**Effect of Caging System on a MHV-model of Experimental Infection**

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## ABSTRACT

**OBJECTIVES**
The primary objective of this study was to determine if the caging system influences the symptomatology of experimentally infected mice. The secondary objective was to evaluate the efficacy of these caging systems to contain the infection.

**METHOD**
Three caging systems were compared: Static Microisolators (SMI), Ventilated Microisolators (VMI) and *Microenvironmental comfort Isolation Containment Enrichment* cages (M.I.C.E.®), a low-velocity ventilated microisolator. In each system, two cages of 5 Balb/c mice/cage were infected intranasaly with $10^6$ pfu of MHV-A59. Daily for 28 days, animals were observed and clinical conditions were scored using a Visual Analog Scale (VAS), with 0 being normal and 10 being the worst condition (moribund/dead). The daily VAS scores were compared between the three groups. Death was not an intended endpoint.

**RESULTS**
Although the kinetic of the infection was comparable among the three groups, for the acute phase of the infection, the degree of discomfort was equivalent between the SMI and the VMI, but the VAS scores were lower for the M.I.C.E.®. Six animals abruptly reached a score of 10: 3, 2, and 1 for SMI, IVC and M.I.C.E respectively. The occurrences of scores >4 (and relative percentages) were 95 (45%), 88 (42%), and 27 (13%) for SMI, IVC and M.I.C.E respectively.

**CONCLUSIONS**
The M.I.C.E.® cages did not exacerbate the symptomatology compared to SMI and IVC. We concluded that clinical signs and survival are affected by the caging system. The caging system should be considered a significant factor contributing to the severity of an experimental infection. For each caging system, cross-contamination of the sentinels was prevented for the duration of the study as serological tests performed on the sentinel Balb/c nu/+ mice were negative, as well as PCR and histopathology.

## INTRODUCTION

Many studies have looked at the effect of caging systems on microenvironmental conditions. But the "cage effect" has not been assessed in many experimental applications. We have evaluated the effect of three caging systems on the clinical response of mice experimentally infected with mouse hepatitis virus (MHV). In parallel, we have studied the relative capabilities of three caging systems to contain the infectious agent.

## MATERIAL AND METHOD

### CAGING SYSTEMS

Three types of caging systems were assessed: static microisolators (group 1-SMI, ACE), high flow/high velocity positive-pressure ventilated microisolators (group 2-VMI, ACE), and low flow/low velocity negative-pressure microisolators (group 3-M.I.C.E., Animal Care Systems) as illustrated in Figure A. Cages were changed one at time under a biosafety cabinet. Non infected cages were changed first (1 cage/group). Animals were transferred using alternatively two pairs of forceps. Forceps were sanitized between each cage using a disinfectant (Spore-clenz™). Cages were changed weekly (group 1), to biweekly (group 2 and 3), according to common husbandry practices. As for environmental enrichment, each cage received a PVC tube and a Neslet™.

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**Figure A: Room Setup.**

**Figure 1 Group 1: Static Microisolator (SMI) - Front View.**

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**Figure 2 Group 1: Static MicroIsolator (SMI) - Lateral View**

**Figure 3 Group 2: Ventilated MicroIsolator (VMI) - Front View**

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**Figure 4 Group 2: Ventilated MicroIsolator (VMI) - Lateral View**

**Figure 5 Group 3: *Microenvironmental comfort Isolation Containment Enrichment* (M.I.C.E.®)- Front View**

**Figure 6 Group 3: Microenvironmental comfort Isolation Containment Enrichment (M.I.C.E.®)- Lateral View**

**Table 1**

| Group | System | # of cages | Frequency of bedding change |
|---|---|---|---|
| 1 | Static Microisolator (SMI) | 3 | Weekly |
| 2 | Ventilated Microisolator (VMI) | 3 | Every 2 weeks |
| 3 | MICE | 3 | Every 2 weeks |

**ANIMALS**

This study was conducted in an AAALAC accredited facility, and the protocol was approved by the Institutional Animal Care and Use Committee. Animals were housed in autoclaved cages with autoclaved aspen wood shavings as bedding. Irradiated diet (LM485, Harlan Teklad) and autoclaved acidified water were provided *ad lib*. Three female Balb/c nu/+ mice (6 weeks old, CRL) and 2 female Balb/c nu/nu mice (6 weeks old, CRL) were used as sentinels for each group. Ten female Balb/c mice (6 weeks of age) per group were infected intranasaly under isoflurane anesthesia with $10^6$ PFU of MHV-A59 (ATCC) in 30 L.

