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A rodent health classification system – key to maintaining a healthy rodent colony

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Abstract

A well-defined health status classification system helps veterinarians and researchers collaborate on planning experiments and aid them in defining facility procedures such as animal receipt and housing. This article will describe a rodent health classification system that has been used by Bristol-Myers Squibb in the U.S. and India to manage and maintain disease-free rodent colonies for research.

Key words: health, classification, rodents

Introduction

There are many potential pathogenic rodent viruses, bacteria, and parasites that can contaminate an animal facility and cause detrimental effects to ongoing research studies. While some of these agents do not present outward clinical signs in the rodent population, an infection can adversely affect study animals and impact ongoing research efforts (Table 1). For this reason, every effort should be made to maintain research colonies that are free of disease.

This article will review a health classification management system that has been used by Bristol-Myers Squibb in the US and India to maintain disease free research animal colonies. This system is used to maintain a Class I facility (free of rodent diseases), while also managing Class II and Class III events (use of unknown animals and disease outbreaks respectively).

Having a well-defined health status classification system facilitates planning discussions among veterinarians and research staff in our company. The assigned health

classification drives the receipt procedures for the rodent shipments, allows investigators to schedule their experiments based on release of animals, and has become an effective communication tool for our investigators and veterinary staff.

Disease Impact on Research

There are many potential pathogens including viruses, bacteria, and parasites that can contaminate an animal facility and cause detrimental effects to ongoing research studies (Jacoby and Lindsey, 1998; Lussier, 1988). While many of these agents do not present any outward clinical signs in the rodent population, they can cause adverse effects in study animals and impact ongoing research efforts (Table 1). Having a general understanding of some of the more common infectious agents or parasites in laboratory animal populations is important in maintaining a healthy research colony and instituting a health classification system that serves as a foundation for all animal experimentation in a good facility.

According to Clifford and Watson, many of the agents that infected rodent colonies almost 20 years ago, still continue to impact research. The reasons why these agents remain in animal facilities vary by each specific pathogen. The increase in shipping laboratory animals globally as well as the use of genetically modified animals has contributed greatly to the continued presence of rodent pathogens and frequent outbreaks. The adverse effects of these various agents in laboratory animals can cause unexpected results and increase variability in experiments. One such infectious agent that has an ongoing presence in animal facilities today is Mouse Hepatitis Virus (MHV). Mouse Hepatitis Virus is a corona virus that is highly contagious and according to 2009 prevalence data, MHV was present in 1.59% of the serum samples tested at their diagnostic laboratories (Pritchett-Corning *et al.,* 2009). MHV infections can cause immunosuppression as well as an increased susceptibility to other infections. MHV can negatively impact research studies in immunodeficient strains such as nude mice and SCID mice.

Table 1.: Rodent Pathogens and Research Impact

AGENT	HOST	ADVERSE EFFECTS/RESEARCH IMPACT
Mouse Hepatitis Virus (MHV)	Mouse	Immunosupression, compromised CNS and gastrointestinal tract, unexplained deaths
Sendai Virus	Mouse, rat, hamster, Guinea pig	Immunosuppression, neonatal and adult deaths, respiratory lesions, interruption in breeding
Minute Virus of Mice and Mouse Parvovirus (MVM, MPV)	Mouse	Immunosuppression, low ascites production, impact on lymphocyte cultures
Rat Parvovirus, Kilham's rat virus, Rat Minute Virus and Toolan's rat virus (RPV, KRV, RMV, H-1)	Rat	Immunosuppression, impact on lymphocyte cultures and oncology studies
Theiler's murine encephalomylelitis virus (TMEV, GDVII)	Mouse, rat	Immunologic and CNS impact
Enzootic Diarrhea of Infant Mice (EDIM)	Mouse	High mortality in young mice less than 2 wks old, diarrhea, alters intestinal absorption
Mouse Adeno Virus (MAD, MadV-1, K-87)	Mouse, rat	Kidney lesions, causes wasting in nude mice
Pneumonia Virus of Mice (PVM)	Mouse, rat, hamster, gerbil, Guinea pig	Pulmonary interference, wasting disease in immunodeficient animals
Helicobacter sp.	Mouse, rat, gerbil	Inflammatory response, gut and liver impact in susceptible strains
Mycoplasma pulmonis	Mouse, rat	Respiratory issues, immunosuppression, animals appear clinically ill
Pasteurella pneumotropica	Mouse, rat, hamster, gerbil, Guinea pig	Reproductive issues and low producing breeders, Respiratory, eye, genital tract and skin infections
Pinworms (Syphacia sp. and Aspicularis sp.)	Mouse, rat, hamster, gerbil	Marker of inadequate biosecurity, rectal prolapse, poor condition, rough hair coats and reduced growth rates

MHV can cause clinical disease in nursing pups and may prove fatal in some cases.

