Renovation of the air-conditioning system in the animal facility at the CARD, Kumamoto University

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Our animal facility was built at the Center for Animal Resources and Development, Kumamoto University in 2000. The animal facility is located on the 6th to 10th floors of the building, with approximately 30,000 mice kept on the 8th to 10th floors. The room temperature and humidity in the animal facility is maintained at a constant 22±2°C and 40-80% using an air conditioning system. There are six air conditioners in our facility: four circulation systems on the rooftop (two systems on the north side and the south side respectively, for the 7th-9th floors) and two outside air introduction systems in the 10th floor machine room (one system for the 10th floor). The air-conditioning system and three deodorizers operate continuously year-round. This year, we plan to renovate the air-conditioning system with the aid of a 2019 National University Corporation Facility Maintenance Works grant. In this presentation, we will explain the outline of our plan to renovate the air-conditioning system at CARD and share our experiences thereof.
The 67th Annual Meeting of Japanese Association for Laboratory Animal Science

P2 Syphacia spp. infections in mice and rats in conventional facilities of NIRS.

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The microbiological monitoring is performed by the different frequency at conventional facilities in NIRS. In those facilities, regular monitoring is being checked 4 times a year in Translational Research Building, but these are being checked only twice in PET Facilities. We found eggs of rat pinworm, Syphacia muris, by cellophane tape test for monitor rats in Translational Research Building, in June, 2018. Then, that we decided to check pinworm about PET Facilities where management practice in facilities is similar. However, this facility is different from Translational Research Building, and mice and rats are raised in the same room. Therefore we checked pinworms about both of mice and rats. As a result, we found eggs of mouse pinworm, Syphacia obvelata, from mouse sample. For the identification of Syphacia muris, Syphacia obvelata and Aspiculuris tetraptera, also PCR tests were utilized as well as microsoopic examination. We have settled those microbiological contaminations for more than 1 year.
Verification of decontaminative effects in clean laboratory rooms with vaporized hydrogen peroxide

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Vaporized hydrogen peroxide (VHP) has an effect of a wide spectrum of decontamination on pathogenic microorganisms. The purpose of this study was to verify decontaminative effects in a clean laboratory room (10-20°C, 100 m³) using VHP. A biological safety cabinet (BSC) in this room was also decontaminated with VHP. The procedure for decontamination was as following: 1. dehumidification, 2. conditioning, 3. decontamination and 4. aeration. The effects of decontamination with VHP were determined by biological indicators (BIs) including *Geobacillus stearothermaphilus* spores and enzymatic indicators (EIs). We confirmed that VHP immediately decontaminated microorganisms throughout the room. VHP infiltrated into every corner of the room and all over the narrow portions of the BSC. Repeated VHP decontamination caused no damage to the precision instrument in the BSC. BI data provided evidence that exposure to VHP could have beneficial effects on decontamination in this room. In contrast, EIs indicated inadequate reactivity to VHP. Usual decontamination with VHP takes considerable long time to complete a series of processes. Under experiment conditions in the BSC, we have succeeded in shortening the decontamination time by reduction in aeration. Our results verified that VHP had the potential to decontaminate quickly and adequately the clean laboratory rooms accompanied by BSCs.
Demonstrate the practicality of the water leakage sensor for laboratory animals

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The accidental water leakage in the laboratory mice cages result in the malfunction of water supply nozzles, deterioration of packing or mouse water playing. Water leaking accidents cause the hypothermia of animals even leading to death. Due to it is difficult to predict the water leaking accidents, confirm each mouse cage during daily routine inspection and check water supply nozzles regularly as countermeasures. In this present study, we try to develop a new water leakage sensor for laboratory animals with environmental enrichment mouse house, and demonstrate the practicality of early detection of water leaking accidents. The water leakage detecting system consists of a water leakage sensor with environmental enrichment mouse house, a sensor pad, a transmitter and a computer for system management. Four week-old female Slc:ICR mice were used for the water leak detection test. The water leakage sensor was collected, when the alarm notification be notified. To confirm the practicality of water leakage sensor under the normal breeding condition of mice, the tests was continuously conducted for 6 months. These results suggested that this new water leakage sensor system as a strategy for preventing the accidental water leakage in the mice cages.
During breeding experimental animals in individually ventilated cages, the occurrence of abnormal animals must be detected as soon as possible to prevent the spread of its causes and resulting damages. Previously we developed an animal watcher using only one optical sensor per cage. Moving patterns of mice differ depending upon mouse conditions. Decrease in the movement should indicate the occurrence of abnormal mice. It is essential to estimate the number of abnormal mice in the case of multiple (up to 5) mice. We looked for a hot spot where single sensor could detect mouse number-dependent movement. Finally, a specific site just below the drinking port of a water bottle with several cm depth was found to be such a hot spot. A positive correlation was observed between the frequency of optical path blocking and the number of animals. In contrast, when the sensor was set at lower positions several cm above the bedding, no such a correlation was obtained; reaching a steady level or decreasing tendency.
Guinea pigs prefer solid floors with bedding to wire-mesh flooring

