Mucosal Cheek Pouch Tattoos Allow Repeat Assessment of Experimentally-Induced Oral Tumor Development in Syrian Hamsters (Mesocricetus auratus)

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ABSTRACT

Tattooing of research animals as a means of individual identification has become prevalent in the research community. An established animal model of oral carcinogenesis utilizes hamsters due to the presence of immunologic privileged cheek pouches. Typically, carcinogen compounds are applied topically or “painted” onto the cheek pouches. Due to the extensive size of the cheek pouches, it can be difficult to determine where the compounds were originally applied, which can impact reapplication of a compound or collecting tissues for assays. The objective of this project was to determine if mucosal cheek pouch tattoos could delineate an area to reliably quantify and monitor neoplasia development. Hamsters were anesthetized and the cheek pouches were everted with a 3cc syringe plunger to access the tattoo site. A reproducible tattoo margin of 1cm by 1cm was created using plastic acetate. Four distinct dots were applied at each corner of the plastic acetate using a tattoo device. Four distinct dots were applied at each corner of the plastic acetate using a tattoo device. Hamsters were anesthetized and the cheek pouch tattoos were reevaluated on day 7, 21, 42, and 124. Day 124 was not as fully visible as day 42, but the tattoos, though faded, could delineate an area to reliably quantify and monitor neoplasia development. This project has proven to be a reliable method to reassess tumor development on the mucosal surface of hamster cheek pouches. To the authors’ knowledge, this is the first report using mucosal cheek pouch tattoos as a method of localization for refined application and evaluation in the Syrian hamster oral carcinogenesis model.

PROBLEM

• The research staff found it challenging to access the previous experimental location in the cheek pouch leading to inconsistent results.
• The objective of this project was to determine if mucosal cheek pouch tattoos could delineate an area to reliably quantify and monitor neoplasia development.

BACKGROUND

• The hamsters were maintained in solid bottom microisolator caging with corn cob bedding (Harlan) on a ventilated rack (Tecniplast, PA USA).
• Animals were provided pelleted hamster chow (Harlan 8640) and automated reverse osmosis water ad libitum. Cages were changed weekly.
• Certified polycarbonate rat huts ( Bioserv: Frenchtown, NJ) and nesseslets were provided for environmental enrichment.
• The housing room was maintained on a 12:12-h light:dark cycle in accordance with the “Guide” recommendations for humidity and temperature controls for hamsters.

MATERIALS AND METHODS

• Equipment: The Aims™ Tattoo System (Figure 1) was used to tattoo the cheek pouches.
• Template: Before anesthetizing the hamsters and starting the process of tattooing, a square marker (1cm by 1cm) was created to delineate the reproducible tattoo margin using plastic acetate (Figure 3). The acetate was measured to fit on the end of a 3cc syringe to serve as the guide for tattoo placement as indicated by the dots.
• Anesthesia: 8 hamsters were anesthetized individually with isoflurane anesthetic utilizing an induction chamber. After the hamsters were anesthetized, they were taken out of the chamber and a cocktail of ketamine 100 mg/kg/xylazine 10 mg/kg was administered subcutaneously to maintain the anesthetic plane. Cheek pouches were then everted with a 3cc syringe plunger (Figure 4) to access the tattoo site. Four distinct dots were applied at each corner of the plastic acetate using the ink tattoo device (Figure 5). The acetate was removed and placement and saturation was verified (Figure 6). Thermal support with Delta Phusel® heat pads were placed under one side of the empty recovery cage at the conclusion of the procedure. The hamsters recovered uneventfully before being returned to their cages.

DISCUSSION

• The tattoo penetrated both the cheek pouch and the cheek mucosa, resulting in 8 dots that lined up easily. Complications were observed in 2 of 8 hamsters one week following the procedure. They developed small, distinct circular lesions on the nape of their necks where the cheek pouches were linked to the tattoo internally. When the cheek pouch was everted at the day 7 time point, it was noted that the tattoo needle had punctured through the skin causing the observed lesions. This complication is easily preventable with careful attention to needle depth when tattooing.

CONCLUSION

• This project has proven to be a reliable method to reassess tumor development on the mucosal surface of hamster cheek pouches. To the authors’ knowledge, this is the first report using mucosal cheek pouch tattoos as a method of localization for refined application and evaluation in the Syrian hamster oral carcinogenesis model.

