

FOUNDATION: 2001 Grant Reports Title: Pathogenesis and Transmission of Enterotropic Mouse Hepatitis Virus (MHV)

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Mouse hepatitis virus (MHV) is the most prevalent viral infection of mice and the majority of MHV strains which infect contemporary mouse colonies are enterotropic (E-MHV). Previous studies had shown that concurrent *Helicobacter* and MHV infection altered the pathogenesis of the virus in immunocompromised mice, including strains that are gamma interferon deficient. The objectives of these studies were to determine the pathogenesis of enterotropic MHV in *Helicobacter*-free immunocompetent and immunodeficient mice and to correlate pathogenesis with virus shedding and transmission of infection. Studies examined a prototype E-MHV (MHV-Y) infection in BALB/c, C57BL/6, and B cell and T cell deficient mice using molecular methods of viral detection. We hypothesized that mouse strain and immune status influence pathogenesis, shedding pattern and transmission, and that detection of virus by fecal PCR correlates with transmission of infectious virus.

The pathogenesis studies in *Helicobacter*-free BALB/c and C57BL/6 mice demonstrated that MHV-Y infection is subclinical, enterotropic (limited to intestinal tract and mesenteric lymph nodes), and not associated with lesions. These results are consistent with previous MHV-Y studies in BALB/c mice, confirming that endemic *Helicobacter* infection of immunocompetent mice does not substantially alter MHV pathogenesis. Antibody isotyping of peripheral blood in BALB/c and C57BL/6 mice indicate that the dominant isotype is IgG2a suggesting that MHV results in a Th1 response.

The immune status of the mouse altered the pathogenesis of MHV infection. T cell deficiency resulted in a chronic, progressive, systemic MHV-Y infection, that included viremia, granulomatous inflammation, necrosis, and clinical disease. B cell deficiency resulted in a subclinical chronic enteric MHV-Y infection which was not cleared until post infection day 102. Approximately 50% of the T cell deficient mice mounted a weak IgM response.

Concurrent transmission studies confirmed that BALB/c and C57BL/6 mice transmitted MHV-Y to sentinel mice for four and two weeks, respectively. BALB/c index mice showed consistent transmission for 28 days after one day sentinel exposure by contact or soiled bedding. These studies also demonstrated that detection of MHV-Y RNA in the feces of infected immunocompetent mice correlated with transmission of infection. Only a small number of BALB/c and C57BL/6 mice had detectable RNA in feces after transmission had ceased. In contrast, B cell deficient mice transmitted MHV-Y to sentinel mice for more than three months, although no viral RNA was detected in feces during the last month. Thus, mice with partial immunity developed a controlled but persistent enteric infection with extended viral shedding.

The conclusions from these experiments will improve colony management by establishing recommendations for diagnosing, assessing and controlling MHV-infection. The results from these experiments also underscore the importance of sentinel mice in detecting enterotropic MHV infection in immunodeficient mice for whom fecal shedding, seroconversion and clinical disease may not be reliable indicators.

Title: Validation of Bispectral Index as an Indicator of Surgical Anesthetic Depth in Pigs

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The goal of this study was to validate a technique for determining adequacy of anesthetic depth in pigs, especially when given a paralyzing agent during a surgical procedure. To this end, we studied the utility of a monitor of brain wave activity, known as bispectral index (BIS), used extensively to determine the level of anesthetic-induced CNS depression in people. The objectives of this study were to determine the relationship of BIS values to depth of anesthesia when using techniques that are common for surgeries in pigs. Six isoflurane-based anesthetic protocols were each studied at 5 levels (0.8, 1, 1.3, 1.6, and 2 MAC). MAC is the minimal alveolar concentration of inhaled anesthetic that prevents purposeful movement in response to a noxious stimulus in 50% of a population.