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The guide for the care and use of laboratory animals describes that rodents prefer solid floors with bedding to wire-mesh flooring, whereas we are still using wire-mesh flooring for guinea pigs due to the risk for flooding of bed and no preference study of flooring in guinea pigs. The aim of this study was to find watering methods with low risk for flooding and to clarify whether guinea pigs prefer solid floors with bedding. Guinea pigs housed on wire-mesh flooring were watered by an automatic watering equipment or water bottles with ballpoint nozzles, and the dropped water was collected. In 4 weeks housing study, body weight gains and food intakes of guinea pigs in cages with bedding materials and water bottles were compared to those in cages with wire-mesh flooring and automatic watering equipments. In preference study, a guinea pig was placed in a cage floored with a wire mesh and paper bedding materials by halves. Dwelling time for each flooring was calculated. Dropped water from water bottles was much less than that from automatic watering equipment. Meanwhile body weight gains and food intakes were comparable between two groups in 4 weeks housing study. In preference study, dwelling time for bedding materials was significantly longer than that for wire mesh, suggesting that guinea pigs prefer solid floors with bedding to wire-mesh flooring. Based on these results, we decided to change flooring for guinea pigs to solid floor with bedding.
Search of the aging marker about the humane endpoint setting of naturally aged mice

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The mortality rate and incidence of specific pathologies in naturally aged mice to use for gerontology and geriatric researches vary due to various genetic and environmental factors. In the present study, we will report about the setting of the humane endpoint of the naturally aged mice. Male and female C57BL/6 (C57BL/6NCrSlc(B6N), C57BL/6J(B6J)) mice (4-weeks-old) were purchased from Japan SLC and Charles River Japan every three months and kept over their lifetime. Physiological (body weight, body temperature and survival rates), biochemical (CORT), and morphological (autopsy) analyses were performed. Body weight showed rapid decrease at around 23 M in B6N male mice, and 24M in B6J female mice. Survival rates of B6N mice started to decrease from 18 M, while in B6J mice, decreased from 21 M. CORT levels were relatively higher in female mice and tend to increase with age in both B6 mice. The product of body temperature and body weight of individuals with high CORT values fluctuated more than 10% during the last 2 weeks before death. It was suggested that this would be a suitable marker for the humane endpoint of naturally aged mice.
Evaluating environmental enrichment for mice and rats by scoring system

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(Background and purpose) Environmental enrichment (EE) is expected to improve animal welfare and relieve stress of rodents in laboratory. Here, we evaluated the effectiveness of EE for mice and rats in our facility by setting score for the use of tissue, Safe Harbor Mouse retreat (SH) and paper tube (PT).

(Method) I: tissue: 1) Crlj:CD1(ICR) mice (3 dams, 45 pups/cage ; 20 cages), 2) Crl:CD(SD) rats (1 dam, 15 pups/cage ; 20 cages). II: SH: 3) C57BL/6J mice (15 males/cage; 20 cages). III: PT: 4) C57BL/6J mice (8 females/cage; 18 cages). Animals were housed in solid bottom plastic cages with bedding and EE. Food and water were provided ad libitum. EE was scored by 1 minute of observation executed between 9am to 3 pm.

(Result) 6 point was set for scoring tissue. 5-complete nest, 4-flat nest, 3-shredded, 2-partly shredded, 1-not touched, and 0-showed aversion. Complete nest was observed in all cages of group 1) and average score was 5. The score for group 2) was 2.8. For SH and PT, 6 point score was set based on time animals were inside EE. 5-always, 4->80%, 3->50%, 2->30%, 1->30%, 0-EE was destroyed on the first day/ showed aversion. The score for group 3) and 4) were 3.3 and 3.8 respectively. The use of SH as a shelter is higher than PT. From these results, it is indicated that the effectiveness of EE can be evaluated by scoring with visual observations.
Effectiveness of habituation to restrainer evaluated by stress marker in rabbits

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Recently, refinement is especially important among 3 Rs. To implement refinement, plasma glucocorticoid levels is often used for evaluating stress in animal. Whereas it has been reported stress responses in rats and dogs, there are few studies in rabbits. In response to customers’ request, rabbits are trained for habituation to restrainer in Kitayama Labes Co.Ltd. As the effectiveness of this training is not clear, we examined the effects of habituation to restrainer on plasma levels of corticosterone in rabbits. Five male and 5 female Japanese-white rabbits (Kbl: JW, 10 weeks of age) were habituated to restrainer (w135×D600×H230 mm, designed by Kitayama Labes Co. Ltd.) for 3 days. Rabbits were restrained for 1 hour (Day1 and Day2) or for 6 hours (Day3). We obtained blood samples from ear vein at the time just after the onset of the restraint, after 30 minutes, 1 hour (Day1, Day2 and Day3), and 6 hours (Day3), and measured plasma levels of corticosterone. As the results, plasma levels of corticosterone increased by restraint in male and female rabbits. Both basal levels and peak levels of corticosterone declined Day2 and Day3 compared to Day1. These results suggest that rabbits might get used to restrainer during 3 days training, and these procedures are effective for reducing stress and for refinement based on scientific evidences.
Our company started Beagle R program as one of Public Engagement from July 2016. Re-homing transfer from research facility to home, and we gave total 30 dogs until 2019. Our Beagle program protect each information of research facility and home each other. In US and Europe, many academia and pharma give retired dogs to home directly. Thus, we expect it will also operate without our company in Japan in near future. But most of them should have some concerns before starting. We managed survey about satisfaction and concerns against re-homing dog in 2019 and got 15 feedback. Satisfaction level was 9.27. 92% of homes decided to receive dog within one month. Family discussion is very important to prevent cancellation at the last moment. 73% of dogs inside of house and are taken walk twice a day. Most of difficulty was feces problem. One home trained dog who could remember place of his rest box within one week. Marshall Beagle is impressed as familiar, no bark, and friendly. But each dog is expressed very different after housing and handled in home. Conversations related the necessary of laboratory animal in the home, is same with Increasing and No change. That is probably based on many home owners worked for research facility or were already interested in animal research. Through this survey, we heard bad impression against animal research, and recognized the importance to disclose correct information in public. And we understood re-homing program in research facility, is very useful for satisfaction and trouble prevention with researchers as well as home.