

## 2003 ACLAM FOUNDATION GRANT REPORTS

### Evaluating the Accuracy and Sensitivity of Bedding Transfer

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Serological monitoring of sentinel mice after transfer of soiled bedding is a common method to detect infections in mice. Because protocols for bedding transfer vary, the sensitivity of this method has not been adequately documented. This study examined the efficacy of bedding transfer at various times during acute infections with mouse parvovirus (MPV) or mouse hepatitis virus (MHV). Aim one of this study demonstrated that 150 ID<sub>50</sub> of MPV or 3000 ID<sub>50</sub> of MHV added to autoclaved corncob bedding elicited seroconversion in contact-exposed mice for at least seven days in the case of MPV, but no longer than four hours for MHV. These findings demonstrated the relative stability of these two viruses in the environment. Aim two examined the ability of bedding from cages containing virus-infected mice to transmit infection at different time points using three different doses of bedding. Six-week old, female, Swiss Webster (SW) index mice were inoculated oronasally with 30 ID<sub>50</sub> of MPV or 300 ID<sub>50</sub> of MHV. Three, 7, and 14 days post-inoculation (dpi), 25, 50 or 100 ml of bedding was transferred to sentinel cages of SW mice. Sentinels were tested for seroconversion 14 days after bedding transfer by indirect immunofluorescence assay. Viral shedding by index mice also was determined by fecal PCR at each time point. This experiment was performed in both static and individually ventilated cages.

MPV shedding by index mice was detected at dpi 3, 7, and 14. Under static housing conditions, seroconversion was elicited in half of the sentinel mice (3/6) exposed to 100ml of bedding at dpi 3, but none of the sentinels exposed to 25ml or 50ml of bedding. On dpi 7 two thirds (4/6) of the mice exposed to 25ml, five sixths (5/6) of the mice exposed to 50ml, and two thirds (4/6) mice exposed to 100ml of dirty bedding seroconverted. At dpi 14 one sixth (1/6) mice exposed to 25ml, one half (3/6) of the mice exposed to 50ml, and one sixth (1/6) of the mice exposed to 100ml of dirty bedding seroconverted. In individually ventilated cages 5/8 mice exposed to 25ml, 8/8 exposed to 50ml, and 7/8 exposed to 100ml of dirty bedding seroconverted. At dpi 7 all sentinel mice seroconverted regardless of bedding dose. At dpi 14, half of the mice exposed to 25 or 50ml of dirty bedding, and six of seven mice exposed to 100ml seroconverted.

Shedding of MHV from index mice was detected at dpi 3 and 7, and 14. Under static housing conditions, seroconversion was elicited in all (6/6) sentinel mice on day 3 dpi regardless of the volume of dirty bedding transferred. On dpi 7 one third (2/6) of the sentinel mice seroconverted at each bedding dose. At dpi 14 none (0/6) of the sentinels that received 25ml of dirty bedding seroconverted, while one third (2/6) of the mice receiving either 50ml or 100ml of dirty bedding seroconverted. In individually ventilated cages all sentinel mice seroconverted at each time point regardless of bedding volume transferred.

These findings suggest that under static housing conditions transfer of soiled bedding detects infection reliably only during peak shedding of MPV (dpi 7) and MHV (dpi 3).

Even during peak shedding, seroconversion to MPV failed to reach 100% regardless of the volume of dirty bedding transferred. When the mice were housed in individually ventilated cages, a higher rate of seroconversion was seen in sentinels at all time points and bedding volumes. Even under these conditions, detection of MPV was most reliable at dpi 7, at which time seroconversion in sentinels reached 100% regardless of bedding volume transferred. Seroconversion to MHV reached 100% at all timepoints regardless of bedding volume.

