the environmental parameter increased, indicating a worse welfare status.

Slide 16: Study Design (continued)
And that makes sense, as we compare the environments that we are able to provide in breeding colonies versus the caging for high containment studies, which are generally smaller, and designed to protect the health of the personnel working in the lab. On this slide you can see the highly-enriched outdoor corrals that breeding colonies are often maintained in, with plenty of space, manipulanda and foraging opportunities, and the ability to form and maintain complex social structures.

Slide 17: Study Design (continued)
Contrast that with these cages, which were specifically designed to house marmosets in an ABSL-3 lab. The cages are smaller, they're individually HEPA-filtered and kept under negative air pressure with fewer opportunities for con-specific interaction. In some studies, animals may have to be singly housed due to the design of the study; so for example, in a study that is evaluating aerosol transmission between animals, you don't want animals interacting with each other and potentially spreading the disease through direct contact.

Slide 18: Study Design (continued)
This is an example of a play cage used at my institution for ferrets in conventional housing. So you can see there are multiple levels, with a variety of environmental enrichment, such as hammocks for sleeping, manipulanda, and even a small pool at the bottom. Ferrets are housed in pairs or trios in this caging. Then you can compare that play cage with the ferret caging that is commonly utilized in high containment for influenza research.

Slide 19: Study Design (continued)
This graphic demonstrates differences in exposure to influenza virus between two established ferret transmission models. The naïve ferrets are the white silhouettes. They are either co-housed with inoculated ferrets on the top in the direct contact model, or they are placed adjacent to inoculated ferrets on the bottom, in the respiratory droplet model. Areas of potential exposure to influenza are depicted in yellow, and then the arrows indicate dispersion of respiratory droplets that are being expelled from the inoculated ferret. As you can see, bulky enrichment items, like a hammock, could potentially impact the respiratory droplet transmission and experiments, so they cannot be used, due to this particular study design. Having said that, once a transmission model is well-characterized, you could then run a pilot study to evaluate the impact certain enrichment has on transmission dynamics. If no, or a minimal impact is seen, you then have a really strong argument for updating the enrichment plan as a study refinement to increase animal welfare. The same can be done with pathogenesis studies, which often prohibit the use of analgesics or anti-inflammatories, since they can impact the immune response being evaluated in those studies.

One of my colleagues, Victoria Marantz, actually performed a pilot study in collaboration with our influenza researchers here, and she evaluated whether buprenorphine affected influenza pathogenicity in ferrets. Her results, published in Comparative Medicine in February, show that the duration and location of viral replication, the lymphohematopoietic changes, and the clinical signs were comparable across all groups at all time points; so collectively, those findings support the continued evaluation of buprenorphine as, again, a refinement for animals in this study design.

Slide 20: Training
High containment and barrier facility animal use protocols tend to be more complicated than conventional animal use protocols. Many of the differences deal with the agent being tested, the species of the animal, the biocontainment facility, the complexity of the research being performed, the expertise and training of the research staff and veterinary personnel, and the PPE required to accomplish the approved protocol tasks. It can be exceptionally challenging to ensure that IACUCs have members with the expertise needed to provide thorough protocol reviews and recognize potential gaps in research proposals that take place in high containment and barrier facilities. IACUC members, especially the non-affiliated member, may not have the training or medical requirements necessary to enter and inspect high containment or barrier facilities. And in that case, how are these spaces and programs being effectively evaluated and inspected?

Personnel working in high containment and barrier facilities also require more comprehensive training than those who work in conventional facilities. For example, facilities with dedicated staff overseeing the enrichment program and behavior management of the animals may not expect their care staff or research personnel to routinely perform behavior assessments, but behaviorists might not have the required medical, psychological, or security clearances to enter biocontainment rooms, and/or they may not wish to enter those labs. So, in those cases, care staff and research personnel may need training to perform the observations and assessments usually undertaken by behaviorists. All personnel who enter and interact with research animals in high containment or barrier facilities should be trained so that they are proficient performing animal welfare evaluations, perhaps utilizing a standardized scoring criteria, such as the ferret grimace scale seen here, and comfortable assessing whether humane endpoints have been reached.

