Abstract

Although some previously common infections, such as Sendai virus and Mycoplasma pulmonis, have become rare in laboratory rodents in North American research facilities, others continue to plague researchers and those responsible for providing biomedical scientists with animals free of adventitious disease. Long-recognized agents that remain in research facilities in the 21st century include parvoviruses of rats and mice, mouse rotavirus, Theiler’s murine encephalomyelitis virus (TMEV), mouse hepatitis virus (MHV), and pinworms. The reasons for their persistence vary with the agent. The resilience of parvoviruses, for example, is due to their resistance to inactivation, their prolonged shedding, and difficulties with detection, especially in C57BL/6 mice. Rotavirus also has marked environmental resistance, but periodic reintroduction into facilities, possibly on bags of feed, bedding, or other supplies or equipment, also seems likely. TMEV is characterized by resistance to inactivation, periodic reintroduction, and relatively long shedding periods. Although MHV remains active in the environment at most a few days, currently prevalent strains are shed in massive quantities and likely transmitted by fomites. Pinworm infestations continue because of prolonged infections, inefficient diagnosis, and the survivability of eggs of some species in the environment. For all of these agents, increases in both interinstitutional shipping and the use of immunodeficient or genetically modified rodents of unknown immune status may contribute to the problem, as might incursions by wild or feral rodents. Elimination of these old enemies will require improved detection, strict adherence to protocols designed to limit the spread of infections, and comprehensive eradication programs.

Key Words: mouse hepatitis virus; parvovirus; pinworms; rodents; rotavirus; Theiler’s murine encephalomyelitis virus (TMEV)

Introduction

Many infectious agents, such as Sendai virus, Mycoplasma pulmonis, and cestodes, have yielded to modern management practices and are now rare in laboratory rodent facilities (Clifford and Cosentino 2006a,b; Livingston and Riley 2003), although undoubtedly extant in wild (Becker et al. 2007) and pet rodent populations as well as possibly in biological material archived in freezers. However, several other infectious agents—such as some of the parvoviruses, mouse rotavirus (epizootic diarrhea of infant mice, EDIM), Theiler’s murine encephalomyelitis virus (TMEV), mouse hepatitis virus (MHV), and pinworms—continue to bedevil laboratory animal managers and investigators and occasionally even large commercial vendors. In this article we examine some of the reasons why these old enemies still haunt us.

Philosopher and writer George Santayana (1863-1952) famously said, “Those who cannot remember the past are condemned to repeat it” (Santayana 1905). Accordingly, a brief look at changes in laboratory animal disease prevalence in the past few decades may be instructive.

Changes in Prevalence Since the 1980s

In the late 1980s and early 1990s (Casebolt et al. 1988; Jacoby and Lindsey 1998; Lussier and Descoteaux 1986), viruses prevalent in laboratory mice included Sendai virus, MHV, EDIM, and the only parvovirus of mice known at that time, minute virus of mice (MVM). In rats the picture was similar, with prevalent viruses including Sendai virus, coronavirus (also called sialodacryoadenitis virus, SDAV), and the rat parvoviruses that were known at that time. In addition to the viruses, pinworms were prevalent in both mice and rats.

As of 2007, only two of the agents (Sendai virus and Mycoplasma pulmonis) that were prevalent in North America 20 years ago had essentially disappeared. Coronavirus in rats also appear to be uncommon now, although sporadic outbreaks occur. Parvoviruses remain prevalent in both mice and rats, and the previously known serotypes have been joined by those more recently discovered. Also

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1Abbreviations used in this article: MHV, mouse hepatitis virus; TMEV, Theiler’s murine encephalomyelitis virus
still prevalent are EDIM, TMEV, MHV, and pinworms (Clifford and Cosentino 2006a,b; Livingston and Riley 2003).

Why have Sendai virus and M. pulmonis more or less vanished while the others remain? General reasons for the continued prevalence of infectious agents, despite knowledge of their existence and of their likely or confirmed interference with research, may be somewhat arbitrarily divided into institutional reasons and reasons intrinsic to the biology of the specific agents. Before a more detailed discussion of each agent, a brief overview may be helpful.