ACKNOWLEDGEMENTS

• Special thanks to Dr. Christopher Weghorst and the ULAR husbandry staff.
A spray pattern “power film” (Figure 1) was found on the inside lid of ventilated rat cages. The cause was partially or completely occluded air levels. were found to be free of all typical laboratory pathogens. Rats of 13.6±0.35 months were obtained from CRL and housed individually in 4 different groups with varying degrees of ventilation occlusion. Rats of 13.6±0.35 months in a standard cage wash without removing plastic air valves (AVs) was found on the inside lid of ventilated rat cages, and investigation of the cause revealed AV rack ports that were partially or completely occluded with debris. Sanitation practices were amended to include: AVs removed, soaked in 10% bleach for 10 minutes, rinsed, and washed in a standard cage wash. It was hypothesized that occluded AVs would negatively impact animal health. Sprague-Dawley rats were housed in four groups: 100% AV-open, 100% AV-occluded, 50% AV-occluded inlet air supply, 50% AV-occluded outlet air supply. Ammonia levels were measured daily for 14 days or until ammonia concentration reached >25 ppm. Half of cages with 100% AV-open or 50% AV-occluded remained on study until day 14. All cages with 100% AV-occluded reached >25 ppm ammonia by day 8 (p=0.001). Airflow and pressure was measured for all treatment conditions with a Techniplast® monitor cage with the absence of animals. Airflow was significantly decreased in cages with 50% (p=0.01) and 100% (p=0.001) occluded AVs. Air pressure significantly increased when AVs were occluded (p=0.001). AVs were cultured for bacterial type and load before and after cage wash. Bacillus spp. was consistently cultured from AV before and after cage wash due to temps only reaching 160°F. Bacillus and Enterobacter species require temps above 212°F for an extended period of time (5 min-212°F for Bacillus249.8°-15-30min for Enterobacter) before they are inactivated. It is recommended to check ventilation ports for damage, occlusion, and debris on a monthly basis or no later than 3 months after rack sanitation to ensure that proper ventilation is being provided to the rats in their cages.
Effects of Influenza Infection on Murine Alveolar Type II Cell Function
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INTRODUCTION
Alveolar type II pneumocytes (ATII cells) are small cuboidal epithelial cells that comprise approximately 15% of the total cells lining the alveoli but only 5% of alveolar surface area. The much larger and flatter alveolar type I (ATI) pneumocytes cover approximately 95% of the surface area of the lung and are the primary site of gas exchange. Surfactant protein C (SP-C) is a key component of pulmonary surfactant that is produced exclusively by ATII cells. Following lung injury, ATII cells can produce inflammatory mediators and will also differentiate into ATI cells to repair the damaged epithelium. During differentiation, ATII cell surfactant lipid and protein production progressively declines. We hypothesized that, as a known cause of severe lung injury, influenza A virus infection will significantly alter ATII cell function. Moreover, we proposed that ATII cell SP-C production would decrease and that ATII cells would increase their capacity to repair the damaged epithelium. During the course of infection, we also noted a decrease in SP-C-positive ATII cells from a mean of 40.7% to 24.8%.

MATERIALS & METHODS
Transgenic mice that express a GFP transgene under the control of the ATII cell-specific surfactant protein-C promoter2 were infected with influenza A/WSN/33 (a mouse-adapted H1N1 strain) for 2-6 days. Lung digests were processed and ATII cells isolated. Cell suspensions were stained with R-Phycocerythrin (PE) conjugated antibodies for KC/GROα, TGFβ1, INFγ, and IL6 and analyzed by flow cytometry. ATII cells were isolated by gating on the high GFP population. Expression of these molecules is indicated by red histograms and autofluorescence of non-stained cells is shown as blue histograms.

RESULTS
Influenza infection resulted in a progressive decline in SP-C-positive ATII cells from a mean of 50% in uninfected mice to 24% at 6 days post-infection (Fig. 1). Quantitation of whole-lung GFP fluorescence by using an in vitro imaging system (IVIS) confirmed this decline in SP-C production by ATII cells from mice expressing GFP under the cell-specific SP-C promoter. ATII cell homogeneity has developed will increase our ability to determine specific effects of influenza infection on ATII cell function and the role of these cells in the post-infection inflammatory cascade and recovery from lung injury. They will also allow us to generate highly-purified ATII cell preparations by cell sorting, which can be used for analysis of influenza effects on ATII cell gene expression.