Another infectious agent prevalent in animal facilities around the globe is Theiler's Mouse Encephalomyelitis Virus (TMEV). This virus is also known as GDVII, or George's disease 7, named after Theiler's laboratory technician. The prevalence of TMEV/GDVII in serum samples according to Pritchett-Corning, *et al*. is 0.26% for mice and much higher at 1.43% for rats. This virus does not present any outward clinical disease in rodents however, the adverse effects of the virus can be significant. Since the virus infects macrophages, immunology studies can be affected. Additionally, TMEV/GDVII affects a variety of cells in the central nervous system so unexpected experimental results in animals infected with the virus can be anticipated.

According to prevalence data, one virus that occurs very frequently in mouse and rat facilities is parvovirus. Viruses in this category include MPV, MMV/MVM, H-1, RV/KRV, RMV and RPV. According to data presented by Pritchett-Corning, *et al.* Mouse Parvovirus 1 and 2 was present in 1.86% of mouse samples tested at their facility and Rat Minute Virus (RMV) in 1.46% of rat samples. Parvoviruses, in general, do not present clinical signs of disease, but they can affect research. Mouse parvoviruses are lymphocytotropic and disrupt research through their effects on the host immune response (CRL Technical Sheet – Mouse Parvoviruses, McKisic *et al.,* 1998). It is important to note that parvoviruses can persist for up to nine weeks in the tissues of immunocompetent mice, can be shed for long periods of time, and are stable in the environment (Jacoby *et al.,* 1995; Clifford and Watson, 2008). Thus, they have the potential to remain persistent in animal facilities or on equipment.

There are other viruses that have a lower prevalence rate in laboratory animal facilities, but they can have a large impact due to their ability to spread to multiple rodent species and affect research. These viruses include Sendai, Pneumonia Virus of Mice (PVM) and Adenovirus (MAD, K-87). Sendai virus is an important pathogen of rodents as it can spread to mice, rats, hamsters and even Guinea pigs. Sendai virus replicates in respiratory epithelium and is very contagious. It is transmitted primarily by contact although aerosol transmission may occur. According to Baker (1998), few clinical signs are observed with Sendai virus infection, however it has been reported that mice will display teeth chattering, dyspnea, prolonged gestation and poor growth. Research implications include immunosupression, effects on local respiratory defense mechanisms, and interruption in breeding (Brownstein, 1986).

Another virus which causes respiratory complications is Pneumonia Virus of Mice (PVM). Again, this virus can infect mice, rats, gerbils and Guinea pigs. Immunocompetent animals infected with PVM do not typically present with outward clinical signs, but immunodeficient models can exhibit an interstitial pneumonia with wasting disease (Charles River Technical Sheet, PVM). Because of the pulmonary dysfunction and wasting presentation, these animals are unsuitable for research. Adenovirus of mice and rats has been reported but has a very low prevalence (Pritchett-Corning *et al.,* 2009). Adenoviruses present no clinical signs in euthymic mice and rats with natural infection. When experimentally infected, suckling mice exhibit a hunched posture, rough hair coat and lethargy while immunodeficient mice display wasting disease and scaly skin. No lesions have been noted in rats experimentally infected with adenovirus. MAdV-1 has been reported to increase susceptibility to *E. coli*-induced pyelonephritis (Charles River Technical sheet, Mouse Adenovirus).

In addition to viral infections, bacterial agents can interfere with ongoing research studies, and *Mycoplasma pulmonis* is one such agent. Typically a pathogen of mice and rats, these bacteria can cause clinical disease and make animals unsuitable for studies (NRC, 1991). Mice often present with a ruffled hair coat, hunched posture, weight loss, and respiratory sounds that resemble "chattering." Rats also show outward signs of disease similar to mice and display porphyrin staining as well as "snuffles."

While viral agents of rodents are of great concern for contaminating laboratory animal facilities, other agents such as pinworms can negatively affect research studies. Pinworms can infect many rodent species including mice, rats, gerbils and hamsters. Generally, pinworm infections do not cause any outward clinical signs however; they do raise questions regarding the biosecurity of the animal facility. Some reports have indicated that animals infected with pinworms can show poor condition, rough hair coat or reduced growth rates. According to the Charles River Technical Sheet, mice infected with pinworms had a greater incidence of autoimmune disease and nude mice and rats had an increase in lymphoma prevalence. Detection and treatment to eradicate pinworms, while it may seem trivial, can have a direct impact on the validity of research studies.

Health Classification System

Protecting the health of the resident animal colonies begins with establishing an internal standard for the acceptable health status. Once this is established, the program must assure that incoming animals, regardless of source, meet that health status. It must also assure that the resident animals remain at that standard while housed in the facility. Even though vendors regularly screen their colonies for common murine pathogens, it is useful to periodically perform a health assessment on vendors' barriers from which your facility routinely receives animals.