## **Effects of Housing Density on FVB/J and NOD/LtJ Mice**

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Many standards in the *Guide for the Care and Use of Laboratory Animals* (the *Guide*) are based on best professional judgment, but current efforts are directed toward replacing these with data-driven standards. We demonstrated earlier that young adult C57BL/6J male and female mice could be housed with half the floor space recommended in the *Guide* without discernable negative effects (Smith AL, Mabus SL, Stockwell JD and Muir C. 2004. Effects of housing density and cage floor space on C57BL/6J mice. *Comp Med*, in press). This report extends that work by examining optimal housing densities for young adult male and female BALB/cJ (BALB), NOD/LtJ (NOD) and FVB/NJ (FVB) mice. The eight-week studies were initiated with three-week-old BALB and NOD mice and 3-5 week-old FVB mice housed in three commercially available cages having varying amounts of floor space. By adjusting the number of mice per cage, we housed them with the floor space recommended in the *Guide* (approximately 12 in<sup>2</sup> per mouse) and three smaller areas (down to 5.6 in<sup>2</sup> per mouse) equivalent across cage types. Injuries and aggressive behavior were assessed daily, and food and water consumption and body weights were determined weekly. The cage micro-environment (in-cage ammonia and carbon dioxide concentrations and temperature and relative humidity) were measured weekly just prior to provision of clean bedding. Urine samples were collected at study initiation and bi-weekly thereafter for determining testosterone levels. Early onset aggression was noted only among FVB male mice housed at all densities in cages having 51.7 in<sup>2</sup> (duplex cages) or 112.9 in<sup>2</sup> (weaning cages) of space. The aggression was severe enough to be considered an animal welfare issue and these mice had to be euthanized and, therefore, could not be included in the data analysis. FVB male mice housed in shoebox cages (67.6 in<sup>2</sup>) did not exhibit aggression until the fifth week of the experiment. Some micro-environmental parameters varied with housing density. In some cases, in-cage temperatures exceeded those recommended in the *Guide* but the mice suffered no apparent ill effects. Testosterone output varied by sex but was housing density-independent and cage type-independent except for male BALB mice housed in shoebox cages. In that case, testosterone concentrations steadily decreased with increasing density. Surprisingly, testosterone levels for male and female FVB mice housed in shoebox cages were similar despite the fighting that commenced among males at week 5. Based on these results, we conclude that young male and female BALB and NOD mice and female FVB mice can be housed with half the floor space specified in the *Guide*. Male FVB mice were extremely aggressive. This may have been due to the age span of mice in the study (adequate numbers of three-week-old mice were unavailable from the vendor), although this factor did not impact negatively on the female FVB mice.

## Controlled Release of Enrofloxacin in Mice

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Laboratory animals such as rodents and rabbits are susceptible to several different types of bacterial infections and often require antibiotic treatment.

Enrofloxacin is a broad-spectrum antibiotic that is safe and effective for treating bacterial infections in rodents and rabbits. Although the benefit of enrofloxacin for the treatment of infections is well demonstrated in these animals, the current method of administration of enrofloxacin is inconvenient and labor intensive due to the stress of repeated handling for dosing (oral or parenteral). For example, currently recommended dose for mice is 10 mg/kg bid for up to 14 days. Therefore, a controlled release biodegradable long-acting formulation of the antibiotic would be a logically alternative to the presently available oral as well as parenteral dosage forms of the antibiotic. A biodegradable gel was proposed for the development of a long acting enrofloxacin formulation.

To rationally design the proposed gel formulations, the physicochemical properties of the drug (enrofloxacin) need to be understood. Accordingly, preformulation studies such as solubility of enrofloxacin in phosphate buffer saline and various plasticizers were performed first. The results of the stability study of enrofloxacin showed that it was stable in PBS (pH 7.4) at 37°C for at least 3 days. Therefore, PBS was used as dissolution medium to perform the *in vitro* release studies.