Slide 21: Overcoming Challenges
All right, so now that we have identified some of the challenges, we can talk about how we overcome them.

Slide 22: Evaluating Animal Welfare
Optimizing animal welfare requires a team approach, so the refinement of husbandry and procedures to reduce animal suffering and improve welfare is an essential component of humane science. Successful refinement depends on the ability to assess animal welfare effectively, and detect signs of pain or distress as rapidly as possible, so that suffering can be alleviated.

The article this table is pulled from provides some practical guidance on setting up and operating effective animal welfare assessment. It describes the components of an ideal welfare state, along with examples of the indicators associated with them. For example, an animal in an ideal psychological state displays species-appropriate behavior. An indicator of an animal's psychological state would be a change in use of enrichment, the development of stereotypies and/or an increase in aggression towards con-specifics. This article does a really nice job setting out general principles for objective observation of animals, recognizing and assessing indicators of pain or distress, and then tailoring those to individual projects. They also review systems for recording indicators including scoresheets, and set out some guidance on how to determine practical monitoring regimes that are more likely to detect signs of suffering. So, if all of this guidance is intended for all staff required to assess or monitor animal welfare, including animal technicians, veterinarians and scientists, because again, evaluating animal welfare is a team sport.

Slide 23: Evaluating Animal Welfare (continued)
The use of validated behavior assessments increases inter-observer reliability, so in mice strains that are robust nest builders, the time to integrate into nest tests, or TINT, is an objective observation of animal welfare. To conduct the test, you take a small amount of nesting material and you add it to the mouse cage, and the nesting behaviors that occur immediately thereafter are observed. The test reveals a positive result when a mouse integrates the new testing material into the main test site within 10 minutes. Failure to interact with the nesting material is defined as a negative result.

Likewise, research has shown that changes in facial expression provide a reliable and rapid means of assessing pain in mice and rats. Grimace scales were first developed for these species based on changes in a number of facial action units, such as narrowing of the eyes, or changes in the position and shape of the whiskers. Grimace scales have since been developed for other species. But a word of caution when using them -- where grimace scales are used to assess pain in real-time at the cage side, each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare. And similarly, grimace scales should only be used in awake animals.

Slide 24: Protocol Review
The protocol review is perhaps the biggest opportunity we have to identify and overcome the animal welfare challenges that work in high containment and barrier facility posts. It's imperative that we, as a research community, ensure that these protocols are conducted with the utmost scrutiny for the welfare of the animals, as well as the health and safety concerns of the individuals conducting the

studies. Both the welfare of the animals and the health and safety of the research staff must be balanced with the integrity of the science being performed.

The specific areas of protocol reviews seen here come from a great ILAR article by Curtis Klages called "IACUC and Veterinary Considerations for Review of ABSL-3 and ABSL-4 Research Protocols." As you can see in the graphic, stakeholders like veterinary staff, researchers, and any institutional health and safety officials represent each side of the triangle with each of them having a critical area of the protocol. Some stakeholders are experts in protecting personnel, while others focus on the integrity of the science. And ideally, all of your stakeholders are balancing the welfare of the animals on the study. Each study should be considered holistically to ensure that the project meets regulatory requirements. Questions for the IACUC to consider are:

- How and how often will clinical observations be performed?
- What, if any, supportive care will be provided?
- What are the scientific endpoints of this study?
- What are the euthanasia criteria?
- Are the criteria specific to this species and this agent?
- How and how often will animals be manipulated?
- What will be documented, and where will those documents be kept?
- If documentation is not electronic, will veterinary staff or other personnel be able to access documentation when they need it?
- What training to personnel have?
- As I mentioned on the training challenges side, research personnel and animal care technicians may need training to perform the observations and assessments usually undertaken by veterinary or behavior staff.
- What contingency plans are in place to protect animal welfare in case of a disaster?
- Are there any specific security concerns that need to be addressed?
- How will items utilized in these studies be decontaminated before and/or after use?

