



**National Center for
Research Resources**

NATIONAL INSTITUTES OF HEALTH

**Final Workshop Report of the
Detection, Impact and Control of
Specific Pathogens in Animal Resource
Facilities Workshop
April 23 – 24, 2009**

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Executive Summary

The workshop entitled *Detection, Impact and Control of Specific Pathogens in Animal Resource Facilities*, sponsored by the National Center for Research Resources (NCRR), National Institutes of Health (NIH), and co-sponsored with the National Institute of Aging (NIA), took place at the NIH Lister Hill auditorium on April 23 – 24, 2009.

The purpose of the workshop was to identify current problems and future challenges relevant to the control, detection, research interpretation and zoonotic potential of emerging and re-emerging pathogens in animal resource facilities. The event represented a unique opportunity to assemble veterinarians and scientists with specific expertise in aquatic models, nonhuman primates (NHPs), and rodents to interact with each other.

Keynoted by Dr. Stephen Barthold, who discussed emerging infections and future challenges, the workshop was divided into six sessions. Each session was moderated, featured invited presenters and allowed ample time for discussion by the participants.

Overall, there were 24 presenters, including session moderators. The workshop was attended by 91 participants, including NIH-supported extramural and intramural researchers, NIH program staff, USDA and FDA scientists and veterinarians, as well as representatives from commercial companies.

At the end of each session and following the last session on each day, wide-ranging discussions were moderated by members of the workshop advisory committee and workshop organizers. Participants also were asked to provide comments and suggestions on the current and future challenges related to the identification and control of emerging and re-emerging pathogens in animal resource facilities.

Purposes and Objectives of the Workshop

- Assess current control strategies for emerging and re-emerging pathogens in the following animal models: NHPs, rodents and zebrafish.
- Identify current problem areas regarding pathogen detection in the target species.
- Identify gaps in current scientific knowledge, such as the role of the environment (water), in susceptibility to infectious diseases in aquatic models as well as characterization of mechanism of pathogenesis of recently identified pathogens in mice (norovirus) and *Pseudoloma* in zebrafish.

- Identify novel and emerging technologies that may increase the sensitivity and specificity of diagnostic tests, including the ability to accurately detect polymicrobial infections.
- Identify the most promising areas for potential research support.

Major Recommendations

- Support research to measure the impact of emerging and re-emerging pathogens on animal models in order to make informed decisions.
- Encourage the continuation of whole genome sequence of emerging and re-emerging pathogens from animal resource facilities.
- Support the improvement of communications of disease outbreaks and potential risks for animal models for the scientific community.
- Encourage the training of veterinary specialists (aquatic models, NHPs and rodents) through partnership with industry and NCRR centers of excellence.
- Encourage the training of veterinarians to become independent, successful investigators.

Summary of Presentations and Discussion

Session I. Summaries of Common Practices

The first session consisted of three presentations that provided an overview of current knowledge and practice related to infectious disease management in aquatic models, NHPs and rodents.

Challenges in Nonhuman Primate Infectious Diseases and Colony Management: It is difficult to eliminate infectious diseases from NHP colonies. Similar approaches (e.g., cesarean births and isolation of offspring) — as used in rodents and other species — have been difficult to achieve on a large scale in NHPs. Challenges in NHP infectious diseases and colony management can be divided into four broad overlapping categories. The first is epizootic infections of wild or captive primate populations with demonstrated overt pathogenic potential such Ebola, cowpox and *Shigella flexeneri*.

A challenge to the primate centers is the importation of animals from areas of the world with a high prevalence of animal/human infection (*M. tuberculosis*, *M. bovis* and primate malarias).

The next category is re-emerging agents that are well described but seen in increasing incidence or in association with unusual pathology as a result of experimental manipulation or immune modulation (simian parvovirus, simian varicella virus and cytomegalovirus).

Finally, there are recently recognized infectious agents with poorly defined or unknown disease effects in the normal host. These infections (GB virus, rhesus rhadinovirus and *Helicobacter spp.*) may be widespread, but little is understood about their impact.

Although not possible to eliminate all pathogens from NHP colonies, defining the full spectrum of microbial agents present within a population, as well as understanding how these agents may

impact their health and experimental work, is a key requirement. Microbial quality control will not eliminate all pathogens, but it can address some of the problems. All facilities with NHPs should implement comprehensive microbial quality control programs and seek methods to integrate and bridge disease surveillance across primate facilities.

Rodent Pathogens — An Overview of Current Knowledge and Practices: Several newly emerging pathogens cause — in most cases — subclinical infections that are assessed through histopathology and, more recently, through perturbations in gene expression through microarray or PCR techniques.

Before testing colony animals, facilities should ensure that they are immunocompetent (i.e., able to develop an immune response) and available for monitoring. Sentinel animals are commonly used in rodent facilities to monitor the health status of colonies. Still another model/system is needed to successfully monitor and eliminate most pathogens from rodent facilities.

