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Creating a Stable Short-term Housing Environment for Rabbits in a Cargo Van

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A stable environment is a prerequisite for animal research. The absence of a suitable laboratory rabbit environment at the gyrotron facility, with the nearest housing being 6.3 miles away, made it challenging to investigate ocular damage induced in rabbit eyes due to exposure to high-frequency millimeter waves. Because rabbits are prone to transportation stress, it was vital to keep them on-site during research. Here we describe the creation of the stable environment necessary to perform reliable and reproducible animal experiments, by using a cargo van parked at the gyrotron facility. To control the interior environment, we placed a window air conditioner, humidifier, dehumidifier, and photocatalyst deodorizer inside the cargo area without altering the original configuration of the vehicle. Rabbits were housed in individual cages for a maximum of 6 d. Microbial contamination in the air was evaluated by using a passive sampling method. The average numbers of bacterial and fungal colony forming units per dish were 0.2 and 4.7, respectively, indicating that the van was as clean as a nonbarrier animal facility. The average temperature was 20.5 °C (range, 17.8 to 22.6 °C), and the average relative humidity was 49.4% (range, 36.2% to 63.2%). The concentration of ammonia was consistently below the detection limit of 0.5 ppm. Other environmental conditions were within appropriate levels. Rabbits lost 6.4% ± 2.2% (n = 52) of their initial body weight during the 13- to 14-h transport but recovered the lost weight within 48 h after arrival.

Abbreviation: MMW, millimeter waves

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Millimeter waves (MMW) have broad applications in high-data–rate communication systems (40 to 48 GHz), automotive radar sensors (77 GHz), high-resolution sensors for airport security (100 GHz), and spectroscopy (20 to 80 GHz).[4,6,14,21] Moreover, the recent trend in wireless communications is to use MMW with frequencies greater than 100 GHz (>100 GHz MMW) to transfer larger amounts of digital data.[1] The rapid development of technology has raised concerns about the possible influence of MMW on human health. We previously investigated elevations in ocular temperature after the exposure of Dutch-belted rabbit eyes to quasi- and millimeter waves (18 to 40 GHz) and acute ocular damage caused by 40 to 95 GHz MMW.[9-11] We found that exposure to MMW induced a rapid elevation in ocular temperature, causing various types and levels of thermal injury.[9-11] MMW at frequencies exceeding 100 GHz are speculated have different thermal damage thresholds.[8] However, few scientific data regarding the effects of >100 GHz MMW on eyes are available.

Acute ocular damage induced by >100 GHz MMW is difficult to determine because of limitations in generating sufficient output power to cause acute thermal effects. We, therefore, used gyrotrons at the Research Center for Development of Far-infrared Region (University of Fukui, Japan), which can generate MMW at frequencies ranging from 162 to 265 GHz, with a maximum output power exceeding 1 kW.[19] Because the gyrotron system is composed of large and immobile equipment, the rabbits had to be brought to the gyrotron facility to perform experiments. However, no laboratory animal facility for rabbits was available at that site, with the nearest one being 6.3 miles away at another UF campus. Because rabbits are prone to transportation stress, it was vital to keep them on-site during research.[18] It was not possible to build a new animal facility for this project alone. Therefore, an alternative housing system was needed that provided a stable and adequate environment for laboratory rabbits, was easy to set up for a short period (1 wk), and was affordable.

This study describes the creation of a stable and adequate environment for laboratory rabbits at the gyrotron facility that allowed the performance of reliable and reproducible animal tests. A suitable environment was created in a cargo van rented from a local agency, without changing the original configuration of the vehicle. Environmental factors were monitored to ensure that they met the criteria for conventional animal facilities. The adaptation period required by the laboratory rabbits was also assessed.

Materials and Methods

This experiment was conducted at the Research Center for Development of Far-infrared Region, University of Fukui, Fukui, Japan. The animals were managed in accordance with practices and experimental protocols reviewed and approved by the Animal Research Committee of the University of Fukui.