And I'd like to spend a little extra time discussing a couple of these specific questions, although I'd really encourage you to read the article in full, because it is great.

Slide 25: Clinical Scoring

First, clinical scoring. Clinical scoring guides identify critical indicators in a species and agent-specific way. They often incorporate an animal's appearance, body condition score or weight, behavior, and their responsiveness to the observer. In order to develop or evaluate a clinical scoring guide, IACUCs and veterinary staff need to fully understand the animal model as much as currently known, as well as the infection and disease progression of the agent being studied. Understanding the agent and the animal model should be balanced with the overall goal of the study. This table from the article on defining and

implementing protocols for welfare assessments in laboratory animals that I mentioned previously lists some of the questions that researchers and IACUCs can ask to develop clinical scoring systems.

- What indicator may be seen in this species, with this agent?
- How frequently should animals be monitored, and at what time to observe that indicator?
- How can that indicator be assessed in an objective way?
- Can environmental indicators be used? So to give an example, and indicator of a certain disease in mice is decreased activity. When should I observe the animal to evaluate whether activity is decreased? I would expect mice to be sleeping during the day, so the best time to truly observe their activity level would be overnight. Can that activity be assessed in an objective way? Perhaps through observing interactions with environmental enrichment. So yes, I can use the time to integration into nest tests, or maybe quantitatively assess their use of a running wheel, if it's present in the cage.

So not all studies need an extensive scoring sheet. Some projects may use a single clinical sign to identify the endpoint for the animal. As an example, if it was found in a pathogenesis study that a 15% weight loss in an animal signifies that the animal will not survive, then that can become the initial criteria for euthanasia, and each animal having a 15% or greater weight loss will then be immediately euthanized.

Slide 26: Clinical Scoring (continued)
Publishing the detection and reporting of clinical signs of a specific agent in a specific species potentially improves the welfare of all animals enrolled in similar studies, by making it easier to develop robust clinical scoring guides, as well as humane endpoints. You can see here a list of common clinical signs of influenza virus infection in ferrets, and I want you to note that the author, Dr. Belser, actually noted in these clinical signs several that are frequently used in criteria from humane euthanasia because of the development of severe disease with those signs. So those signs are weight loss, lethargy, and neurologic signs.

Slide 27: Euthanasia Criteria
And speaking of euthanasia criteria, the criteria should be tailored, again, to the specific agent and species, just like the clinical scoring guides. Knowledge of disease progression determines the frequency of observation, as you can see in this example, right? And this particular scale was developed for a paramyxovirus in hamsters. So there's an emphasis on neurological and respiratory signs. As the disease progresses in these animals, they begin having clinical signs, and they get higher scores, which increases the number of health checks. If 24-hour monitoring is not available, and an animal is scoring very high on a pain score, it is very worth considering whether or not to pre-emptively euthanize that animal, and that is, of course, up to the veterinarian's discretion, although please do include your researchers in those conversations. As with clinical scoring guides, once euthanasia criteria are established and well-validated, please publish them. The ARRIVE Guidelines recommend reporting animal care and monitoring information, including any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress, any expected or unexpected adverse events, as well as the human endpoints established for the study, the signs that were monitored, and the frequency of monitoring. Adding this information to the body of literature builds a portfolio of opportunities for future possible interventions to better guard the welfare of the animals enrolled in these studies.

Slide 28: Minimally Invasive Monitoring
There are several forms of minimally-invasive monitoring that allow personnel to perform health checks and collect data without having to enter the space or handle the animals. Cameras can be set up in front of cages to allow remote visual assessments. Another option is telemetry, where small, implanted devices permit automated and wireless measurements of parameters like temperature, blood pressure, and brain activity with data then transmitted to a receiver outside of the animal's cage. The ability to take automated physiological measurements wirelessly from these animals reduces the labor burden and increases staff safety, as well as reducing stress on the animals. As an added benefit, wireless and automated recordings permit much more extensive data collection over long periods of time, and under more natural conditions because you're not having to handle the animals.