DISCUSSION
The flow cytometric analysis techniques which we have developed will increase our ability to determine specific effects of influenza infection on ATII cell function and the role of these cells in the post-infection inflammatory cascade and recovery from lung injury. They will also allow us to generate highly-purified ATII cell preparations by cell sorting, which can be used for analysis of influenza effects on ATII cell gene expression.

REFERENCES

ACKNOWLEDGEMENTS
Many thanks to Alice Gaughan and Lisa Joseph for their assistance and technical support and to the late Dr. Jo Rae Wright for providing the SPC/GFP mouse line. Funding provided by National Heart, Lung, and Blood Institute RO1 HL102469 to I.C.D.
Refinement of Swine Enrichment via Customization of Foraging Balls Results in Increased Duration of Play

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ABSTRACT
Providing research animals with appropriate environmental enrichment helps to encourage species-specific behavior. In swine, this includes behaviors such as rooting and foraging. One of the ways to achieve this goal for swine in the research setting is to place food enrichment into a foraging ball—a hard, hollow, plastic toy which has pre-drilled holes in it to engage the swine in removing the food. Many of the commercially available foraging balls for swine have multiple small holes throughout the surface. This requires the use of small food items, which poses the problem of food falling through the grates, elevated flooring that swine are housed on in our facility. There are also commercially available foraging balls that have few extremely large holes in the surface; and in our experience, they do not present enough of a challenge for the swine. In an effort to provide the best possible enrichment for our swine, we created customized foraging balls from herding balls—a hard, hollow, plastic ball with no holes. The herding balls were customized by drilling varying sizes and numbers of holes. This allowed larger food enrichment choices which would not fall through the flooring, but still provided more of a challenge for the animals. The swine were observed playing and investigating the foraging balls for longer periods of time, approximately 30-45 minutes while there was food in the ball. These customized foraging balls created a more challenging experience for our swine, thus extending play time and providing a better enrichment experience in our facilities.

BACKGROUND
• Rooting is well-documented as an essential foraging behavior in pigs. Studies show that domestic pigs spend 51% of their day investigating and rooting in their surroundings.
• This natural behavior can become destructive to the pig itself or to the enclosures they occupy unless the behavior is redirected.
• When pigs were observed in an enclosure with enrichment, they spent their time rooting, biting, and chewing the available enrichment. Pigs in enclosures devoid of enrichment spent their time rooting, biting, and chewing the floors and walls of their enclosure.
• To date, the University Laboratory Animal enrichment program for swine include daily food enrichment placed in the food bowl once daily and toys rotated in the cage once a week.
• The purpose of this study was to develop an enrichment device that was efficient and effective at enhancing the food enrichment they were already receiving while also promoting healthy normal behaviors.

RESULTS
Figure 1. The original herding ball (top) and the customized foraging balls (29mm, 35mm, or 44mm). Figure 2. Hole saws used to modify the herding balls. Figure 3. A carrot and a grape on the standard flooring in our facilities. Figure 4. Pig interacting with modified (29mm hole) foraging ball. Figure 5. The video camera used to collect data fixed to the top of a run. Figure 6. Pig drinking ignoring the standard flooring in our facilities. Figure 7. Scored amount of interaction with a ball in five minute intervals.

3. The foraging ball as a quick and easy enrichment device for pigs (Sus scrofa); Mary E. Hantaehnery, MA, Delaine Charles, MS, Kristina M. Adams, MS, and James L. West, PhD; Lab Animal, Vol. 37, No. 9, page 413.