**SENTINEL CAGE:**

For each group, one cage of sentinel mice was placed beside and below the two infected cages in an inversed "L" disposition (

Figure 7 Disposition of cages).

**Figure 7 Disposition of cages**

| | |
|---|---|
| | Infected |
| Infected | Sentinel |

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**EXPERIMENTAL INFECTION:**
Ten mice per group (5 mice per cage) were infected intranasaly under isoflurane anesthesia with $10^6$ PFU of MHV-A59 (ATCC) in 30 μL.

**VIRUS**
Mouse hepatitis, strain A59 was propagated in CCL 9.1 from the American type culture collection (ATCC). Viral titers were determined by limiting dilution in the same cell line according to the formula of Reed and Muench. The final titers were reported as the number of tissue culture infective doses able to infect 50% of cultures per ml of culture fluid ($TCID_{50}$/ml).

**PRE-INFECTION SCREENING**
To confirm the mice were not infected with MHV prior to the experiment, fecal samples for PCR were collected from sentinel cages a few days prior to experimental infection.

**POST-INFECTION SCREENING**

**Sentinel cages**:
For each group, at the end of the study, serology (ELISA) was performed on serum samples from the three sentinel Balb/c nu/+ mice, fecal PCR for MHV was conducted. Histopathology was done on the two sentinel Balb/c nu/nu mice for each group.

**Infected cages:**
For each group, serology (ELISA) was performed on serum samples from 2 mice/cage.

**OBSERVATION AND SCORING**
Animals were observed twice a day during the more severe phase of the infection (day 5 to day 9), and otherwise, once a day. The general condition of infected animals was scored using a Visual Analog Scale, ranging from 0 (no clinical signs) to 10 (worst clinical condition: moribund/dead).

## RESULTS

**PRE-INFECTION SCREENING**
Fecal samples for PCR collected from sentinel cages the day before the experimental infection were negative.

**POST-INFECTION SCREENING**

**Sentinel cages:**
For each group, cross-contamination of the sentinels was prevented for the duration of the study as serological tests performed on the sentinel Balb/c nu/+ mice were negative, as well as PCR and histopathology.

**Infected cages:**
For the three groups, infected animals were serologically positive for MHV at the end of the study.

**OBSERVATION AND SCORING**
Daily scores were summed up and compared for each group. Although the kinetic of discomfort was comparable for the three groups with a peak between Day 10 and Day 20, scores were higher for group 1 (SMI) and group 2 (VMI) than for group 3 (M.I.C.E.). Scores were comparable between groups 1 (SMI) and 2 (VMI) (Figure 10).