Slide 29: Minimally Invasive Monitoring (continued)
And telemetry is not just an option for rodents. The authors of this article utilized telemetry for their study of Rift Valley Fever in marmosets to measure temperature and activity every 15 minutes. In the telemetry data shown here, two marmosets were infected with Rift Valley Fever. The animal on the left survived for the duration of the study, while the other met the criteria for euthanasia nine days after inoculation. You can see that both animals' temperatures show the characteristic diurnal variation prior to inoculation with the onset of fever beginning three to four days after infection. Body temperature returned to near normal levels in the surviving marmoset, but remained increased in the animal with severe illness. Both of the marmosets displayed a decrease in activity that coincided with the onset of fever.

There are also a number of products available that provide home cage monitoring systems for animals housed in individually-ventilated cages -- IVC. This technology makes it possible to check for the presence of food and a water bottle, to evaluate the condition of the bedding in the cage by analyzing the moisture content, and to monitor spontaneous animal movement within the cage remotely. This translates into avoiding unnecessary animal handling, supporting daily animal health checks, and preventing animal losses due to cage flooding. And that kind of seems too good to be true, right? There are challenges to implementing those minimally-invasive monitoring systems.

So how can programs get institutional buy-in? Who pays for the equipment and is responsible for ongoing maintenance? Where are the remote monitoring stations set up, and who should have access to them? These are all important questions to answer as you consider these products. If your institution utilizes remote monitoring, I would love for you to let us know in the chat what arguments were most persuasive in getting institutional buy-in, and the resources allocated for the project. I personally have found that combining occupational health and safety so there are fewer people in the suite, which is safer, with animal welfare, and we're better able to evaluate and assess the health and wellbeing of the research animals to be an effective argument in favor of the resource allocation for these systems. And you can also recruit the researchers to your side by educating them on the additional data that they will be able to collect from each animal on these studies.

Slide 30: Animal Selection Considerations We also need to take several considerations into account we are deciding which animals we are going to enroll into studies in high containment or barrier facilities. So, a full medical history review should be conducted prior to selecting animals, and when you are selecting a cohort for a study, it's also important to review records of past behavioral issues and

assessments in order to exclude any animals with self-destructive or aggressive behaviors. The space constrictions we previously discussed limit the ability to move problem animals to different room or cage locations, and it's also much more difficult and potentially dangerous to care for wounds in these spaces. Gender compatibility and group interactions should also be considered; study groups made up entirely of males or groups with too many dominant animals can cause aggressive or self-injurious behaviors, especially in macaque species. And while we are evaluating and selecting these animals, we should also be developing enrichment plans at the same time, to take into account the more limited opportunities that animals in these spaces may have to be socially housed, or to interact with conspecifics.

Veterinarians and researchers can collaborate with behavior personnel to train animals for voluntary participation prior to studies starting. So, in this picture, a rhesus is participating in a voluntary blood draw, and while this particular procedure would probably not be feasible in high containment due to safety concerns, the theory behind it can still be applied. If a study involves daily oral administration of a therapeutic, the animals could be trained prior to study to start voluntarily taking the therapeutic, which would then eliminate the need to sedate the animals every day for gastric intubation.

Slide 31: Animal Selection Considerations (continued)
So as we consider what animals should go into high containment or barrier facilities, we also need to consider characteristics of caging systems to maximize animal welfare and human safety, such as the applicable laws and regulations in the Animal Welfare Act, the *Guide* and PHS Policy. Caging should be large enough to provide required floor space, but small enough to allow, in the case of rodents, the primary housing enclosure to fit into the biosafety cabinets and have laminar flow maintained.

Ventilated caging systems often have solid sides that block light, resulting in a dark cage interior, which can make it difficult to observe the animals, especially if you are trying to perform those observations remotely through a camera. If the cage door must be opened to complete observations, it disturbs the animals within, and it also impacts the cage's negative ventilation barrier. The presence of a squeeze back mechanism in deep caging will facilitate removal of animals from their housing, and then if you have IVCs, those should have battery backups and audible visual alarms to alert when the cage rack is on a battery backup.