MATERIALS AND METHODS
Equipment
• Six inch diameter herding balls were purchased from a commercial vendor and a hole saw from a local hardware store was used to modify the herding balls (Figures 1 and 2).
• Balls were either left unmodified or had a hole(s) drilled into them. Three arbitrary hole sizes were used—29mm, 35mm, or 44mm (Figure 1).
• The hole(s) drilled into the ball were sanded with fine grit sand paper to smooth any rough edges and remove any flakes of remaining plastic.
• No ball had more than three holes in it and all holes in an individual ball were the same size.
• The balls with the hole(s) had enrichment food items such as grapes, strawberries, carrots, cherry tomatoes, or pieces of pear placed in them. Food enrichment was chosen based on size—whether or not it would fall through the flooring (Figure 3). The unmodified balls had no food enrichment.

Animals
• A group of 18 pigs were used for data collection: five pigs to test each of the experimental balls and three for the control ball.
• Pigs were housed in a 90in X 38in run and fed Mazuri mini pig diet twice daily. City water was provided via an automatic watering system (lxk).

Experimental Design
• The pigs were exposed to a ball for thirty minutes and videotaped (Figure 5).
• The videos were then reviewed and scored based on the amount of contact time each pig had with the ball.
• Points were awarded at each 5 minute interval.
• The ball received one point for every minute of time the pig was in contact with it for a maximum score of five points per interval.

CONCLUSION
By customizing a foraging ball to our specifications, we were able to create a more challenging enrichment experience that promoted normal swine behavior in the laboratory setting for an increased duration of time. The 29mm size foraging hole(s) which engaged the animals for the longest duration without loss of food through the run flooring is 29 mm.
Meningocele with a Focal Lipomatous Hamartoma in a Mouse (Mus musculus)

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The Ohio State University, Columbus, Ohio

ABSTRACT

A 7-month-old, female, genetically modified mouse on a C57BL/6 background presented to the Veterinary Service with a 0.25-cm-diameter, soft, elevated mass on the dorsal aspect of the rostral cranium. No other abnormalities were noted during the physical examination, nor were clinical signs of neurological dysfunction observed. Consultation with the investigator revealed that this finding was not an expected phenotype in the colony. At necropsy, the mass was demonstrated to involve the subcutis but also to extend as a fibrous stalk through an orifice in the calvarium (i.e., skull). Histopathologic examination revealed a narrow, irregular cavity lined by a thin meningotheial layer subtended by a variably thick layer of dense connective tissue. Other subtle but notable dysplastic defects in the adjacent brain included the loss of cerebrocortical layer I, agenesis of the corpus callosum, and migration of the habenular nuclei (H) into the third ventricle (III) walls. The meningocele lumen (L) is lined by a thin meningothelial layer, resting on dense connective tissue, which forms a stalk (S) connecting to the underlying leptomeninges. H&E, 100x.

RESULTS

Figure 1. Clinical Presentation. Soft, elevated mass on the dorsal aspect of the rostral cranium (“forehead”) in an otherwise healthy, naive 7-month-old female C57BL/6 Eu-155 transgenic mouse.

Figure 2. Appearance of the extra-cranial mass on the rostro-dorsal calvarium, as seen when the skin has been reflected.

Figure 3. Meningocele (extracranial extension of the meninges) encased within a lipomatous hamartoma. H&E, 20x.

Figure 4. The brain exhibits focal loss of cortical layer I (arrows), agenesis of the corpus callosum, and migration of the habenular nuclei (H) into the third ventricle (III) walls. The meningocele lumen (L) is bounded by a stalk (S). H&E, 100x.

Figure 5. The meningocele lumen (L) is lined by a thin meningotheial layer resting on dense connective tissue, which forms a stalk (S) connecting to the underlying leptomeninges. H&E, 100x.

DISCUSSION

• Pathologic evaluation confirmed the presence of a meningocele, an extracranial extension of the meninges through a persistent foramen in the bone (in this case, the calvarium).

• The pathogenesis of the lesion is an incomplete closure of the neural tube near the rostral neuropore, indicating the defect arose at approximately gestational day (GD) 9.25 (where GD 0 represents the morning on which a copulation plug was used).

• While this was a spontaneous neural tube defect (NTD), there are over 240 mouse mutants (engineered, induced, or spontaneous) and strains that serve as models for human NTD.

MATERIALS AND METHODS

• Mice. Animals were housed in microisolator cages containing ¼ inch corn cob bedding and a cotton nestlet under a 12-12 hr light/dark cycle in accordance with “The Guide for the Care and Use of Laboratory Animals” and experimental use approved by the Institutional Animal Care and Use Committee. Animals were provided ad libitum Teklad 7912 rodent chow and filtered city water.