Animal breeders identify and maintain their breeding colonies at a specific site by a breeding area designation. For example, let us assume that vendor X has a rodent breeding facility in Bangalore and in Hyderabad. Within these facilities they may have different breeding areas for Sprague Dawley (SD) rats. We will call these different breeding areas for Bangalore area 03 (BLR-03) and in Hyderabad breeding area 07 (HYD-07). As a customer, you should assume that the SD rats from each of these areas are a different sub-strain, and you should also assume that each of these areas will have a slightly different health status.

When you consider purchasing SD rats from vendor X, you can choose BLR-03, HYD-07, or both. Regardless of

your choice, you should review the vendor-supplied sentinel data for the breeding area from which you will purchase animals. You should also verify the vendor's findings through your own internal health-screening program, and it is a good idea to occasionally verify health status by using a third-party diagnostic laboratory. All of the modern methods to screen those animals for the presence or absence of unacceptable pathogens can be used. Ideally, these diagnostic evaluations should be performed on retired breeders since they have resided longer in the vendor's colony. The testing should include serology, PCR, microbiology, and parasitology (endo- and ectoparasites). In addition to screening the animals' health status, you should also evaluate the methods for packaging and shipping the animals from the vendor's facility to your facility. The preferred method is by dedicated truck, which transports the animals in a sanitized, climate controlled environment, directly from the vendor's barriers to the research facility.

Based upon the results of the health screen and shipping procedures, a health status classification (Class I, II or III) can be assigned to all animals scheduled for delivery from the breeding area you choose (Table 2). The assigned health classification dictates the receipt procedures for the rodent shipments, allows investigators to schedule their experiments based on release of animals, and has become an effective communication tool for our investigators and veterinary staff (Figures 1 and 2).

Class I animals are free of all known murine pathogens associated with spontaneous disease. For example, these rodents are negative for agents such as Sendai virus, corona virus, parvovirus, pneumonia virus of mice, ectromelia, mouse encephalomyelitis virus, lymphocytic choriomeningitis virus, reovirus, *Helicobacter* spp., CAR Bacillus, *Pasteurella* spp., *Streptococcus pneumoniae*, *Clostridium piliforme*, endoparasites and ectoparasites. Typically, rodents obtained from large, commercial animal vendors and animals purpose-bred in an in-house breeding program for internal supply can be considered Class I animals.

Class I animals must be shipped by ground transport in environmentally – controlled vehicles owned or contracted by the vendor. Each shipment of animals is received and examined by animal husbandry staff for compliance with order specifications and for the absence of overt clinical disease. The transport vehicle must be sanitized according to a standard operating procedure, may only transport other Class I animals, and must be evaluated for cleanliness and proper temperature. Upon receipt, mice are acclimated to the diet, caging, and environment of the facility in the Class I animal rooms or holding rooms for a minimum of 48 hours (Landi *et al.,* 1982) while rats are acclimated for a minimum of 72 hours (Capdevila *et al.,* 2006). These rodents are not required to go through a quarantine procedure. They are observed daily while in their acclimation period, and once it is over, they are released to the investigator for use (Figure 1).

Table 2.: Examples of Health Classifications Decisions

Health Status	Transportation Method	Health Classification
No pathogens detected in vendor breeding area, (Class I)	Vendor owned, environmentally controlled, only Class I animals on vehicle	Class I – acclimation then use
No pathogens detected in vendor breeding area, (Class I)	Vendor owned, environmentally controlled, animals of unknown health status on vehicle	Class II – quarantine, test and release to Class I if clean, move to Class III if infected
No pathogens detected in vendor breeding area, (Class I)	Vendor owned, environmentally controlled, animals held in transfer station for 24 hours	Class II – quarantine, test and release to Class I if clean, move to Class III if infected
No pathogens detected in vendor breeding area, (Class I)	Air transportation from Europe or US	Class II – quarantine, test and release to Class I if clean, move to Class III if infected
Class I colony of research animals, disease detected during sentinel monitoring	NA	Class III – quarantine and re-derive to Class I
Class III animals from breeding source	Shipped with Class I animals	Class III – quarantine and re-derive as Class I. All Class I animals on shipment become Class II.
No pathogens detected in vendor breeding area, limited experience with vendor, currently Class I	Vendor owned, environmentally controlled, animals of unknown health status on vehicle	Class II – quarantine, test and release to Class I if clean, move to Class III if infected
No pathogens detected in vendor breeding area, (Class I), but did not have time to conduct vendor area screen before animals were shipped	Vendor owned, environmentally controlled, animals of unknown health status on vehicle	Class II – quarantine, test and release to Class I if clean, move to Class III if infected

Class II animals fall into 3 categories. 1) They may be Class I animals that are coming from a source that you have little experience with, and you have decided to take extra precautions before releasing them to the investigator. 2) They may be Class I animals that were shipped by some means of transportation that allowed the boxes to be exposed to animals of unknown health status. This may be air transportation, ground transportation in a non-vendor vehicle or on a route where animals are off-loaded and held in common holding rooms at a transfer station, or quarantine facilities at airports. Due to these shipping procedures, you decide to take extra precautions. Even if a new vendor provides sentinel results indicating that their animals are healthy, the animals are considered Class II until diagnostic tests verify their health status. 3) They may be animals that previously had a disease (Class III animals), have gone through a rederivation program, and are awaiting testing to confirm their Class I status.