Significantly higher BIS values were identified for low inhaled isoflurane anesthetic concentrations within each treatment compared to higher concentrations. However, significant differences for mean BIS values were only sporadically observed within anesthetic protocols for pigs maintained between 1.3 and 2 MAC. Pigs maintained at less than 1 MAC isoflurane usually had BIS >60 while pigs maintained at 1 MAC or greater usually had BIS <60. The targeted range for BIS in anesthetized people undergoing surgery is between 40 and 60. Thus, CNS depression in pigs similar to that of adequately anesthetized people is predicted when the isoflurane concentration is maintained at 1 MAC or greater. Administration of the muscle relaxant, atracurium, or the analgesic, fentanyl had no effects on BIS compared to isoflurane alone. Noxious stimulation was associated with significantly different mean BIS values when compared to non-stimulated pigs in only one instance: surprisingly, a lower BIS value was observed in stimulated, compared to non-stimulated, pigs during 1.6 MAC isoflurane combined with atracurium and fentanyl.

Results of this study support use of the BIS monitor to verify adequate CNS depression in pigs undergoing noxious stimulation during isoflurane anesthesia. We conclude that BIS appears reliable for identification of light versus deep isoflurane anesthesia in pigs, but is less useful for determination of the level of CNS depression between 1.3 and 2 MAC.

Title: Behavioral and Clinical Pathology Changes in Fish Subjected to Anesthesia and Surgery with and without Peri-Operative Analgesics

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Use of fish in biomedical research has soared in recent years. Surgical procedures have been performed on fish in research settings for many years, and are increasingly being performed on pet and display aquarium fish. Effects of surgery on fish behavior and physiology that could impact research results are under-investigated. In particular, pain management techniques that could alleviate surgical effects have not been evaluated in fish. We aimed to document behavioral and physiologic changes in fish undergoing surgery, both with and without peri-operative analgesic administration.

Thirty koi carp (*Cyprinus carpio*; 126 +/- 53 g) underwent exploratory celiotomy by veterinary students in a surgery training laboratory. Surgeries were performed using the fish anesthetic tricaine methanesulfonate (MS-222) at 100 - 200 mg/L on a recirculating fish anesthesia delivery system surgery table. Fish were randomly divided into three groups of 10 fish. During skin closure, one group received the opiate butorphanol at 0.4 mg/kg IM, one received the nonsteroidal anti-inflammatory agent ketoprofen at 2.0 mg/kg IM, and the third received physiologic saline IM, all adjusted to equivalent volumes per weight of fish. Preliminary observations had indicated that koi treated with butorphanol at 0.4 mg/kg IM intra-operatively could be distinguished during the first few hours after surgery from those receiving equivalent volumes of physiologic saline IM by their tendency to swim higher in the water column, exhibit greater activity levels, and a more rapid return to feeding. In that small pilot study, observers were blinded to treatment, but fish were grouped according to treatment and could be evaluated in aggregate. In the current study, observers were blinded to both treatment and group, and fish were evaluated for vertical position in the water column, caudal fin beat rate as an indicator of activity level, respiratory rate, and response to food (presentation of a single pellet) at -24, 0.5, 1, 2, 3, 6, 18, 24 and 48 h from the end of surgery. In addition, hematocrit, total solids, plasma cortisol, and complete plasma biochemistry panels were compared between blood samples obtained 2 weeks prior to surgery and 48 h after surgery.

Following surgery, vertical position in the water column and caudal fin beats were reduced at multiple time points (Friedman two-way layout,  $p < 0.05$ ). Fewer fish consumed the pellet at 0.5 and 1 h, and more fish that did feed at those times did so less vigorously than at other time points (Chi Square with correspondence analysis,  $p < 0.05$ ). However, there were no differences between treatment groups at any time (Kruskal-Wallis,  $p > 0.05$ ). Clinical pathology analytes that changed following surgery included significant decreases in hematocrit, total solids, phosphorus, total protein, albumin, globulin, potassium, chloride, and increases in plasma glucose, aspartate aminotransferase, creatine kinase, lactate dehydrogenase and bicarbonate (Wilcoxon matched pairs signed ranks,  $p < 0.05$ ). There were no changes in cortisol, calcium, sodium or anion gap. Between treatment groups the only analyte that differed was creatine kinase, which increased significantly less in the ketoprofen group (median increase of 90 U/L) than in the butorphanol and saline groups (median increases of 2647 and 2267 U/L, respectively; Kruskal-Wallis,  $p < 0.05$ ), suggesting that the anti-inflammatory effects of ketoprofen reduced muscle damage secondary to surgically-induced inflammation. The reductions in potassium and chloride took place despite recovering the koi in water supplemented with 1 g/L sea salts in an attempt to avoid electrolyte imbalances, though the reductions were minor and greater