**Institutional Practices**

Possible reasons relating to institutional practices responsible for the continued prevalence of some infectious agents over the past 15 to 20 years include either an increase in the number of facilities or institutions that conduct rodent disease surveillance or ambivalence toward eradication. Published reports of the number or percentage of research institutions that conduct health surveillance are not available, nor are there reports indicating whether institutions include more of their intramural facilities in surveillance programs. Facilities newly subject to surveillance may turn out to harbor previously unsuspected infectious disease. The current trend toward incorporating additional facilities into monitoring programs could thus foster the impression of continued disease prevalence as detected by the percentage of positive samples processed by diagnostic laboratories. Because data are not available, no conclusions can be drawn; however, any impact of more institutions beginning to monitor will be diluted by the great majority of institutions that already have routine surveillance programs. In addition, there has been no impact from institutions initiating health monitoring programs for other diseases such as M. pulmonis and Sendai virus.

Other possible reasons that some adventitious infections remain prevalent might be apathy or ambivalence about the need to take the steps necessary for eradication, or reappearance despite a good faith eradication attempt. Some investigators simply do not see the necessity of interrupting their research for an attempt to eliminate an infection that may have no impact on their work, especially if the success of the eradication is not guaranteed or if treatment or rederivation may result in animals that could respond differently in their research. Or one group may wish to eliminate an infectious agent while others resist such efforts. As a result, eradication attempts may be half-hearted, doomed to failure, and result in a loss of credibility for the animal management staff.

Finally, the shipping of rodents, especially genetically modified mice, between research facilities has burgeoned in the last decade and is a recognized risk of contamination for receiving facilities. Whereas in the past the primary risk associated with incoming rodents was from occasional contamination at a handful of major vendors, now there is a similar or greater level of risk from each of the dozens or scores of collaborators that ship rodents between institutions (Mahabir et al. 2008).

**Biology and Environmental Persistence**

Agent-specific biology plays a critical role in determining which agents remain prevalent. Of the viruses that persist from decades past, all except MHV remain active in the environment for more than a few days and many can also be shed for prolonged periods (e.g., KRV may be shed for 2 months). Long-term shedding of an agent that remains infectious in the environment for a long time makes elimination very difficult. Other agents, especially MHV, are highly contagious and can spread very rapidly if the type of housing or research manipulation permits lateral transmission. Agent-specific aspects are considered more fully below.

One might ask, “So what? What is the problem with continued low prevalence of subclinical infections, especially if the responsible scientists express little concern?” There are at least two important problems raised by low-prevalence subclinical infections. First, any nidus of infection in any part of an institution poses a risk of contamination to the remainder of the facilities. Second, many research measurements are increasingly precise, and some, such as various cytokines, are exquisitely sensitive to perturbation from even subclinical and transient infection. Given that for many viruses there is a dose-response effect, that not all animals will be exposed to the same dose or in the same week, and that response to infection varies among individual animals, even subclinical infections can increase variability in all parameters they influence. As a result, there is a growing clash between investigators who study subtle variations in metabolic, immunologic, or transcriptional responses and others who are reluctant to endure the cost, research delays, or potential changes in their models that might result from infection eradication campaigns.

**Parvoviruses**

**Why They Persist**

As discussed elsewhere, parvoviruses remain, both individually and as a group, among the most prevalent viruses in laboratory rats and mice (Besselsen et al. 2008; Carby 2008; Clifford and Cosentino 2006a,b; Livingston and Riley 2003). In light of the significant biological similarities among rodent parvoviruses, we consider the reasons for their continued presence in general.

The primary reasons for the ongoing prevalence of parvoviral infection probably relate to the difficulty of eradication rather than reintroduction: they remain infectious for long periods in the environment, are difficult to inactivate, are shed for long periods by infected animals, and are more difficult to detect than many other agents.

Because parvoviruses remain infectious in the environment for long periods (Yang et al. 1995), they may persist
in corners and crevices, on equipment and office supplies, and in machinery cooled by internal fans even after normal cleaning of countertops and cages has removed most or all infectious particles. Although there are no reports of parvoviruses “hiding” in electronic machinery such as computers, freezers, and ultrasound machines, PCR has detected a common arterivirus of swine (porcine respiratory and reproductive syndrome virus) in such machinery (Kaufold et al. 2005), demonstrating the potential for electronic equipment with internal cooling fans to accumulate virus present in animal rooms. Whether such potential reservoirs for viral particles can or do serve as a source of sufficient infectious virions to infect mice or rats is unknown, but the possibility should be considered in programs to eliminate virus from facilities, especially where previous eradication attempts have failed.

Parvoviruses also resist some common disinfectants and may therefore require exposure to relatively high heat or to oxidizing disinfectants (Prince et al. 1991). The efficacy of ionizing irradiation has not been determined for rodent parvoviruses, although 50kGy is effective for inactivation of the human parvovirus B19 (Grieb et al. 2005).