Animals. Dutch rabbits (*Oryctolagus cuniculus*; strain, Kbt:Dutch) were obtained from a commercial breeder (Biotek, Saga, Japan). These SPF animals are guaranteed free of the following pathogens: *Bordetella bronchiseptica*, *Pasteurella multocida*, *Salmonella* spp., *Eimeria intestinalis*, *Eimeria stiedai*, *Mycoplasma*

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Figure 1. The cargo van system provides environmentally stable, short-term housing for laboratory rabbits. (A) Refrigerated cargo van. (B) Standard cargo van with black plastic sheets. (C) Interior of the cargo van equipped with a humidifier (a), air conditioner (b), photocatalyst deodorizer (c), LED lamp (d), rabbit cage (e), data logger (f), and fence (g). (D) The inside of a rabbit cage.

spp., *Clostridium piliforme*, Sendai virus, *Cysticercus pisiformis*, and external parasites (mites and *Psoroptes cuniculi*). However, the SPF status of these rabbits after arrival at the facility was not tested. These Kbt:Dutch rabbits (46 males; age, 11 to 13 wk; body weight, 1.85 ± 0.03 kg) were housed individually in stainless steel housing cages with a slatted floor (385 mm × 450 mm × 400 mm; Toyo-Riko, Tokyo, Japan) for a maximum of 6 d (Figure 1 C and D). Retired Kbt:Dutch rabbits (6 males; age, 15 to 19 wk; weight, 2.24 ± 0.23 kg) were used for the pilot experiment only. Animals were fed 170 g of laboratory-grade chow (Labo R Stock, Nosan, Kanagawa, Japan) once daily. Tap water was given from a 650-mL bottle without restriction. The rabbits were singly housed to prevent them from licking or aggravating any eye injuries in other rabbits that might result after MMW exposure. Rabbits could see or smell each other. Enrichment or hay was not provided to avoid the risk of damaging the eyes due to scratching. Rabbits were weighed once daily by using a pediatric scale (model BD-586-WH, Tanita, Tokyo, Japan). The food and water intakes were checked daily when personnel performed a brief physical health check (fur, skin, ears, and so forth).

Housing. Because the entrance to the gyrotron facility was only 2.5 m high, a cargo van (cargo area, 3000 mm [length] × 1520 mm [width] × 1320 mm [height]; RegiusAce model, Toyota Motors, Aichi, Japan) or equivalent was rented from a local rental agency and was parked in a large air-conditioned room at the gyrotron facility. During the experiments, the van's engine was completely shut down. To control the interior environment, a window air conditioner (model CWH-A1815-WS, Corona, Niigata, Japan), humidifiers (Dyson, Malmesbury, United Kingdom), dehumidifier (MJ180-LX, Mitsubishi Electric, Tokyo, Japan), photocatalyst deodorizer (QOL-MINI-V3, Renatech, Kanagawa, Japan), and LED lamp were placed inside the cargo area without changing the original configuration of the vehicle. The cages were illuminated indirectly by lamps positioned where rabbits could not see them. A maximum of 8 cages could be placed inside the cargo area. Each rabbit's urine and feces were collected by using pet pads (Unicharm, Ehime, Japan) in catch pans. The pet pads were changed and the catch pans cleaned daily to prevent the generation of ammonia. Personnel working in the van wore a gown, a hair bonnet, gloves, and a mask, and the designated animal room was secure, with only authorized personnel permitted to enter.

Environment. The temperature in the cargo van was maintained between 17 and 23 °C, and the relative humidity was between 30% and 70%. Illumination was controlled by an analog timer to set a 12:12-h light:dark cycle. The air in the cargo van was sanitized and deodorized by using UV-irradiated nano-oxide titanium in a deodorizer before the air conditioner exhausted air outside of the van. The interior environment (temperature, humidity, and CO_2 concentration) was monitored and recorded by using a data logger (model TR-76Ui-H, T and D Corporation, Nagano, Japan). The environmental noise level was monitored by using a sound-level meter (model NL-14, RION, Tokyo, Japan). Airspeed and illumination at cage level were measured by using an air-velocity meter (model 6004, KanoMax Japan, Osaka, Japan) and an illuminance meter (model T-1M, Konica Minolta, Tokyo, Japan), respectively. The level of ammonia in the interior air was measured by using an ammonia detector tube (no. 3L, Gastec, Kanagawa, Japan).