Enrichment devices in these spaces should be disposable, autoclavable, and/or able to withstand surface decontamination. So, disposal enrichment products are usually made of paper or cardboard. The cardboard huts, as you can see here, tubes, or bedding with paper pieces are often used with rodents, and then after use these items can be decontaminated and then just disposed of. For reusable enrichment items, it's ideal if they could be decontaminated first, and then processed through cage wash machines prior to being reused. Enrichment devices that are traditionally provided to non-human primates hold up well to sanitization in a cage washer, but they're not always autoclavable. So autoclavable devices, such as puzzle feeders made of polycarbonate or polysulfone or metal devices are available, and they can usually be reused if they are sterilized. Then, of course, food treats are also a viable enrichment option for some species, depending on your study design.

Slide 32: Additional Resources
All right, finally, some additional resources for future reference.

Slide 33: The Biosafety Level 4 Zoonotic Laboratory Network
The Biosafety Level 4 Zoonotic Laboratory Network was established in 2016 as a network of government-mandated organizations with national level responsibility for protecting animal and human health by working together to enhance knowledge, competency, and capacity to meet current and future high containment needs. The steering committees and working groups have published several articles that may be of interest to you. And one of the major strategic focus areas of the network is strengthening laboratory personnel training, which thus far has been accomplished through several training workshops.

Slide 34: The National Centre for the Replacement, Refinement, & Reduction of Animals in Research
The National Centre for the Replacement, Refinement and Reduction of Animals in Research was established in 2004, and you can see their mission here. Their website has a wealth of information on grimace scales, experimental design and reporting, husbandry, and new research methodologies.

Slide 36: References
There are some absolutely fantastic papers out that talk about a lot of the things that we discussed today. I also want to encourage everyone to check out the Animal Welfare Information Center. They have some great resources for environmental enrichment, especially for non-human primates. Most of the articles on this slide are great, and have to do with grimace scales. I want to again point out the Klages article, IACUC and veterinary considerations for review of ABSL-3 and ABSL-4 research protocols. I always have to cite the BMBL.

Slide 37: References (continued)
Then, if your institution has non-human primates, I would encourage you to check out the National Primate Research Center Behavior Management Consortium; they have some truly excellent trainings and other resources for animal welfare assessments and enrichment for non-human primates.

If you're interested in telemetry but don't know too much about it, there's this great overview of telemetry for small animals in lab animal medicine.

Slide 38: References (continued)

Slide 39: Questions?
And with that, what questions do you have for me?

>> Dr. Cate Pritchard: Hi, Dr. Tansey. We don't have any questions at this particular time. Oh, one just came through.

Question 1: Do you have any tips for IACUCs conducting remote - so, live video inspections? And any areas of special emphasis for close-up viewing, or any requests to make of a videographer?
>> Dr. Cassandra Tansey: Oh, any requests to make a videographer. Okay, so some of this is just going to depend on how your facility is laid out. I think having a checklist and having all of your IACUC members contribute to that, and really review ahead of time what you think are the most important welfare indicators is really important. If there are -- a lot of the times in high containment facilities, you can try to schedule those inspections while the suite is down, so there are no infectious agents present and the suite has been decontaminated so that you can actually safely walk into those spaces. Obviously, the trade-off with that is that animals are not present, so you are really looking at the space and materials, and everything else and potentially doing a record review. But sometimes that is -- that's the happy medium between safety and still being able to go into the suite.

I personally always pay attention when I'm in these suites, or doing video assessments, I like to look at the anesthetic agents that are being utilized, pay close attention to calibration and any expiration dates. Look at if there are sterile or clean equipment that's being used, look at the dates that those were autoclaved to make sure that everything is still in date. I usually pay pretty close attention to any feed bins to make sure that the food is in date, and to see that the date that those bins were last sanitized. So those would be some of my helpful hints.

>> Dr. Cate Pritchard: We have another -- a few questions.