Infectious Diseases in Aquatic Models: Zebrafish use in biomedical research has been growing exponentially in areas such as developmental genetics, toxicology, oncology and infectious diseases.

Aquatic models face significantly higher susceptibility to infectious disease than mammalian models. In their environment (water), they are constantly surrounded by potential pathogens. Opportunistic pathogens have not been well defined and studied in aquatic models.

Husbandry and infectious disease control studies and techniques in aquatic models are just in the process of being developed or shared from aquaculture or ornamental fish.

Diagnostics in fish are limited, because several infectious diseases/agents have not yet been discovered. The most common infection in aquatic colonies is microsporidiosis (*Pseudoloma neurophilia*), which is associated with emaciation and skeletal deformities and has been found in most aquatic facilities. Additionally, *Mycobacterium spp.* has been also commonly detected.

Present strategies for control of infectious diseases include quarantine, eggs-only disinfection, monitoring and sentinel programs, specific pathogen-free fish, ultraviolet (UV) disinfection, cross-contamination prevention and restricted access.

Session II. New Technologies

The second session focused on new technologies for detection of emerging and re-emerging pathogens. The first presentation described a staged strategy for pathogen discovery and virus detection using three different tools: Mass Tag PCR panels, GreeneChip Arrays and shotgun sequencing. A second presentation described new serological techniques and interpretation. The third presentation described discovery of new viruses by utilization of sequence-independent genome amplification.

Mass Tag PCR: Mass Tag PCR is a new technology for sensitive, highly multiplexed, rapid differential diagnosis of a pathogen by signal analysis. Currently, it is possible to generate a panel that will test up to 30 agents at the same time and determine polymicrobial infections.

GreeneChip Arrays: The Greene Chips system offers a highly multiplexed, differential diagnosis of infectious agents and has been used for virus surveillance detection of respiratory pathogens, influenza serotyping, virus discovery and panmicrobial surveillance.

High-Throughput Sequencing: High-throughput sequencing will solve problems in pathogen surveillance and discovery that other techniques cannot, and it can be complementary to other sequencing technologies.

Session III. Environmental Quality and Infectious Diseases

The third session featured two presentations on the subject of environment quality and infectious diseases. The first presentation described the indoor/outdoor housing considerations for NHP and their effect on pathogen transmission and host susceptibility. The second presentation described the key factor that water plays in an aquatic environment regarding the susceptibility to a myriad of pathogens.

Indoor/Outdoor Housing Considerations for NHP: Housing and environmental considerations constitute a key factor to susceptibility/exposure to several pathogens in NHP colonies. Typical housing strategies for NHPs are designed to encourage social contact, but that can limit the ability to control the spread of infectious agents.

Indoor and outdoor housing present some challenges and particular attributes that can be considered advantages or disadvantages as they pertain to infectious disease control. Indoor housing allows more strict infectious disease control and monitoring of environmental conditions. The major difference between rodent and NHP indoor housing is that rodents are contained in cages, and NHPs are contained in rooms.

Challenges to environmental quality are numerous. NHP use in biomedical research has increased and is expected to continue this upward trend. Shortage of domestic NHPs will continue to result in increased importation, which can lead to reemerging diseases coming from source countries.

The Key Factor that Water Plays in an Aquatic Environment Relevant to Pathogen Susceptibility: The health and survival of aquatic organisms is closely tied to the dynamic and interdependent chemical and physical characteristics of the water in which they live. Aquatic organisms can tolerate a wide range of water quality parameters; however, optimal environmental conditions are species-specific and also may vary with the life-stage.

Each species of fish has specific water quality requirements that support optimal growth, reproduction and survival. Fluctuations in some of these requirements can result in growth inhibition, reproductive failure and reduced resistance to disease.

Different facility designs — including basic one-way flow-through and closed recirculating systems — have been developed with the goal of maintaining optimal water quality.

Obligate pathogens and opportunistic organisms can overlap and have a severe effect on fish living in poor quality water or under stress. Adverse impacts of sublethal chronic exposures to unfavorable water quality are poorly understood and generally believed to predispose fish to infectious disease. Numerous opportunistic bacterial agents are ubiquitous in aquatic

environments, making it impossible to exclude them from culture facilities and can cause disease in association with environmental stress.

Session IV: Normal Microbial Flora in Health and Disease

The fourth session was comprised of two presentations on the subject of normal microflora in health and disease. The first presentation focused on the relationship of the macaque gut microbiome in health, lentiviral infection and chronic enterocolitis. The second presentation concentrated on current efforts and status of the human microbiome project which will provide a base of knowledge of all microbes that inhabit the human body.

The Macaque Gut Microbiome in Health, Lentiviral Infection and Chronic Enterocolitis: Data were presented showing current efforts related to the sequencing of the macaque gut microbiome in health and disease, utilizing 454 pyrosequencing. The technique allows a single person to sequence a full bacterial genome in a day. Investigators also characterized 141,000 sequences of 16S rRNA genes obtained from 100 uncultured GI bacterial samples. Several bacterial species were detected, with significant differences in the ecology of these populations over time and diet changes but not during the course of infection.