Microbial air monitoring. After the interior of the cargo van was cleaned by using 70% ethanol, settle plates (culture dishes [diameter, 9 m]) containing trypticase soy agar with 5% sheep blood (catalog no. 251239, Nippon Becton Dickinson, Tokyo, Japan) and plates containing Sabouraud dextrose agar with chloramphenicol and gentamicin (catalog no. 251359, Nippon Becton Dickinson) were placed on the floor at the front, center, and rear (1 plate of each type per location) of the cargo area without rabbits present and exposed to air for 30 min through the passive sampling method.[16] The trypticase soy agar plates, for monitoring general bacteria, were incubated for 48 h at 37 °C, and the Sabouraud dextrose agar plates, for monitoring fungi, were incubated for 5 d at 24 °C. The total

	Japanese standards guideline[5]	ILAR guideline (8th edition)[7]	This study (mean [range])
Temperature (°C)	18–24	16–22	20.5 (17.8–22.6)
Relative humidity (%)	40–60 (must be >30 and <70)	30–70	49.4 (36.2–63.2)
CO_2 (ppm)			548 (442–802)
Air velocity (m/s)	0.2	Direct exposure of animals to air moving at high velocity should be avoided	0.1–0.2
Microbial contamination of air	≤ 3 cfu (barrier facilities) ≤ 30 cfu (nonbarrier facilities)	not specified	bacteria, 0.15 cfu fungi, 4.65 cfu
Ammonia (ppm)	≤20	not specified	≤0.5
Illumination (lux)	150–300 (40 to 85 cm above floor)	130–325 (cage level)	64–116 (cage level)
Noise (dB)	≤ 60	not specified	48.5
Cage size (per rabbit; weight, <2 kg)	floor area, 0.14 m²; height, 40.5 cm	floor area, 0.14 m²; height, 40.5 cm	floor area, 0.17 m²; height, 40 cm

Figure 2. Criteria for environmental factors in laboratory rabbit facilities. Temperature, relative humidity, and CO_2 concentration were monitored every 5 min. Values of air velocity and illumination are expressed as the range of the mean levels at each cage. Ammonia and noise levels are mean values for each experiment. Compiled from data in references 5 and 7.

number of colonies was divided by 3, resulting in the number of colonies per dish.

Statistical analysis. Commercial software (Excel 2010, Microsoft, Redmond, WA) was used to perform the data analysis. Student *t* tests were used to compare rabbit body weights before shipping with those after arrival (day 0, 1, or 2).

Results

Rabbit housing system using a cargo van. We used either a refrigerated van (Figure 1 A) or a standard van (Figure 1 B), the glass of which we covered with sheets of black plastic (Figure 1 B). The interior temperature was controlled by using a window air conditioner (Figure 1 C), and the interior humidity was controlled by using a dehumidifier or humidifier, depending on environmental conditions. Illumination was controlled by an LED light and analog timer, and odors were minimized by using a photocatalyst deodorizer (Figure 1 C). Metal storage racks were used as fences to prevent rabbits from escaping (Figure 1 C). Because the vans had to be returned to the rental agency after each experiment, vehicles were not permanently customized or altered.

Cleaning the interior of the cargo van. According to Japanese standards guidelines, 90-mm culture dishes exposed to air for 30 min should have fewer than 30 cfu of microbial contaminants for nonbarrier facilities without animals (Figure 2). We used 70% ethanol to clean the van interior and then exposed settle plates for 30 min. The plates yielded an average of 0.2 cfu for bacteria and 4.7 cfu for fungi, indicating that the interior of the van was as clean as a nonbarrier animal facility (Figure 2).