• Pathologic Evaluation. The mouse was euthanized via CO2 asphyxiation. After the gross postmortem evaluation, tissues were fixed in neutral buffered 10% formalin for 24 hours, processed by routine methods and embedded in paraffin. Sections (4 µm) were stained with hematoxylin & eosin (H&E) and evaluated by light microscopy.

REFERENCES


Figure 6. Similarities between human and mouse neural tube defects. The underlying brain exhibits a number of subtle structural changes consistent with defective neural tube closure. The minimal nature of these neural defects would not have affected neurological function during life.

• While this was a spontaneous neural tube defect (NTD), there are over 240 mouse mutants (engineered, induced, or spontaneous) and strains that serve as models for human NTD.

Figure 2. Appearance of the extra-cranial mass on the rostro-dorsal calvarium, as seen when the skin has been reflected.

Figure 6. Diagrammatic cross-section of cranial neural fold elevation.
The Health Management of a Rice Rat (Oryzomys palustris) Colony within a Laboratory Animal Facility

ML Nicolaus and JM Hickman-Davis
University Laboratory Animal Resources

ABSTRACT
Animal care and use programs within academic institutions need to provide appropriate health management conditions for a variety of animal species. The rice rat (Oryzomys palustris) was selected by an investigator to be used as an animal model for periodontal disease. In preparation of using this animal model, University Laboratory Animal Resources’ Quality Assurance Laboratory needed to develop a standard of care for a colony of animals acquired from an outside source. The aim was to determine the appropriate health monitoring requirements for the animals, along with establishing baseline data bank of blood chemistry values for this species. Animals were initially quarantined and monitored for excluded pathogens using parasitologic and serologic methods by direct testing of the colony animals and sentinel animals associated with the veterinary care program which will help provide high quality research subjects in the future.

BACKGROUND
Oryzomys palustris: Rice Rat
- Weight: 40-80 grams
- Gestation: 25 days
- Litter size: 1-7
- Diseases: Argentinian Hemorrhagic Fever, zoonotic

The fur of the rice rat varies from gray to brown with blackish hairs intermixed. The ventral side and feet are whitish. The ears are small and the tail is as long, or longer, than the head and body.

Research Uses
- Arboviruses
- Copulatory behavior
- Congenital erythrocytic porphyria
- Dental development
- Periodontal disease

Quarantine Guidelines for Import of Rodents
The Ohio State University
- Quarantine will be set up for all animals coming in from sources not on the OSU approved Rodent Vendor listing
- The quarantine time frame is 6-8 weeks for full testing unless there are health issues
- Initial testing of colony animals upon arrival
- Sentinel placement with individual quarantine groups

MATERIALS AND METHODS

Ectoparasites
- 20% of the incoming colony animals were tested for fur mites via tape test
- Positive for Radfordia affinis

Treatment
- Occurs during cage changes
- Add ½ tsp of ATGARD® (Dichlorvos) granules to each cage
- Change cages weekly with new ATGARD® added at each cage change
- Continue to add ATGARD® to cages weekly for 6 weeks
- At end of treatment-change cages (no ATGARD® added)
- 20% of the incoming colony animals were tested for fur mites via tape test

Endoparasites
- 20% of the incoming colony animals were tested for pinworms via tape test and fecal float
- Positive for Syphacia murtis

Treatment
- Treatment initiated using Harlan™ Teklad Global 18% Protein Diet medicated with Fenbendazole and placing Atgard® (Dichlorvos) Swine wormer in the bedding. Results were the successful elimination of both pathogens. At the end point of the study, blood was collected via cardiac stick and serum was submitted for complete blood cell count and serum chemistry to the Comparative Pathology and Mouse Phenotyping Shared Resource at The Ohio State University. Standard baseline rice rat values were determined by comparing the results to each other as well as the standard rat values for serum chemistry.