imbalances have been observed previously with recovery water lacking salt supplementation. Potential adverse effects of butorphanol (respiratory depression, lethargy) or ketoprofen (hemorrhage, kidney or liver damage) were not evident at the single doses used in this study.

Heart rate spectral analysis is a technique that uses beat-by-beat fluctuations in heart rate to gauge sympathetic and parasympathetic tone, which may be influenced by pain. Because of the electrical conductivity of water, heart rates of fish can be measured and recorded without direct contact by use of a specially designed tubular electrocardiogram (ECG) chamber. In this study, koi proved to have insufficient ECG signal for recording in the ECG chamber, while tilapia (*Oreochromis niloticus*) generated a robust signal. Tilapia not undergoing surgery and treated with butorphanol, oxymorphone and naloxone at several doses exhibited none of the expected differences in autonomic tone by heart rate spectral analysis. Because neither butorphanol nor ketoprofen altered post-surgical behaviors examined in this study, and no effects on heart rate spectral analysis were observed in butorphanol-treated tilapia without surgery, effects of these analgesics on post-surgical heart rate spectral analysis was not pursued at this time.

Implications for laboratory animal veterinarians--

Several behavioral and clinical pathology measures were altered significantly from pre-surgical values within the first 48 h of surgery, though time to complete resolution was not determined in this study, and measures for koi treated with butorphanol at 0.4 mg/kg IM or ketoprofen at 2 mg/kg IM peri-operatively were no different from saline-treated controls (with the exception of creatine kinase which increased significantly less for ketoprofen-treated koi). However, neither were there any observed adverse effects from attempted analgesic intervention. Pre-surgical clinical pathology values generated in this investigation can be used as reference intervals for koi, and represent an expansion in sample size and number of analytes previously available for comparison. Post-anesthesia and surgery electrolyte imbalances in koi may be alleviated but not eliminated by recovery in water supplemented with 1 g/L sea salts. While this study did not conclusively show beneficial effects of peri-operative analgesic intervention, it should not be taken as evidence that analgesics are of no value in fish, particularly in light of the marked behavioral changes observed following surgery. Either different behavioral or physiologic measures, or different analgesic protocols, may prove more effective in demonstrating analgesic effects in fish.

Title: Enhancement of Rodent Health Monitoring in Ventilated Cage Racks

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Housing of rodents in individually ventilated caging (IVC) presents opportunities for development of new, more effective microbiological monitoring methods. Currently, sentinel mice exposed to soiled bedding are most commonly used to monitor mice housed in IVC, but this method is labor and time intensive and efficacy varies depending on the infectious agent.

This study compared the efficacy of exhaust air monitoring using sentinels exposed to exhaust air from the IVC rack and PCR analysis of filters placed in the exhaust airstream of the IVC rack with the efficacy of traditional monitoring of sentinels exposed to soiled bedding or sentinels by contact with infected mice. Groups of 12 Swiss Webster mice were experimentally infected with mouse hepatitis virus, mouse parvovirus, murine rotavirus and *Helicobacter*, and transmission of infectious agents from these mice were monitored for 12 weeks. These agents were chosen because they are the most prevalent infectious agents of mice and differ in their size, infectivity, environmental stability, route by which they are transmitted and duration of infection they cause.

Mouse hepatitis virus is a large RNA virus, that is highly infectious, is unstable in the environment and causes an acute intestinal infection. Mouse hepatitis virus was transmitted to all contact, soiled bedding and exhaust air sentinels and mouse hepatitis virus RNA was detectable on exhaust air filters for 40-60 days.