Infected animals shed parvoviruses for relatively long periods, with shedding of MVM for up to 4 weeks (Smith 1983), MPV for at least 6 weeks (Smith et al. 1993), and, in rats inoculated in infancy with Kilham rat virus (KRV), for more than 2 months (Jacoby et al. 1991). The duration of shedding for rat parvovirus (RPV) and rat minute virus (RMV) is unknown.

Detection of low-prevalence parvovirus contamination among groups of animals can also be difficult for several reasons relating to their biology and unfortunately compounded by modern management practices. In certain circumstances common to most contemporary research facilities, parvoviral infection can have a lower prevalence than many other infections. As rats and mice mature, they are less susceptible to parvoviral infection and must be exposed to a larger dose in order to seroconvert (Besselsen et al. 2000, 2006). Also, one of the most commonly used strains of mice, the C57BL/6, is relatively resistant to infection, requiring an infectious dose of 10- to 100-fold the dose for a BALB/c or CD-1 mouse (Besselsen et al. 2000; Shek et al. 2005).

Last, parvovirus may be reintroduced at institutions that have conducted successful eradication campaigns. It is difficult to quantify the risk of reintroduction from sources external to an institution (e.g., from vendors or collaborators, or from feed or other supplies) relative to the risk from internal reservoirs (e.g., materials in freezers, wild or feral rodents, or contaminated equipment or even heating, ventilation, and cooling systems). Although it is always appropriate to examine all possible sources, such efforts often do not reveal the “smoking gun”; the most prudent approach to prevent a recurrence is to address as many of the potential sources as is feasible, not just the one or two considered most likely responsible for the latest contamination.

For the future, the best hope may be the development of tools for better detecting low-prevalence paroviral infection—for example, improved monitoring technology for racks of individually ventilated cages. Initial work in this area has begun (Compton et al. 2004b,c; Smith et al. 2007), although the sensitivity of such approaches is as yet too low to serve as more than a supplement to the standard soiled bedding sentinel system.

Exposure and Prevalence

Because, presumably, distribution of infectious paroviral virions is not uniform in the environment or in cages, animal exposure also is not uniform. Thus it is possible that only an occasional animal is exposed to an infectious dose, even though an entire group of animals may seem to have had the same exposure history. Furthermore, transfer of small amounts of soiled bedding in a sentinel program may only sporadically transfer infection, and individual cage-level bioexclusion systems, such as individually ventilated cages, severely inhibit cage-to-cage transmission.

Effective exposure requires moving an infectious dose of parovirus to the cage of a sentinel that may be past the age of peak susceptibility, then hoping the infected fecal pellet finds its way to the top of the bedding and is consumed by the sentinel. Notwithstanding the challenges, transfer of infection to sentinels is the critical step necessary for paroviral detection, although an occasional sentinel may test positive without confirmation of infection in any of the colony animals. For more detailed discussion of the difficulties of infection monitoring of animals housed in cage-level bioexclusion systems, see the articles in this issue by Peterson (2008), Shek (2008), and Watson (2008).

Very low prevalence of paroviral infection in animals housed in cage-level bioexclusion systems leads to sporadic positive results in sentinels and makes management decisions very difficult. The problem is compounded by the fact that infection of one animal in a cage does not always lead to enough viral shedding to infect cagemates, as is true with MPV (Besselsen et al. 2007). Testing every animal may be necessary to exclude the possibility of infection, although the slight increase in paroviral detection may not justify the increased costs. A low prevalence of sporadic positive results in sentinels without confirmatory positives in the source colony can lead to questions of false positives and whether the sentinels were truly negative on arrival; others (e.g., Shek 2008) have addressed these issues.

Uncertainty from monitoring results can make it difficult for a laboratory facility manager to gain the political capital necessary to effect paroviral eradication—with the requisite testing, environmental disinfection, procedural changes, delays to research, and costs of testing—especially as success is difficult to guarantee. Because paroviruses are difficult to detect and inactivate, incomplete eradication attempts are unlikely to succeed; and once an eradication attempt fails, it may be difficult to muster sufficient interest, commitment, or funding for a second attempt.
Effects on Research

Research effects of parvovirus infection of rats and mice include well-demonstrated and long-lasting effects on the immune system, oncology studies, and lymphocyte cultures (Besselsen et al. 2008; Ellerman et al. 1996; Jacoby et al. 1996; MCKisic et al. 1995). But despite the substantial literature of parvoviral interference with research, it is sometimes difficult to garner the necessary commitment from principal investigators. A gain, the low prevalence of paroviruses is an important factor: in many instances, only a very small minority of animals are infected, and among these the systemic effects are often subtle and relevant to only a few research areas. Effects of the virus on infected cells may be more significant (productive infection is cytopathic), but very few cells are productively infected so the overall effect on a tissue can be small. Therefore, for most research, any individual animal is unlikely to be affected, and effects on a small percentage of animals in a group are unlikely to change overall study conclusions.