Class II animals are received into a quarantine area that is separate from the Class I animal holding and acclimation area. They are typically housed in semi-rigid isolators or an equivalent isolation system. The transport vehicle and shipping containers are thoroughly evaluated when the animals are received into the facility. A veterinarian evaluates the animals upon receipt, and samples of blood, tissue and feces are collected for serologic, microbiologic, parasitologic and PCR evaluations. In many cases it is helpful to order additional animals for testing. If the results are negative, the animals remain in quarantine and the serologic testing is conducted three weeks later since it takes approximately 21 days for rodents to produce a detectable level of antibodies to viral pathogens. If the results are negative, the animals can be released to the Class I area for investigator use. If the results are positive, the animals are assigned Class III status and are moved into the Class III quarantine area (Figure 1).

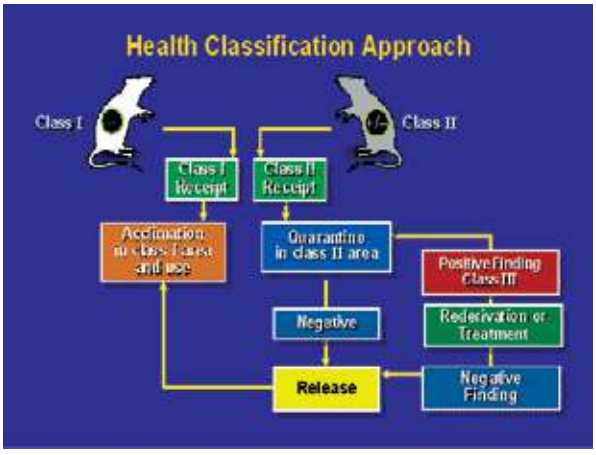

Figure 1. A schematic drawing of the process for receiving Class I and Class II animals into an animal facility.

Class III animals are those that are known to harbor significant pathogens and should not knowingly be received into a Class I or II animal holding or quarantine area. Class III animals should be received directly into a quarantine facility or area that can perform rederivation (Figure 2). The animals should be rederived and offspring should be tested to ensure that they are free from known murine pathogens. Once their pathogen-free status is confirmed, they can be transferred to the Class I area or released to the investigator for use. If the Class III animals must be used for research prior to rederivation, the study must be conducted in an isolator or in the quarantine facility. The issue of using Class III animals in research must be thoroughly evaluated as it might compromise research.

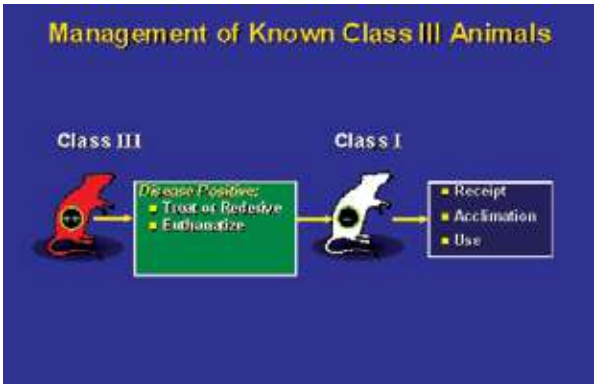

Figure 2. A schematic drawing which outlines the process of receiving known Class III animals (disease positive). Class III animals may not be received directly into Class I animal facilities. They must be received into a separate quarantine area and undergo a treatment or rederivation process.

Conclusion

High quality research requires high quality animals. Many if not all of the common rodent diseases can alter the animals' physiology, resulting in unwanted variables in research. Therefore, it is imperative that the health status of the animals be maintained at the highest standards possible.

The rodent health classification system described in this paper has been used to establish internal standards for health status, identify preferred delivery methods, manage acclimation and quarantine of animals, facilitate discussions with our research staff about animal receipt and release procedures, and align our response to sudden changes in the health status within the facility.

In discussions with our investigators, we reference the health classification of the animals, and they immediately know how their animals will be managed in our facility. If we indicate to an investigator that we have identified an infection in our colony, they realize that their animals have gone from Class I to Class III, and that this new classification imposes an entirely new set of management procedures on the access and use of their animals.

An effective, regular health monitoring and surveillance program, along with an established health classification system, makes an animal facility and associated research very credible.

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