Stability of the interior environment. To evaluate whether the cargo van system provided sufficiently stable environmental conditions, we individually housed 6 male Dutch rabbits in the cargo van for 6 d. The average temperature was 20.0 °C (range, 18.6 to 21.7 °C), and the average relative humidity was 54.8%, ranging between 41.6% and 66.1% (Figure 3). In addition, the ammonia concentration was typically below the level of detection (0.5 ppm), and we smelled virtually no animal odor (Table 1).

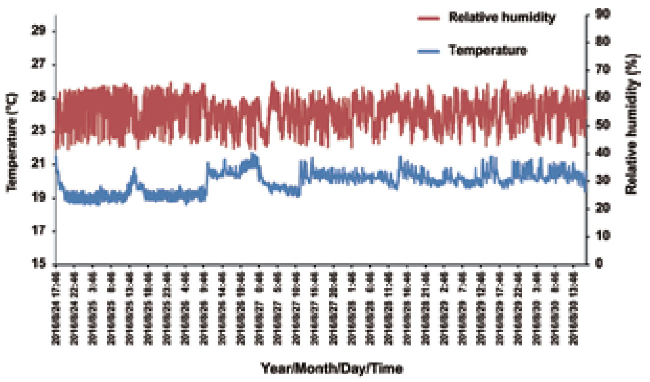

Figure 3. Temperature and relative humidity in the cargo van over 6 d. Six male Dutch rabbits were housed individually, and interior temperature (blue line) and relative humidity (red line) were monitored.

From November 2016 through January 2018, we performed 6 experiments to evaluate acute ocular damage after using the gyrotron, with 4 to 8 male Dutch rabbits housed in the cargo van during each experiment. Environmental data (Table 1) indicated that the environment in the cargo van for rabbit housing was sufficiently stable and met both Japanese governmental and ILAR guidelines (Figure 2).

Period of rabbit adaptation. Rabbits are prone to transport stress, rarely eating or drinking during transportation.[18] Typically, our rabbits left the animal vendor around 0500, arriving at the gyrotron facility between 1500 and 1600 on the same day. These rabbits were placed singly in divided boxes without food or water for shipment by air-conditioned truck and air cargo. Upon arrival, their health condition was examined briefly, and their weight was recorded. All rabbits lost body weight during transfer (day 0) without any signs of health problems and had regained the lost weight within 1 d after arrival, with weight becoming stabilized by 2 d after arrival (Figure 4). We further evaluated weight changes in 52 rabbits during the adaptation period. During shipping, these rabbits lost 6.4% ± 2.2% (mean ± 1 SD; $P < 0.001$) of their body weight before transfer (day 0) in the absence of any clinical signs (Figure 5). Relative to their

Table 1. Environmental parameters in the cargo van rabbit housing system

		August 2016	November 2016	December 2016	January 2017	November 2017	December 2017	January 2018	Mean
Temperature (°C)	Mean	20.0	20.4	20.3	21.6	20.2	21.1	19.7	20.5
	Maximum	21.7	22.6	21.5	24.8	21.0	23.4	23.4	22.6
	Minimum	18.6	17.1	18.8	18.8	18.8	16.8	16.0	17.8
Relative humidity (%)	Mean	54.8	48.2	44.7	42.9	55.0	50.0	50.1	49.4
	Maximum	66.1	70.8	54.6	51.3	72.6	62.3	64.5	63.2
	Minimum	41.6	33.2	33.1	27.3	40.6	39.1	38.6	36.2
CO_2 (ppm)	Mean	637	525	516	500	589	516	553	548
	Maximum	884	818	785	733	904	673	818	802
	Minimum	466	458	424	439	406	442	456	442
Ammonia (ppm)		BLD	BLD	BLD	BLD	BLD	nt	nt	BLD
Air velocity (m/s)	Maximum	<0.01	nt	nt	0.23	0.1	nt	nt	0.17
	Minimum	<0.01	nt	nt	0.1	0	nt	nt	0.1
Illumination (lux)	Maximum	320	nt	103	80.6	74.5	71.6	46.7	116
	Minimum	280	nt	25	41.3	15.2	14.1	10.8	64
Noise (dB)		45.1	nt	49.9	50.4	nt	nt	nt	48.5