Minimal impacts naturally lead to ambivalence among scientists about the need for parvoviral eradication, as such measures are highly disruptive to research and almost certainly, if temporarily, more so than the parvovirus “problem” itself. This ambivalence is unfortunate, because interdisciplinary research is increasing and the impact of parvoviral (and other) infections at the molecular level is being increasingly explored in many research areas. Parvovirus infection may not only directly confound research results but also inhibit collaboration as veterinarians attempt to limit the spread of infection within and between institutions.

Rotavirus

Mouse rotavirus infection, caused by a group A rotavirus and also known as epizootic diarrhea of infant mice (EDIM), was widespread in previous decades (Carty 2008) and remains moderately prevalent in research facilities (Clifford and Cosentino 2006a,b; Livingston and Riley 2003). The reasons for its continued prevalence resemble those for rodent parvoviruses: rotavirus are nonenveloped and remain infectious in the environment, with relative resistance to many disinfectants; as with parvoviruses, the use of oxidizing disinfectants is recommended (Prince et al. 1991).

But the epidemiology of rotavirus differs significantly from that of parvovirus (Ward et al. 2007). Although all ages of mice are susceptible to infection, disease occurs only in mice infected before 2 weeks of age and only if no maternal antibodies have been transferred in the milk. Because of this narrow window of susceptibility, disease from rotavirus infection is rare in mice. In addition, the virus is shed in large amounts but for less than 1 week after infection in immunocompetent mice (viral clearance is dependent on both B and T cell function).

Although the environmental hardness of rotavirus contributes to its continued presence in research facilities, the impression at one major rodent diagnostic laboratory is that mouse rotavirus appears in sporadic outbreaks rather than simmering as a low-level presence, as is often the case for parvoviruses (CBC personal communication with WR Shek, Charles River Laboratories, May 2007). If true, this suggests that the introduction, or reintroduction, of mouse rotavirus in research facilities may occur with some frequency. In contrast, parvovirus, as discussed above, may persist in facilities primarily because of incomplete eradication, compounded by difficulties in detection.

Rotavirus can be introduced into an animal facility by imported mice, by wild or feral mice, by contaminated feed, bedding, or other supplies, or by personnel who handle infected rodents elsewhere. It is impossible to eliminate any of these possibilities, but some can be somewhat discounted. A natural, and reasonable, early consideration in investigating the source of a sudden rotavirus outbreak at a research facility is the health status of mice imported from vendors or other institutions. Although it is important to examine and reexamine sources of incoming mice, it is easy to screen immunocompetent mice for seroconversion to rotavirus, as such seroconversion is rapid and there is excellent cross reaction among group A rotaviruses (Ward et al. 2007). In addition, because infected mice shed for only a few days, this route of facility contamination is unlikely unless actively shedding mice are imported.

The exception is for immunodeficient mice (Ward et al. 2007). Recent work has indicated the necessity of both T and B cells for normal rotaviral clearance. Severe combined immunodeficient (SCID) mice also have prolonged shedding. Thus it is possible that immunodeficient mice that do not undergo screening either by fecal PCR or by immunocompetent sentinel mice could introduce the infection into a facility.

Wild and/or feral mice can also serve as the source of rotaviral infection, but this is probably not a major risk factor. If the wild or feral population is endemic to a facility, then mice are likely to have mild infection while still partially protected by maternal antibody and thus clear the virus before weaning. Foraging mice are less likely to shed rotavirus than many other agents, although they could certainly act as fomites. A sudden rotavirus contamination concomitant with other infections common in wild mice and either rare in laboratory mice (such as mouse cytomegalovirus, lymphocytic choriomeningitis virus, or LCMV) or otherwise absent in the facility might suggest contamination from wild or feral mice, but rotaviral contaminations most often occur as single-agent events. Nonetheless, the cost of targeting or preventing wild or feral mouse incursions is considered money well spent.