**Figure 8 Examples of clinical signs**

**Figure 9 Examples of clinical signs**

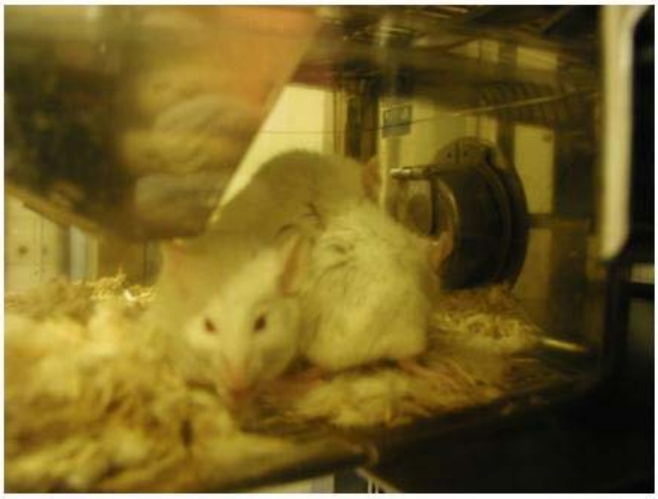

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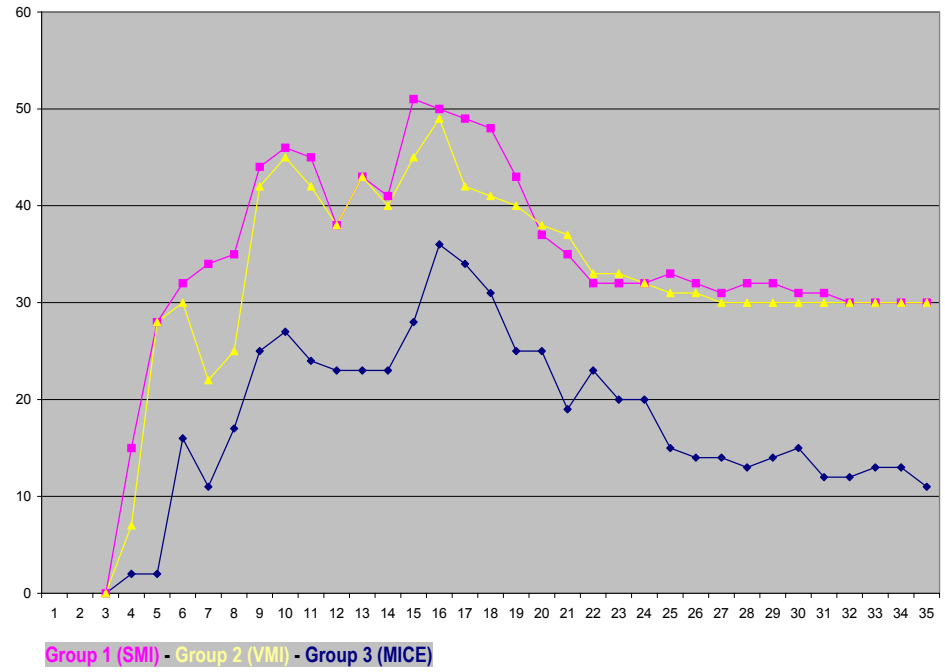
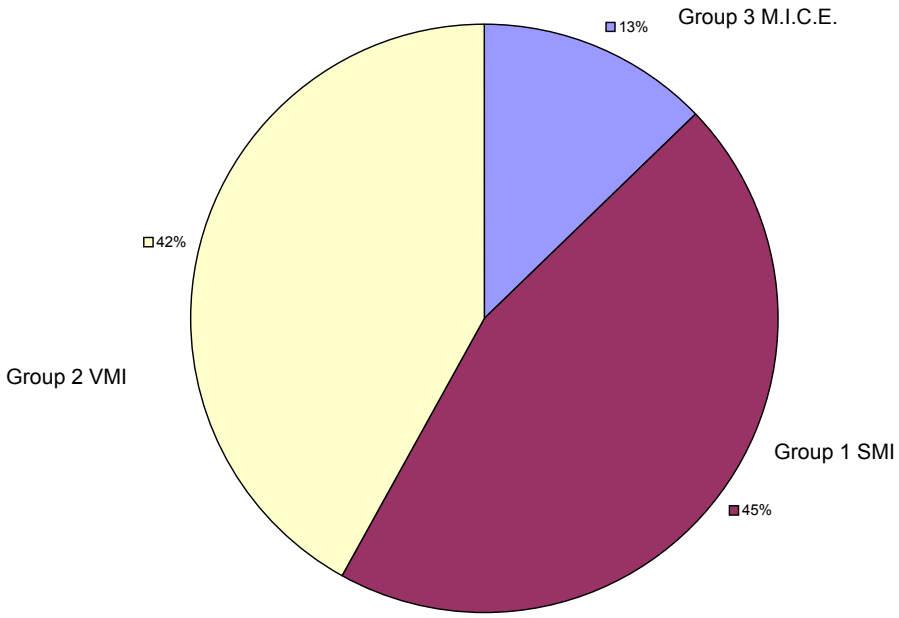
**Figure 10 Cumulative scores vs days**




The number of times a score equal or higher than 4 was observed was compared for the three groups. The occurrences (and relative percentages) were 95 (45%), 88 (42%), and 27 (13%) for group 1, 2 and 3, respectively. Occurrences were comparable for group 1 and 2, but greatly lower for group 3 (Figure 11).

**Figure 11**

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## DISCUSSION

This study suggests that the caging system can impact on the clinical manifestation of an experimental infection. The three systems differed in many aspects: their ventilation rate and air velocity, their floor surface area, volume, and the intra-cage structure. It can be hypothesized that the cage environment may contribute positively or negatively on the stress level of the animals. A more stressful environment could make the animals more susceptible to the infection, resulting in an increased severity of the clinical signs. A high air velocity is considered to cause discomfort but also can contribute to hypothermia in diseased mice. Poor ventilation can cause accumulation of air contaminants, such as ammonia and dust particles in the microenvironment. These contaminants have been shown to negatively affect the immune response. The density affected by the floor surface area is a factor of social stress. The cage infra-structure dividing the space into separate zone and the complexity of the environment allowing expression of normal behaviors can prevent or reduce social stress, especially in a situation where the stability of the social hierarchy is affected by morbidity and mortality.

Further studies are required to confirm this trend and to assess the effects of these specific parameters on the clinical response. Although it may appear beneficial not to exacerbate the clinical signs on animal welfare standpoint, in studies where the outcomes is directly based on morbidity and mortality, this could result in a reduction of the measurable effect and a consequent increase in the number of animals required.