BLD, below the limit of detection (0.5 ppm); nt, not tested
Values represent the mean of the acquired data using a data-logger, except for ammonia concentration (single value), air velocity (maximum and minimum), and illumination (maximum and minimum) and noise (single value)

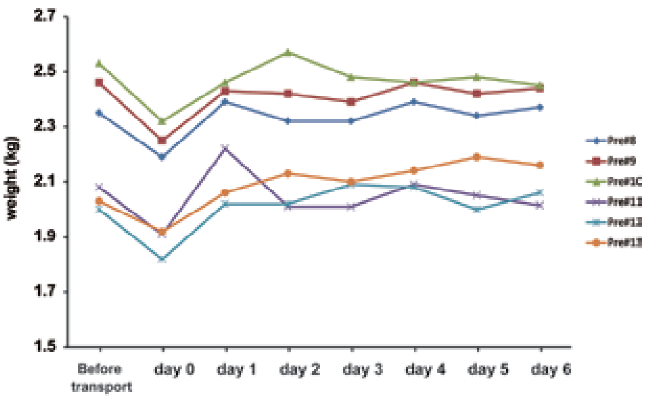

Figure 4. Body weights of rabbits housed in the cargo van. Six rabbits (Pre#8, Pre#9, Pre#10, Pre#11, Pre#12, and Pre#13) from Figure 2 arrived on day 0 and were weighed daily until day 6. Weights on the day of shipment (before transport) were provided by the vendor.

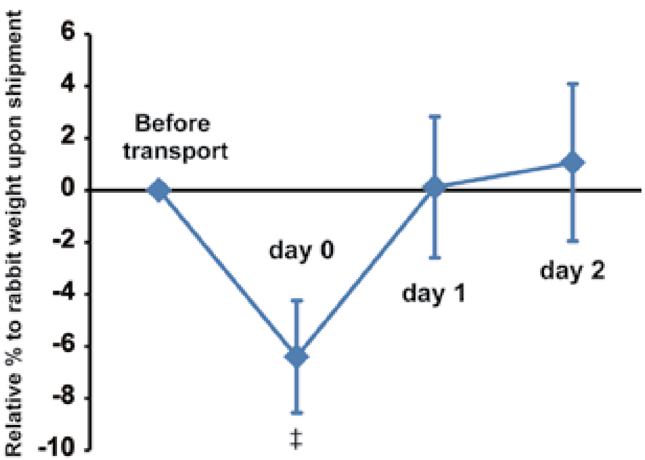

Figure 5. Adaptation of rabbits in the cargo van. Rabbits (n = 52) were weighed on arrival (day 0) and on days 1 and 2 after arrival. Values are shown as the change in weight (mean ± 1 SD [error bars]) relative to the weight on the day of shipping (before transport). ‡, Value is significantly ($P < 0.001$) different from that before shipping.

initial body weight, their weight on day 1 was 100.1% ± 2.7% and on day 2 was 101.1% ± 3.0%, respectively (Figure 5), with all 52 rabbits regaining the lost weight within 2 d after arrival; therefore, all tests evaluating acute ocular damage in rabbits were performed after a 2-d adaptation period. Together, our results indicate that the cargo van housing system provided a stable and reliable environment for laboratory rabbits.

Discussion

We report that the conventional housing system we devised by using a cargo van can be a practical choice for short-term rabbit housing. The interior environment was controlled by using additional equipment and without changing the original configuration of the vehicle. Microbial contamination, odor, and noise levels and the narrow temperature and relative humidity range all conformed to Japanese standards for nonbarrier animal facilities. Furthermore, all rabbits regained any weight lost during shipment within 2 d after arrival.