The Human Microbiome Project: The largest collection of human microbes resides in the intestine (approximately 10 – 100 trillion organisms), and aggregate genomes of these gut species (microbiome) may contain 100-fold more genes than the human genome. The microbiome is an integral part of the human genetic landscape and genetic evolution.

The Human Microbiome Project is part of the NIH Roadmap project and of interest to several NIH Institutes and Centers. It will provide a base of knowledge that will justify funding future hypothesis-driven research. The goals of the project are to catalog the microbes that inhabit the human body, examine whether changes in the microbiome can be related to health and disease, and generate a community resource to support and enable metagenomics-based projects that investigate the role of microbial communities in human health.

The strain distribution so far shows nearly half (49 percent) localized in the GI tract, 18 percent on the skin, 14 percent in the urogenital tract, 13 percent in the oral cavity and 6 percent in the airways.

Session V: Genetics and Infectious Diseases

The fifth session consisted of two presentations on genetics and susceptibility to infectious diseases. The first presentation described the importance of the Major Histocompatibility Complex (MHC) class I genotyping in rhesus by massively parallel pyrosequencing. The second session described how the genetic background of inbred mice could be determinant in the susceptibility and disease severity to an infectious agent.

Major Histocompatibility Complex (MHC) Class I Genotyping: MHC class I genetics present challenges for macaques MHC genotyping. MHC typing is relevant for NHP breeding, infectious disease pathogenesis studies, and vaccine development. MHC class I functions molecules present SIV/SHIV-derived peptides to cytotoxic T lymphocytes (CTL), and the repertoire of MHC alleles determines the specificity of CTL responses. High polymorphisms provide a library of MHC class I alleles in a population. Every macaque has a pair of MHC

class I haplotypes, and extensive polymorphism in allelic content has been observed. Use of 454 pyrosequencing has revolutionized the field of macaque genotyping, and it is hoped that it will be available for a wide variety of infectious agents.

Session VI: Specific Infectious Agents and their Impact on Research

The sixth session consisted of presentations on specific pathogens that cause disease in aquatic models, specifically zebrafish, NHPs and rodents. The zoonotic potential of several infectious agents was noted.

Mycobacterial Infections: Mycobacteria are animal and human pathogens that can be acquired through the intestinal and respiratory tract. Mechanism of transmission is well known for *M. tuberculosis* but unknown for non-TB mycobacteria. The majority of mycobacteria are environmental microbes. Some atypical mycobacteria can cause devastating disease. These are acquired from the environment by immunosuppressed hosts and result in pulmonary disease, lymphadenitis, skin and soft tissue disease or disseminated disease. *M. avium* complex is the most common disseminated bacterial infection in simian AIDS.

Mycobacteriosis constitutes one of the most significant disease problems in zebrafish facilities and encompasses a number of different species of mycobacteria. Mycobacterium is ubiquitous in aquatic systems and can be exacerbated by sharing of fish between facilities. Specific and sensitive tests are needed to correctly identify mycobacterial infection in aquatic models.

Simian Retroviruses: Retroviral infections are persistent. The integration of proviral DNA explains the persistence and also the establishment of latent infections, which are in a dormant state but always have the potential for reactivation and transmission. Among simian retroviruses, there is a broad spectrum of clinical and pathological outcomes with subclinical carriers being very common.

Rodent Parvoviruses and Noroviruses: Norovirus has been found to be much more prevalent than parvovirus in rodent colonies. Murine parvovirus have been better studied than norovirus.

Although norovirus infection results in lethal infection in mice deficient of innate immunity, their potential confounding effect on research results is just beginning to be understood. Several diagnostic assays are available for both viruses, mainly serology and PCR.

Helicobacter Infections: More than 30 helicobacter species have been identified, and more than 35 recently found Helicobacter are waiting to be named. In general, Helicobacter infections tend to induce T helper type 1 immune responses in mice.

Disease outcome can be multidimensional, unlike acute infections in which "one microbe-one disease" is often the case. Chronic infectious agents more likely represent interactions between infecting microorganisms and a multitude of host and environmental factors, including interactions with other infectious agents (coinfections). Mounting evidence from human and animal studies shows that prior or concurrent infections with unrelated pathogens can modulate immunopathogenic responses.

Protozoal Infections: NHPs are infected with a wide range of protozoal agents which can have an adverse impact on NHP health and research programs. Protozoa can directly impact animal

and colony health causing overt morbidity and mortality, or they can be nonpathogenic in the normal host but cause zoonotic disease in humans.

There is a diversity of protozoan parasites in fishes that have not been well studied. The most common diseases include white spot disease (caused by *Ichthyophthirius multifiliis*), velvet disease (caused by the parasite dinoflagellate *Piscinoodinium pillulare*) and skinny disease (caused by the microsporidian *Pseudoloma neurophilia*).

Contact Information

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