Although cell lines, serum, and other biologically derived reagents have been the source of parvoviruses, LCMV, and other viruses, these materials do not seem to be a source of rotavirus and have not been detected among several thousand such samples tested by PCR at one major diagnostic laboratory (CBC personal communication with KS Henderson, Charles River Laboratories, May 2007), possibly because rotaviruses are present only briefly before the start of infection.
being cleared and because of their highly restricted cell tropism (only mature enterocytes at the tips of villi).

A more likely explanation for the sudden occurrence of mouse rotavirus at a research institution is the contamination of supplies or introduction by personnel who have handled or been exposed to contaminated material. For example, standard processing of feed and bedding may inactivate many viral agents, although it does not result in a sterile product. Contamination of entire lots of feed and bedding seems unlikely, given the generally localized nature of rotaviral contaminations (i.e., a single institution at a time), whereas contamination of entire lots of feed might reasonably be expected to result in nationwide outbreaks. A more probable, although unproven, mechanism of contamination is exposure of individual bags or pallets of feed or bedding to infected wild or feral mice. Mice have a keen sense of smell, and stored feed must be a powerful lure. Mice nesting on or near stored feed could easily contaminate the bags or contents with multiple agents. Nonenveloped viruses would be among the most likely infectious agents to survive time and desiccation, especially those shed in high quantities such as rotavirus. However, no published reports could be found to support either this hypothesis or the theory of introduction by humans carrying infectious material from other mice.

The impact of adventitious rotavirus infection on adult mice is probably low, although Baker (2003) has described actively infected mice that showed several alterations in intestinal physiology.

Unlike efforts with parvoviruses, rotavirus eradication at a facility is more likely to be successful. Because much of the prevalence of mouse rotavirus is due to sporadic outbreaks at individual institutions, a major goal in reducing rotaviral contaminations should be outbreak prevention. This can be effected by careful screening of the health status of incoming mice, and by either autoclaving all feed and bedding immediately before use or by purchasing irradiated feed and disinfecting the external wrapping before use. At a minimum, it is advisable to disinfect the external surfaces of feed and bedding containers. However, based on a recent abstract describing incomplete inactivation of parvovirus on a cardboard surface (Lee et al. 2007), spraying paper bags of feed and bedding with common disinfectants does not necessarily eliminate all risk. Rotaviral prevention strategies should also include personnel considerations such as personal protective equipment (PPE).

**Theiler’s Mouse Encephalomyelitis Virus (TMEV)**

Also known as GDVII (George’s disease 7, named after Theiler’s laboratory technician), TMEV is caused by a nonenveloped, single-stranded RNA virus. Its taxonomy has recently been clarified as one of two distinct serotypes of the species Theliovirus; the other is Encephalomyocarditis virus (genus Cardiovirus, family Picornaviridae). Theliovirus sp. consists of at least four viruses, all probably distinct serotypes: TMEV in mice, Theiler’s-like virus of rats (TLV or RTV), and two viruses of humans, Vilyuiisk human encephalomyelitis virus and Saffold virus (Jones et al. 2007). We focus on TMEV,2 of which GDVII is one isolate and the most frequent source of antigen for TMEV serology assays. Although these assays are sometimes called GDVII, they actually detect all TMEV strains as well as RTV.

TMEV remains moderately prevalent in laboratory mouse research facilities, as evident in serology results compiled by major diagnostic laboratories (Clifford and Consentino 2006a,b; Livingston and Riley 2003). There are no recent reports of clinical disease in immunocompetent mice due to TMEV infection, however, and the paralysis due to demyelination is essentially the result of experimental intracerebral inoculation with the virus.

Picornaviruses are moderately resistant to inactivation in the environment but susceptible to rapid inactivation at 50°C or by UV irradiation (Lipton et al. 2007). Oxidizing disinfectants can also be effective (Prince et al. 1991).

Reasons for the continued presence of TMEV are difficult to pinpoint. Infected immunocompetent mice may shed TMEV in the feces for as long as 53 days; the duration of shedding by immunodeficient mice has not been reported but is likely longer. As with the previously discussed viruses, it may be that imported mice serve as a source of facility contamination; certainly the long shedding period of TMEV would make it likely that mice infected near weaning may still be shedding at the time of shipment. Other infected populations of mice that could serve as occasional sources of TMEV contamination include wild or feral mice, pet mice, and mice raised as food for carnivorous reptiles, birds, and mammals. As with rotavirus, contamination of feed or bedding, especially the external surfaces of bags, could also be a source of TMEV infection. It is appropriate to test cell cultures and biologically derived reagents for TMEV as a matter of course, but PCR testing of thousands of cell cultures and other biological reagents at one major diagnostic laboratory did not find any evidence of TMEV contamination (CBC personal communication with KS Henderson, Charles River Laboratories, May 2007).