The Architectural Institute of Japan provides guidelines regarding laboratory animal facilities and equipment (Figure 2).[5] Although not legally binding, these guidelines serve as recommendations for appropriate facilities and are based on the NIH Design Policy and the ILAR guidelines for the care and use of laboratory animals.[5,7] The Animal Research Committee of the University of Fukui required that: the cargo van would have a system to control both temperature and humidity; the interior of the van could be cleaned and sanitized both before and after use; measures would be taken to reduce odor and to prevent animals from escaping; persons working in the van would wear designated personal protective equipment; and the designated room where the van was located would be secure, with only authorized personnel allowed to enter. Our rabbit housing system using a cargo van was approved by the Animal Research Committee of the University of Fukui.

We monitored the interior environment of the van during our ocular research for more than 1 y and found that the temperature, relative humidity, and CO_2 concentration could be regulated by using house appliances. We minimized animal odor by using pet pads and a photocatalyst deodorizer. Although the noise level was close to the recommended maximum, perhaps due to the sound of the air conditioner, the mean noise level was within Japanese guidelines. Commercially available music for pet rabbits played on a CD player reportedly lowered the stress levels in a colony of individually housed male rabbits.[17] Implementing such musical enrichment might be considered for future experiments. Both the Japanese and ILAR guidelines provide similar general recommendations regarding appropriate illumination for rodents, rabbits, cats, NHP, and dogs, but no specific requirements for rabbits are described.[5,7,13,15] Because our research involves evaluating ocular damage due to MMW, we set the maximal level of illumination to be lower than the guideline recommendations. Excessive illumination might cause adverse effects to injured rabbit eyes; we need to assess this possibility further.

Social housing of laboratory rabbits is a topic that has frequently been discussed.[2,12,24] Because laboratory rabbits (*Oryctolagus cuniculus*) are a naturally gregarious species, social housing of rabbits has been introduced to facilities.[12,23] Successful social housing reportedly reduces the incidence of abnormal behaviors in rabbits, and no available evidence suggests that immune responses, stress levels, or growth rates are altered by social housing.[12,22] However, several factors (for example, animal sex and strain, experimental design) need to be considered when using social housing for rabbits. Male rabbits are usually more aggressive than females, and Dutch rabbits are known to be more aggressive than New Zealand white rabbits.[2,23] Neither physical damage to eyes due aggression nor wound-licking behavior could be prevented if rabbits are housed together. Because our research focuses on ocular damage by MMW, we chose to house our rabbits individually to avoid such eye injuries.

The temporary housing system was created to keep rabbits on-site during animal research. Rabbits are prone to transport stress, rarely eating or drinking during shipment.[18] All 52 rabbits demonstrated significant weight loss upon arrival but gained back the lost weight within 48 h, consistent with the recommended adaptation period for laboratory animals after transport.[18] Laboratory rabbits have been reported to develop various symptoms immediately after shipping, including anorexia, hyperglycemia, neutrophilia, lymphopenia, and elevated plasma cortisol concentrations.[20] Rectal temperature, glucose and lactate concentrations, and PCV are also found to increase immediately after transport.[3] In both reports, these physiologic changes returned to normal within 48 h.[3,20] Because our project was designed to test the effects of MMW on rabbits, stressful procedures such as blood collection were avoided whenever possible. Examinations of the rabbits prior exposure to MMW confirmed that the frequency of animals with abnormal eye conditions was below reasonable levels (data not shown).

In conclusion, we have demonstrated that our cargo van rabbit housing system was as environmentally stable as a conventional animal facility, at least for short-term housing. Although a stable environment is a prerequisite for research, animal housing facilities for rabbits are not always available, especially outside biology and medical science-related facilities. Animal research is important to evaluate the risks of technology on human health. Although not problematic when laboratory equipment and devices are movable and small enough to be placed in animal facilities, some equipment, like the gyrotron, are too large to relocate, and study animals must be transferred to the facility where the equipment is located. We found that our cargo van housing system provides practical and environmentally stable short-term rabbit housing when permanent facilities are unavailable.

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