Serology
- Colony animals were placed under minimal anesthesia using isofluorane
- Blood was collected via the submandibular vessel
- Animals were monitored until sternal, then returned to cages
- Serum was sent to Charles River Laboratories
- Tested for pathogens using the Rat Assessment Plus panel

Post Treatment
- Samples were collected two and four weeks post treatment from all colony cages
- Positive tests required re-treatment and follow-up testing until negative

RESULTS

Complete Blood Cell Count

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<td>WBC 2.9-20.9 K/µL</td>
<td>RBC 7.45 M/µL</td>
<td>PLT 1162.66 K/µL</td>
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<tr>
<td>MCH 19.11 pg</td>
<td>HCT 53.39 %</td>
<td>BA 0 %</td>
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<td>MCV 71.56 fL</td>
<td>RDW 15.66 %</td>
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<th>Serum Chemistry</th>
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<td>GLU 148.46 mg/dL</td>
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<td>CREAT 0.43 mg/dL</td>
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<td>ALT 10.0-35.0 u/L</td>
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<td>TBILI 0.18 mg/dL</td>
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<td>CREAT 0.5-2.2 mg/dL</td>
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CONCLUSIONS
The establishment of baseline serum chemistry values and complete blood cell counts, along with successful treatment of two pathogens, has contributed to the formation of a veterinary care program which will help provide high quality research subjects in the future.

ACKNOWLEDGEMENTS
Photographs of rice rats, fur mites, pinworms, Atgard®, serology tubes, and reagents courtesy of the Quality Assurance Laboratory, ULAR, The Ohio State University. White blood cell diagram courtesy of webmd and white blood cell smear courtesy of kumc.edu.
A Retrospective Examination of the Impact of Removing Shoe Covers from Standard Personal Protective Equipment within the Animal Facility

ML Nicolaus, JM Petty, DM Harrison, VK Bergdall, and JM Hickman-Davis
University Laboratory Animal Resources

ABSTRACT

Historically, shoe covers were considered an integral part of the bio-containment process for maintaining a “clean” animal room. With the introduction of microisolator caging and ventilated rack housing, it became necessary to re-evaluate the contribution of different elements of PPE utilized to maintain animal and human health. A study was designed to test the effectiveness of shoe covers for bio-containment at both the animal room floor level and on personnel utilizing fluorescent powders. Experiments were performed both with and without shoe covers and a black light was used to survey fluorescence around the room, on the rack, on inner and outer surfaces of cages, and on personnel. While there was no difference in the spread of fluorescence in the presence or absence of shoe covers at the room level, it was determined that the application of shoe covers offered a direct potential for contamination of personnel from contact with shoe bottoms. In August 2010, shoe covers were removed from standard PPE for all ULAR facilities except BSL3 and housing, it became necessary to re-evaluate the contribution of different elements of PPE utilized to related time savings of $9,000 each year. Results indicate that the elimination of shoe covers did maintain animal and human health. A study was designed to test the effectiveness of shoe covers and on personnel. While there was no difference in the spread of fluorescence in the presence or absence of shoe covers at the room level, it was determined that the application of shoe covers used to survey fluorescence around the room, on the rack, on inner and outer surfaces of cages, and on personnel. Since the final removal of shoe covers in August 2010, two pathogens, pinworms and MHV, have been identified in one vivarium. Subsequent sentinel testing for the July-September 2012 quarter was negative for all restricted pathogens. The health data retrieved over the past 24 months through the sentinel program shows that the removal of shoe covers did not impact disease prevalence in ULAR facilities. The identification of pinworms in 2011 and MHV in 2012 is consistent with the OSU history and we feel confident that this does not have any relationship with the discontinuation of the use of shoe covers. The removal of shoe covers from the standard required PPE for rodent room entry in ULAR facilities has resulted in significant projected savings each year of $18,000 in supply costs and an estimated $9,000 a year in staff-related time savings.

RESULTS

Sentinel Program in ULAR Rodent Facilities

All ULAR rodent facilities have a cage of two sentinel assigned to each ventilated rack up to 140 cages. Mouse sentinel cages consist of two, 3-4 week old CD-1 heterozygous nude immune competent mice. Sentinels are exposed to 100% pooled dirty bedding from colony animal cages at each cage change. Quarterly both sentinel animals have samples collected and processed for diagnostic testing. Sentinels are replaced biannually. Testing for serology and helicobacter is through Charles River Valley (CRL) and remaining testing is in house.