The next step was to evaluate the stability of enrofloxacin in the stabilizers. The solubility of enrofloxacin in all the solvents tested was higher at 37°C than that at room temperature (25°C). The drug was most soluble in TEC and least soluble in PBS. The rank of solubilities of enrofloxacin in plasticizers and PBS followed the following descending order: TEC>>PEG 400>ATEC>PBS. The results of compatibility study for the drug in PEG 400, TEC and ATEC showed that enrofloxacin was stable in all 3 plasticizers at three tested temperatures for more than 4 weeks. More than 90% of the drug was recovered from PEG 400, TEC and ATEC samples after 4 weeks at each tested temperature. Therefore, all 3 plasticizers could be used to formulate the biodegradable long acting enrofloxacin gel formulations for further studies.

Prescott *et al* have reported that the highest MIC<sub>100</sub> of enrofloxacin for 13 different veterinary bacterial pathogens was less than or equal to 1.0 µg/mL, hence, 1.0 µg/mL was set as the desired *in vivo* plasma concentration that needed to be maintained in this study. Based on the results of preliminary studies, the dose of the enrofloxacin injected in mice plays a key role in maintaining plasma concentration of enrofloxacin at or above MIC for prolonged period of time. Therefore, the effect of dose injected on the plasma concentration profile of enrofloxacin was investigated. The ratio of TEC and PEG was chosen to be 1:4 for optimizing gel formulations for the rest *in vivo* studies, since toxicity (LD<sub>50</sub>) of TEC in mice is higher than that of PEG. The concentration of PLGA was chosen to be 5% in the gel formulations; the loading of enrofloxacin was chosen to be 40% in the gel formulations. The dose of enrofloxacin could be adjusted by varying the volume or weight of the gel formulation injected.

The initial plasma concentrations of enrofloxacin in mice from different doses was as follows: 40 mg > 30 mg > 20 mg. The plasma concentration of enrofloxacin in mice could be maintained at or above 1 µg/mL for more than 5 days after a single subcutaneous injection of gel containing 40 mg dose. The plasma concentration of enrofloxacin in mice could be maintained at or above 1 µg/mL for only 2 days after a single subcutaneous injection of gel containing 20 mg dose. The plasma concentration of enrofloxacin in mice could be maintained at or above 1 µg/mL for approximately 4 days after a single subcutaneous injection of gel containing 30 mg dose. Therefore, a gel formulation with 30 mg dose could be the formulation of choice for maintaining plasma concentration at or above 1 µg/mL for 3 to 5 days as indicated by the objective of this project.

The *in vivo* release profile of enrofloxacin from the gel formulations was estimated based on the dose administered and the amount of drug remaining at the injection site. The *in vivo* release profile of enrofloxacin from the gel formulation with 40 mg dose is shown in Figure 11. Approximately 96.51% of the dose of enrofloxacin injected was released by day 9. The *in vivo* release profile of enrofloxacin from the gel can only be used as an estimation due to the difficulty of completely recovering all the gel residue at the injection site.

The gel residue is expected to completely disappear from the injection site with time due to the biodegradable nature of the gel composition. There was no sign of inflammation on subcutaneous tissue in contact with the gel at the injection sites.

**Conclusion:** The results of this study showed that it is feasible to develop a long acting controlled release biodegradable injectable gel formulation for enrofloxacin to treat bacterial infectious diseases in laboratory animals. Enrofloxacin was compatible with all 3 plasticizers and stable in the promising gel formulation tested in the study. The *in vitro* and *in vivo* release of the drug from the gel formulations could be modulated by the composition of the gel formulations, especially the drug loading of enrofloxacin in the gel and the dose of enrofloxacin injected. The plasma concentration of enrofloxacin could be maintained at or above the MIC<sub>100</sub> for at least 4 to 7 days from one of the tested formulations. The gel formulation was also biocompatible.

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PEG 400 was kindly donated by Union Carbide Co. (A Subsidiary of Dow Chemical Company) (Oxford, CT).