Question 2: Any ideas on how these areas can also be part of [post-approval monitoring] PAM?
>> Dr. Cassandra Tansey: Yes. So post-approval monitoring -- I do. And again, this is with a caveat that pretty much everything that I say has to do with your specific facility and how it is laid out. There are some cases -- if you have remote video capabilities in your facility, that is great. Our facility has some overhead cameras that are built into the suite, and so our post-approval monitor will go in and actually be sitting there in front of the monitor and watching the activities through that camera live, while we are performing activities in there. There are also a lot of suites that have windows to an outer corridor that is still restricted to two personnel, so you don't just have random people walking around, but you can stop in that window and you can actually look into the animal housing room or the animal procedure room. So again, our post-approval monitor will coordinate with our PIs to find out what days they will be doing animal work, and then sometimes you will go and stand at the window and monitor and kind of watch what they're doing. If he has specific questions, he can kind of ask them to bring cages of animals to the window for him to evaluate so that he can see that. We also occasionally do that with veterinary assessment, so if I have a researcher that's inside and they want an assessment of an animal from a blinded observer, then sometimes our veterinary staff will go to the window and the researcher will bring the cage to the window so that the vet can assess and see if anything else - further treatment or anything else is required.

>> Dr. Cate Pritchard: That's actually interesting because there's a comment in the chat that's talking about the video monitoring and concerns about security. So it says, "Concerns about security, if you're using video monitoring of animals in these spaces, getting IT involved if you aren't potentially having a hacker getting into your video feed. Our institution has a lot of internet security firewalls that must be followed."

>> Dr. Cassandra Tansey: And the other thing that you can also consider with videos is, having it hard-wired into your facility so that the output only goes to one single computer. And that computer is then not connected to the internet, so that is one of the things that we do here for the same security concern. But absolutely, if you guys are looking at remote monitoring and things like that, that would fall under the "special security concerns" under the IACUC protocol review and make sure that you are looping in your IT support, so they can provide appropriate guidance.

>> Dr. Cate Pritchard: This remote monitoring is a really good way to have all of your IACUC members being able to participate in the inspections, not just people who have a specialized experience.

>> Dr. Cassandra Tansey: Absolutely.

>> Dr. Cate Pritchard: All right, we have a lot coming in now. Somebody says, "We went with a hardware option for our high containment facility just for that reason." So, great point.

Question 3: Are ABSL-4 suite decontamination agents of choice still paraformaldehyde?
We have one other question right now is, "Are ABSL-4 suite decontamination agents of choice still paraformaldehyde?"

>> Dr. Cassandra Tansey: Oh, that's a great question. We do not use paraformaldehyde. It is absolutely still -- it is absolutely still a viable option, 100 percent. And there are institutions around the country that do still use that. But there are other choices. Some of that is going to depend on site-specific risk assessments of what agents you are working with, and what -- I mean, it's really very site-specific risk assessments. But if you would like to have that discussion, whoever asked that question, you are welcome to email me, and I'm happy to give you more details.

>> Dr. Cate Pritchard: All right, we'll put your email in the chat just one more time. Let's see, cdc.gov. All right, so we are almost at 2:00. I think we have gotten to everybody's questions. So, we did have some questions during the webinar about whether the slides and the webinar recording is going to be posted -- so they are going to be posted. We do need to process them for 508 compliance, which is taking about a few weeks right now, so we'll get that up as soon as we can. In the meantime, here is a shameless plug to please go and visit our website. We have all of our webinars up there, and you can monitor when we post these slides and the transcript, and the recording on there. We'll also send out a newsflash so that everybody's in the know.

Slide 40: Next Webinar TBD
And with that, I think we are all set for today. So I would like to give you a huge thanks, Dr. Tansey, for coming and presenting with us today and being such a good sport. Thank you also to all of our participants for coming and joining us today. Our next webinar topic is to be determined. It will be this fall, 2022.

So that's all we have for you today. Thank you so much, and goodbye.

>> Dr. Cassandra Tansey: Thanks, everyone!

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