Serologic detection of TMEV by routine assays does not appear to be difficult, as there is good serologic cross reaction among strains. The virus remains active in the environment long enough that transfer of soiled bedding is considered an effective method of sentinel exposure. However, as with parvovirus, it is possible that individual cage-level bioexclusion may keep the prevalence of TMEV infection very low in a contaminated facility, which could complicate detection.

Investigators have recently described research impacts of TMEV infection, including immunologic effects (the 2RTV appears to have a higher prevalence than TMEV, but we do not discuss it here as it is an emerging disease, not an “old enemy.” Control measures are probably similar for both the rat and mouse viruses.)
virus infects macrophages) and, with experimental inoculation or in immunodeficient mice, affects a variety of cells of the central nervous system (Baker 2003; Lipton et al. 2007). Eradication of TMEV from research facilities depends on accurate detection of infected groups of mice, elimination of those that are infected (or shedding), and adequate cleaning and disinfection of contaminated materials. Prevention of recontamination entails the screening of incoming animals, disinfection of imported material, testing of biological materials, and proper use of PPE. Based on its moderate environmental stability, resistance to disinfection, and relatively long duration of shedding, the difficulty of TMEV eradication may be intermediate between that for parvovirus (more difficult) and rotavirus (less difficult). However, as with both parvoviruses and rotavirus, eradication requires commitment by all involved, as half-hearted attempts are likely to fail.

Mouse Hepatitis Virus

Mouse hepatitis virus (MHV) is the name for a group of coronaviruses that infect mice. Known since 1949, it remains among the most prevalent viruses of laboratory mice, following the recently discovered norovirus and the parvoviruses (Besselsen et al. 2008; Clifford and Cosentino 2006a,b; Livingston and Riley 2003).

There are many reasons why MHV should no longer be prevalent. As an enveloped, single-stranded RNA virus, it has little resistance to desiccation, heat, detergents, or disinfectants, and is probably inactivated in the typical animal environment within a few days, although there is some evidence that enveloped viruses can remain active for longer periods in feces. Furthermore, immunocompetent mice clear the infection, so viral shedding ceases within 1 to 4 weeks.

However, MHV is highly contagious and, if transmission is unimpeded by filter tops or other cage-level bioexclusion, spreads rapidly. As a result, unlike the situation with the parvoviruses, detection is facilitated by potentially higher prevalence as well as by excellent serologic cross reactivity among the myriad strains. The prevalence of MHV contrasts with that of the common coronavirus of rats, SDAV, which is now uncommon (Clifford and Cosentino 2006a). Nonetheless, a contamination with SDAV may be intermediate between that for parvovirus (more difficult) and rotavirus (less difficult). Thus, the MHV strains that are shed in large amounts and more readily transmitted by fomites are common, whereas those that require close contact between mice for transmission are uncommon.

MHV can spread like wildfire, although the use of individual cage-level bioexclusion may limit its prevalence in an institution. However, we note that the ease of MHV transmission by fomites was underscored in a recent study that reported the inadvertent transmission of the virus among individually ventilated cages even though the technicians understood it was a virus research study and assiduously followed strict procedures to avoid such transmission (Compton et al. 2004c). In immunocompetent mice, enterotropic MHV causes clinical disease only in nursing pups of MHV-naïve dams, so transmission of the virus by fomites contaminated by animals not known to be infected is likely a major reason for its continued prevalence. This problem is exacerbated by growth in the use of immunodeficient mice as well as in the use of genetically manipulated mice with unrecognized immunologic alterations (Barthold and Smith 2007). To reiterate, a probable major reason that MHV remains a problem in many research facilities is transmission via fomites.

Transmission of MHV by reservoir populations of pet, wild, or feral mice is also possible. The virus is present in wild populations (Becker et al. 2007) and is common in pet mice. In addition to the danger of disease transmission from incursions of wild mice into, or the roaming of feral mice within, research facilities, the high potential for spread of MHV by fomites extends to clothing and other materials brought into the research facility from external locations where they may have been contaminated by contact with infected mice or mouse droppings.

It is also advisable to consider the potential for facility contamination with MHV by means of contaminated feed or bedding, although this possibility seems less likely, given the susceptibility of the virus to inactivation in the environment. MHV is more likely to be spread from a relatively fresh source of contamination.