**Table 2 Caging systems specifications**

| | SMI | VMI | M.I.C.E.® |
|---|---|---|---|
| Air changes/hour | 0.82 | 60 | 20 |
| Air velocity (cm/s) | N/A | 75 to 125 | 2 |
| Floor surface area (cm2) | 483.87 | 483.87 | 545 |
| Volume (ml) | 8,000 | 8,000 | 9,500 |

## CONCLUSION

The M.I.C.E.® cages did not exacerbate the symptomatology compared to SMI and IVC. We conclude that clinical signs and survival can be affected by the caging system. The caging system should be considered a significant factor contributing to the severity of an experimental infection. Caution should be applied when extrapolating data from different caging systems.

For each caging system, cross-contamination of the sentinels was prevented for the duration of the study as serological tests performed on the sentinel Balb/c nu/+ mice were negative, as well as PCR and histopathology.

The performance of caging systems should not only be evaluated for their capacity in preventing cross-contamination and to remove air contaminants but also by assessing their global impact on the welfare of the animals and their response to experimental manipulations.

## REFERENCES

Barnard, C.J., J.M. Behnke, J. Sewell. 1996. Environmental enrichment, immunocompetence, and resistance to Babesia microti in male mice. Physiol. Behav. 60:12223-1231.

Barnard, C.J., J.M. Behnke, J. Sewell. 1994. Social behaviour and susceptibility to infection in house mice (mus musculus): effect of group size, aggressive behaviour and status-related hormonal responses prior to infection on resistance to babesia microti. Paraistology 5:487-496.

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Barnard, C.J., J.M. Behnke, J. Sewell. 1993. Social behaviour, stress and susceptibility to infection in house mice (mus musculus): effects of duration of grouping and aggressive behavior prior to infection on susceptibility to babesia microti. Parasitology 2:183-192.

Baumans, V. F. Schlingmann, M. Vonck, H.A. Van Lith. 2002. Individually ventilated cages: beneficial for mice and men? Contemp. Top. Lab. Anim. Sci. 41: 13-19

Hoglund, A.U., A. Renstrom. 2000. Evaluation of individually ventilated cage systems for laboratory rodents: cage environment and animal health aspects. Lab. Anim. 35: 51-57.

Lipman, N.S. 1999. Isolator rodent caging systems (state of the art): a critical view. Contemp. Top. Lab. Anim. Sci. 38: 9-17.

Memarzadeh, F., P.C. Harrison, G.L. Riskowski, T. Henze. 2004. Comparison of environment and mice in static and mechanically ventilated isolator cages with different air velocities and ventilation designs. Contemp. Top. Lab. Anim. Sci. 43: 14-20.

Lipman R.D., E.T. Gaillard, D.E. Harrison, R.T. Bronson. 1993. Husbandry factors and the prevalence of age-related amyloidosis in mice. Lab Anim Sci 43:439-444.

Perkins, S.E., N.S. Lipman. 1996. Evaluation of microenvironmental conditions and noise generation in three individually ventilated rodent caging systems and static isolator cages. Contemp Top Lab Anim 35:

Quan, N., R. Avitsur, J.L. Stark, L. He, M. Shah, M. Caligiuri, D.A. Padgett, P.T. Marucha, J.F. Sheridan. 2001. Social stress increases the susceptibility to endotoxic shock. J. Neuroimmuno. 115:36-45.

Reeb, C.K., R.B. Jones, D.W. Bearg, H. bBedigian, D.D. Myers, B. Paigen. 1998. Microenvironment in ventilated animal cages with differing ventilation rates, mice populations, and frequency of bedding changes. Contemp. Top. Lab. Anim. Sci. 37:2.

Rivard, G.F., D.E. Neff, J.F. Cullen, S.W.J. Welch. 2000. A novel vented microisolation container for caging animals: microenvironmental comfort in a closed system filter cage. Contemp. Top. Lab. Anim. Sci. 39: 22-27.

Tu, H., L.J. Diberadinis, N.S. Lipman. 1997. Determination of air distribution, exchange, velocity, and leakage in three individually ventilated rodent caging systems. Contemp. Top. Lab. Anim. Sci. 36: 69-73.

Tsai, P.-P., U. Pachowsky, H.D. Stelzer, H. Hackbarth. 2002. Impact of environmental enrichment in mice. 1: Effect of housing conditions on body weight, organ weights and haematology in different strains. Lab Animals 36:411-419.

Van Loo, P.L.P., J.A. Mol, J.M. Koolhaas, B.F.M. Van Zutphen, V. Baumans. 2001. Modulation of aggression in male mice: influence of group size and cage size. Phys. Behav. 72:675-683.