Sentinel Testing Results January 2009-June 2012

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Pinworms visible on Necropsy and signage

Marine norovirus (MNV) is not excluded from any ULAR facility at this time and is considered endemic to the rodent population. A recent study reported that this does not have any relationship with the discontinuation of the use of shoe covers. Subsequent sentinel testing for the July-September 2012 quarter was negative for all restricted pathogens. The health data retrieved over the past 24 months through the sentinel program shows that the removal of shoe covers did not impact disease prevalence in ULAR facilities. The identification of pinworms in 2011 and MHV in 2012 is consistent with the OSU history and we feel confident that this does not have any relationship with the discontinuation of the use of shoe covers. The removal of shoe covers from the standard required PPE for rodent room entry in ULAR facilities has resulted in significant projected savings each year of $18,000 in supply costs and an estimated $9,000 a year in staff-related time savings.

CONCLUSIONS

The health data retrieved over the past 24 months through the sentinel program shows that the removal of shoe covers did not impact disease prevalence in ULAR facilities. The identification of pinworms in 2011 and MHV in 2012 is consistent with the OSU history and we feel confident that this does not have any relationship with the discontinuation of the use of shoe covers. Subsequent sentinel testing for the July-September 2012 quarter was negative for all restricted pathogens. The removal of shoe covers from the standard required PPE for rodent room entry in ULAR facilities has resulted in significant projected savings each year of $18,000 in supply costs and an estimated $9,000 a year in staff-related time savings.

ACKNOWLEDGEMENTS

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MATERIALS AND METHODS

Krypton Powder and Fluorescence Simulation Powder are useful in situations where invisible detection is needed.

A room within the vivarium was equipped with a bio-safety hood and a ventilated rack containing microisolator cages. A thin layer of powder was applied to an area inside of the animal room door. Study participants randomly selected a task to perform (Table 1), donned required PPE including shoe covers, entered the room and performed the task. After the last participant, the room was examined for distribution of powder using a black light. The room was cleaned, and the experiment repeated without shoe covers. Black light assessment revealed no contamination powder within any of the cages or on any of theanimate objects within cages. Powder was visible throughout the room in normal traffic patterns on the floor in either the presence or absence of shoe covers (Fig. 1 and 2). One participant used the lower row of ventilated rack as a step to gain access to top row of cages (Fig. 3).

A thin layer of powder was applied in front of a PPE station. One at a time, participants donned PPE, either with or without shoe covers. Participants entered a room, were examined using a black light, and photographs were taken of any fluorescence. Black light examination showed that all participants that were asked to don shoe covers showed fluorescence in multiple areas. Fluorescence was most notable on gloves and gowns, especially the sleeve and cuff area (Fig. 4). No fluorescence was noted on participants that did not apply shoe covers (Fig. 5).
Histiocytic Sarcoma in a Djungarian Hamster (Phodopus sungorus)

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Abstract

An aged male Djungarian hamster (Phodopus sungorus) presented with bilateral facial swelling, proptosis of the left eye, and blepharoconjunctivitis of the right eye. The animal was a breeder with no prior experimental or medical history. Ibuprofen-medicated drinking water was initiated to manage clinical signs. However, due to the poor prognosis, the hamster was humanely euthanized. Gross necropsy demonstrated a proposed left globe, a ruptured right globe, and marked facial edema and hemorrhage. Microscopic examination revealed large thrombi bilaterally in major veins draining the retrobulbar venous plexus, with large retrobulbar hemorrhages compressing the Harderian glands. Abdominal findings included multiple soft, white, attached and free-floating nodules; a large mass effacing the cranial pole of the right kidney, and red-brown ascites fluid. The white nodules were coalescing foci of histiocytic sarcoma dispersed throughout the abdominal cavity and displacing the parenchyma of major viscera (especially liver). The diagnosis of a histiocytic lineage for the neoplasm was supported by immunohistochemistry to detect the macrophage surface antigen CD163. The kidney mass was a renal cell carcinoma. While not tested directly, a reasonable explanation for the clinical presentation was reduced protein synthesis (including thrombolytic molecules) as a consequence of liver destruction and/or cachexia associated with systemic neoplasia. To the authors’ knowledge, this case represents the first description of disseminated histiocytic sarcoma reported in a Djungarian hamster.