As with all viruses, there is the possibility of transmission by cell lines or biologically derived reagents (Mahabir et al. 2008). However, the cell tropism of enterotropic MHV strains is quite limited, suggesting their infrequent transmission by biological material. This observation seems to be supported by a major rodent diagnostic laboratory that tests more than 1000 cell lines and other biologics annually by PCR and that did not find MHV in any cell line or biological reagent submitted for screening (CBC personal communication with K.S. Henderson, Charles River Laboratories, May 2007). This finding suggests that cell lines and other biologics are rarely responsible for MHV outbreaks. Vigilance is nonetheless required, as a noninfected cell line from an infected mouse may still be contaminated with infectious host material (Barthold and Smith 2007).

Because the infrequent collection of soiled bedding for sentinels allows animals to clear infection before being sampled, and excessively aged soiled bedding allows the
inactivation of infectious material, in both cases infection may escape detection, especially if the prevalence is low. In practice, however, this has not seemed to be a problem and MHV has been transferred by soiled bedding (Compton et al. 2004c).

The research impact of adventitious MHV infection has been recently reviewed (Baker 2003; Barthold and Smith 2007), so we mention it only briefly. Most publications on MHV have dealt with respiratory strains, which are also called polytropic because of their ability to infect a variety of cell types (both in vivo and in vitro) and which have been shown to alter a wide range of host responses. Enterotropic strains typically infect only enterocytes. However, as stressed by Barthold and Smith (2007), MHV strains of intermediate tropism also exist, so the distinction between enterotropic and respiratory, although useful, is not absolute. Persistent and/or disseminated infection of enterotropic MHV in a wide range of mice with immunologic deficits (e.g., athymia, B cell deficiencies, interferon deficits) underscores the interactions with host defense mechanisms by even enterotropic strains (Compton et al. 2003, 2004a).

Elimination of MHV from research facilities is easier than for previously discussed agents because of its lack of environmental persistence. It depends to a great extent on controlling the spread of infection by fomites. Such efforts must be coupled with thorough testing, especially of mice with immune deficits or of unproven immune status such as many genetically modified mice, as well as in areas with breeding or with the introduction of new, potentially naïve mice that could perpetuate the infection. PCR may play an increasing role in detecting infection in immunodeficient or persistently shedding mice. Pest control and careful control of incoming animals are also necessary.

Pinworms

Despite the availability of anthelmintic treatments (Klement et al. 1996; LeBlanc et al. 1993; Lipman et al. 1994; Sueta et al. 2002) and barrier caging systems (Dillehay et al. 1996; LeBlanc et al. 1993; Lipman et al. 1994; Wescott et al. 1976), pinworms remain prevalent in barrier caging systems (Dillehay et al. 1996; LeBlanc et al. 1993; Lipman et al. 1994; Sueta et al. 2002) and laboratory mice and rats (Clifford and Cosentino 2006a,b; Suets et al. 2002) and are handled as a single problem, but the two genera have very different life cycles and thus require different approaches to diagnosis and control. A. tetraptera eggs (an average of 17 eggs per worm per 24h; Phillipson 1974) are shed in fecal pellets and require at least 5 days at room temperature for embryonation (A. nyla 1966a) before they become infectious. Once ingested, they hatch in the cecum and colon within 2 to 6 hours, undergo a period of development in the colonic crypts, and emerge as L3 larvae (A. nyla 1966b; Behnke 1974). By 8 days after ingestion larvae are found in the anterior colon, where they complete their development to adults (A. nyla 1966b; Behnke 1974). The life cycle requires at least 24 days for completion (A. nyla 1966b).

In contrast, eggs from Syphacia sp. are laid directly on the perianal skin and embryonate in 5 to 20 hours, fastest at 37°C and slower at room temperatures (Chan 1952; Taffs 1976). Once ingested, the eggs hatch in the small intestine and cecum within the first 2 hours, then complete their development to adults in the cecum (Chan 1952; Lewis and D’Silva 1986). Gravid females migrate to the anus and lay their eggs on the perianal skin before dying. S. obvelata lay approximately 350 eggs (Chan 1952) and S. muris 450 to 550 eggs per female; the life cycle is complete in as little as 12 days for S. obvelata and 7 to 8 days for S. muris (Lewis and D’Silva 1986). Autoinfection via ingestion of fecal pellets as they exit the anus is common; retroinfection via migration of larvae through the anus is not proven (Chan 1952).