Mouse parvovirus is a small DNA virus that is moderately infectious, highly stable in the environment and causes a persistent systemic infection with viral shedding in the urine, feces and expired air. Mouse parvovirus was transmitted to contact and soiled bedding sentinels, but was not transmitted to exhaust air sentinels. Mouse parvovirus DNA was detected on about a quarter of the exhaust air filters.

Murine rotavirus is a medium-sized double stranded RNA virus, which is highly infectious, highly stable in the environment and causes an acute intestinal infection. Murine rotavirus was transmitted only to contact sentinels.

Sendai virus is a large RNA virus, that is highly infectious, is unstable in the environment and causes an acute respiratory infection. Sendai virus was transmitted to all contact and exhaust air sentinels but not to soiled bedding sentinels. Sendai virus RNA was detected on exhaust air filters for 40 days.

*Helicobacter* are gram-negative spiral bacteria that are highly infectious, are unstable in the environment and cause persistent infection of the intestinal tract and liver. *Helicobacter* was transmitted to all contact, a quarter of the soiled bedding sentinels and none of the exhaust air sentinels. *Helicobacter* DNA was detected on 50% of exhaust air filters.

Uninoculated control mice remained uninfected indicating that husbandry was not a means of infectious agent transmission. Results were similar when the IVC rack was operated at positive and negative air pressure suggesting that relative differences in air pressure in the IVC rack do not substantially impact infectious agent transmission via the exhaust air. Results of this study showed that direct exposure of infected mice to contact sentinels is the only method of monitoring tested which detected all agents. Exposure of sentinels to exhaust air was effective at detecting several infectious agents (mouse hepatitis virus and Sendai virus). Viral and bacterial RNA and DNA on exhaust air filters were detectable for several months. Therefore, exhaust air filters and exhaust air sentinels add two new microbiological monitoring tools for detecting infections of mice housed in IVC racks. The use of sentinels which receive both exhaust air and soiled bedding could greatly increase the efficiency of microbiological monitoring allowing for the detection of all five infectious agents used in this study. Exhaust air filters can be used to

rapidly detect the distribution of an infectious agent being shed and to determine when shedding has ceased.

Title: Cloning of the Guinea Pig Adenovirus Hexon Gene for Development of Serology Testing

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Initial reports of an adenovirus as an infectious cause of bronchial pneumonia in guinea pigs surfaced in Germany (1) and later in North America (2, 3), Australia (4) and additional European countries (5-7). Symptomatic disease was described as a lethal pneumonia. Recently, a DNA amplification test was used in a research study to demonstrate the life cycle of this virus in guinea pigs (8). Most guinea pigs upon exposure to the adenovirus experience transient viral replication in their nasal mucosa for up to 15 days with only an "occasional sneeze" observed, rarely lethal pneumonia. Our laboratory sought to develop a method to distinguish guinea pigs that had survived GPAdV infection during their lifetime.

We successfully cloned the gene coding for the major surface protein of GPAdV (9). We spliced the gene into a human adenovirus and grew the recombinant virus in cell culture subsequently developing two types of serology tests: enzyme linked immunosorbent assay (ELISA) and indirect immunofluorescent assay (IFA). We inoculated two guinea pigs with GPAdV by instilling nose drops extracted from infected tissues, and collected small amounts of blood from the guinea pigs for several weeks. Our serology tests detected the development of antibodies produced by these animals to the GPAdV beginning at one week and reaching maximal levels by four weeks.

Finally, we requested guinea pig serum from our colleagues at other institutions through the listserv known as COMPMED. Six institutions responded sending a total of sixty-two serum samples. We found that five (5) of six (6) institutions sent serum from guinea pigs that had significant antibody titers to GPAdV: 19% of the samples were positive, 78% were negative, and 3% were equivocal. In conclusion, thanks to the funding made available by the ACLAM Foundation, we were able to develop serology tests able to detect antibodies to GPAdV in previously infected guinea pigs. These novel serology tests will be useful in determining if this virus influences the health of guinea pigs and scientific validity of research performed using them.

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NOTE: Since these summaries can not capture the detail of the projects, we look forward to reading the full publications in the literature. We thank the authors and staffs for their efforts to expand the body of knowledge in our field.