Background

Histiocytic sarcoma is a malignant tumor of macrophage lineage that has been reported in humans, dogs, cats, camels, mice, and rats.1,4 The neoplasm may be characterized by widespread involvement of major viscera (especially liver and spleen) in conjunction with characteristic cell features such as pleomorphic nuclei with prominent nucleoli as well as giant and/or multinucleated tumor cells. Spindle cells may be the primary cell type in some areas, and subcutaneous or soft tissue masses may be associated with this neoplasm.1

Histiocytic sarcoma in mice occurs more frequently in animals over 12 months of age and involves extensive infiltration of abdominal organs such as the liver, spleen, and uterus. Animals of the C57BL/6J strain, particularly females,2 appear to be predisposed to this tumor. Findings in mice commonly include hepato-megaly, ascites, enlarged abdominal lymph nodes, and pleural effusion. A single reference characterizes histiocytic sarcoma in hamsters as a locally invasive soft tissue mass or a diffuse lymphomatous neoplasm.1

Clinical History

An aged male breeder hamster presented with a rough coat, profound facial edema, and substantial pericellular swelling affecting the right eye. Clinical improvement was not observed after treatment with ibuprofen-medicated drinking water and the hamster was euthanized.

Gross Findings

Gross findings included moderate edema and hemorrhage of the face, proptosis of the left eye, rupture of the right eye, and many spontaneous lesions including ascites, a large mass effacing the cranial pole of the right kidney, multiple smooth white nodules in the abdominal cavity, and mild focal necrosis of the spleen. The renal cell carcinoma, while quite large, was confined to a portion of one kidney, and would not have impacted renal function. The ongoing effort to support the tumor cell populations and their activities (such as ascites) and the consequences (inflammation in response to tumor-induced necrosis of the visceral parenchyma and degradation of the free-floating neoplastic polyps) would have greatly depleted the hamster’s energy reserves in multiple tissues. Marked, diffuse atrophy of brown fat and difficulty in isolating white fat deposits demonstrated that this animal had been in a physiological state of severe catabolism for an extended period prior to the onset of clinical disease. Additionally, ge-related degenerative conditions affecting the skeleton and the widespread damage to the facial tissue would have contributed to the poor condition of the animal. Repeated hemorrhage with indolent suppurative inflammation and edema of the retrobulbar tissues bilaterally, proptosis of the left eye, and ulcerative keratitis and rupture of the right eye coupled with marked necrosis of the right Harderian gland over time could have limited the hamster’s interest in food consumption and environmental interaction.

Histological Findings

Histologic examination revealed large thrombi bilaterally in major veins draining the retrobulbar venous plexus, with large retrobulbar hemorrhages compressing the Harderian glands. Abdominal findings included multiple soft, white, attached and free-floating nodules; a large mass effacing the cranial pole of the right kidney, multiple smooth white nodules in the abdominal cavity, and mild focal necrosis of the spleen. The neoplastic cells in these structures were highly cellular and pleomorphic (variable in features) as shown by the presence of many multinucleated giant cells were evident in neoplastic foci, and vessels often contained rafts of tumor cells (arrows). The adjacent hepatic parenchyma was degenerating or necrotic.

The most important findings were two major neoplasms: histiocytic sarcoma, which was widely distributed as white nodules in multiple viscera and throughout the abdominal cavity, and renal cell carcinoma.

In the mesenteric, neoplastic cells were present as a widespread infiltrate of the surface and as densely cellular polyps anchored by highly cellular stalks. The neoplastic cells in these structures were more pleomorphic (variable in features) as shown by the presence of a few giant nuclei (arrows) and larger, sometimes multiple nucleoli. The free-floating abdominal nodules were highly cellular but entirely necrotic except for an external layer of simple mesothelium. Their features were consistent with the neoplastic poly described above, suggesting that attached viable nodules required a vascular supply.

The immunohistochemical labeling pattern of neoplastic cells in the liver was consistent with histiocytic sarcoma. The tumor cells were moderately to strongly labeled by anti-CD163 (above), but not by anti-cytokeratin or anti-epithelial membrane antigen (not shown), suggesting that the tumor originated from macrophages instead of epithelium or mesothelium.

Histology Laboratory.

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References