## **Group housing, environmental enrichment and exercise enhance expression of genes involved in neuronal signaling, plasticity and growth in the mouse hippocampus**

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We examined the effects of group housing, environmental enrichment, and voluntary exercise on changes in gene expression in mice. All subjects were compared to individually housed mice. The mouse hippocampus was studied because it is the key region involved in learning and memory and is involved in adult neurogenesis. All treatments induced a significant increase in expression of genes involved in neuronal signaling, plasticity and growth; key genes involved in enhancing brain health. These changes likely result in beneficial changes such as enhanced learning and memory performance and brain structural changes. The most robust changes in gene expression occurred in group housed mice. While group housing, enrichment and exercise are all important and beneficial, group housing of mice appears to be most beneficial with regard to brain health in mice. If group housing cannot be provided to mice, adding another ‘replacement’ treatment, such as a nestlet or an exercise wheel, offers similar, although not as robust, benefits with regard to brain health. Results from this study can be applied to improve the well-being of laboratory mice. They also have broad implications for studies involving neuroscience experiments particularly those studying learning and memory or inducing plasticity and recovery from neuronal injury.

**Table 1:** Percentage change in expression of key genes involved in brain health in group housed, environmentally enriched and exercised mice compared to control (individually housed) mice. Two to four independent samples from each treatment are compared to control gene expression levels within the hippocampus. Change values are averages of the treatments compared to controls. NC represents no significant change.

|                                       | Group | Enrichment | Exercise |
|---------------------------------------|-------|------------|----------|
| PSD-95                                | 139   | NC         | 118      |
| Calmodulin                            | 196   | 181        | 189      |
| Neurogranin                           | 216   | 177        | NC       |
| Cortactin                             | 123   | NC         | NC       |
| N-cadherin                            | 111   | 144        | 77       |
| Integrin B5 subunit                   | 159   | NC         | NC       |
| Neuronal activity-regulated pentraxin | NC    | 125        | 129      |
| BDNF                                  | NC    | 64         | 147      |
| TrkB                                  | 275   | 130        | 115      |

# **A controlled and retrospective population-based survey of zoonotic disease occurrence among laboratory animal workers in the United States**

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The purpose of this study was to estimate the frequency and types of zoonotic diseases transmitted to persons working with laboratory animals or their tissues in the conduct of research, teaching, and testing. Despite the fact that the species collections currently maintained in laboratory animal facilities are generally provided by commercial vendors which have successfully eliminated all or most zoonotic agents from their colonies, we hypothesized that episodes of known or suspected exposure to zoonotic diseases continue to occur and that the frequency of such cases differs significantly by type of worker and by type of institution. Furthermore, in accordance with previous observations within segments of this workforce, we hypothesized that underreporting of work-related exposures was substantial. Hence, this study was intended to provide important information to help refine prevention-oriented educational efforts. Through a statistically valid self-administered cross-sectional survey of laboratory animal workers administered by postal questionnaire, 23 of 1,367 persons surveyed reported 28 cases of infection with zoonotic agents from research animals at their workplace during the past five years. Six persons indicated that their infections were medically confirmed. Based on these data, the annualized incidence rate for work-related transmission of zoonotic agents from laboratory animals was 45 cases per 10,000 worker-years at risk (95% confidence interval: 30 - 65 cases), approximating the rate for nonfatal occupational illnesses in the U.S. agricultural production-livestock industry and for those employed in the health services during 2002. Logistic regression analysis found various characteristics of persons and their employers that were significantly associated with the likelihood of having been medically evaluated for exposure to a zoonotic agent from laboratory animals. Most (95.5% + 1.1%) persons working with laboratory animals or their tissues indicated that they knew whom to talk to at their institution for medical evaluation and care should they be concerned about the possibility of an occupationally acquired zoonotic disease in future. However, occupational illnesses and exposures among laboratory animal workers was underreported, as 10 of the 28 (36%) alleged zoonotic disease cases were not communicated to the employee's supervisor. Lack of concern about the potential significance to their health and the perception of punitive consequences to the employee were some of the reasons cited for underreporting, an issue which must be vigorously addressed in the interests of continuing progress toward zoonotic disease prevention in this field.