Environmental Persistence

Environmental persistence of eggs no doubt contributes to the continued prevalence of pinworm infections. In the absence of an ovicidal disinfectant, cleaning to physically remove as many eggs as possible remains the principal method for routine decontamination—sound advice for eradication attempts for all infections, not just pinworms.

Eggs may contaminate ventilation ducts (Hoag 1961) or shared equipment or procedure areas (Huerkamp 1993) and can recontaminate a colony after the completion of treat-
ment. Knowledge of egg longevity in the environment is important to determine the need for environmental decontamination, but specific data are unavailable. *A. tetraptera* eggs are thought to be long-lived in the environment, remaining dormant for several months at 4°C (Stahl 1966). Anya (1966a) reported, however, that culturing newly shed eggs at 37°C accelerated embryonation, decreased the number of viable eggs, and reduced their longevity. In a study to determine methods to inactivate viable *S. muris* eggs, 100% inactivation occurred only with temperatures of 100°C for 30 minutes and ethylene oxide, although high kill rates with formaldehyde and chlorine dioxide suggested that these chemicals could be successful with adjustments to the protocol (Dix et al. 2004). Huerkamp and colleagues (2000) reported the eradication of *S. muris* without environmental decontamination, suggesting that the eggs in the environment may not have outlived the treatment period (fenbendazole in feed every other week for five treatments). *S. obvelata* eggs appear to be unstable—they are reported to survive only 42 hours under ideal conditions, and may be inactivated by drying or immersion in liquids (Chan 1952; Grice and Prociv 1993).

As noted above, *S. muris* eggs are resistant to most common disinfectants (Dix et al. 2004), and it is assumed that *A. tetraptera* eggs have similar properties. Physical methods (e.g., scrubbing with detergent, steam cleaning, or painting) are thus most likely to be effective for environmental decontamination. Biosafety cabinets used to protect mice from aerosolized pathogens may actually be a route to widespread egg dissemination given that eggs shed in the cabinet are resistant to the routine disinfectants used to prevent transmission of other pathogens between cages.

**Diagnostic Challenges**

Problems with diagnosis are another significant factor in continuing prevalence. Diagnosis in barrier caging systems usually relies on the detection of infection in sentinel mice exposed to soiled bedding (Brielmeier et al. 2006). But there are many opportunities for failure. For instance, the collection of soiled bedding from only a subset of cages may miss infected cages, particularly in nonbreeding colonies housed in barrier caging where the prevalence of patent infection could be low. And when infected colony cages are sampled, fecal pellets in the soiled bedding sample may not contain eggs. Both *S. muris* and *A. tetraptera* exhibit cyclic egg excretion, although there is some question about the period of peak excretion—*S. muris* eggs are shed mostly in the afternoon (Van der Gulden 1967), while *A. tetraptera* eggs are variably reported as shed mainly at dawn (Phillipson 1974) or in the afternoon (Bunte and Nolan 2006). Small bedding samples could easily miss the infected fecal pellets, particularly when one sentinel cage services many colony cages and there is pressure to take small samples of soiled bedding to avoid overloading the sentinel cage.

Stage of infection also affects the numbers of eggs shed. Immunocompetent mice develop resistance to *A. tetraptera* pinworms and harbor fewer worms later in the infection (Behnke 1976, 2007), and mice infected with *S. obvelata* largely cease shedding eggs by 14 weeks (Clarke and Perdue 2004) after infection. Conversely, *A. tetraptera* has a long (minimum 23 days) prepatent period before eggs are shed (Anya 1966b). Even when eggs are present in the bedding sample, they may be too new or too old to infect the sentinel: *A. tetraptera* eggs take at least 5 days to embryonate at 27°C, before which they are not infectious (Anya 1966a), and *S. obvelata* eggs are reportedly inactivated by drying or by immersion in water (Chan 1952).

A further problem with sentinel testing lies in its retrospective nature. A positive sentinel pinworm test indicates exposure to pinworm-infected bedding some time during the exposure period, which typically lasts at least 3 months. By the time of diagnosis, the positive colony cage(s) may no longer be present or may have moved to another area, complicating decisions about the size of the risk group for treatment.

Detection of pinworm eggs in samples presents challenges: it is labor intensive and prone to false negatives. Antemortem testing is the easiest but least sensitive method. Usual methods include flotation of fecal pellets in saturated salt or sugar solution for *A. tetraptera* (Baker 2007) and microscopic examination of clear cellophane tape applied to the anus (tape test) for *Syphacia* sp. (LeBlanc et al. 1993). Although centrifugation dramatically enhances the sensitivity of simple flotation of fecal pellets, many such pellets from mice with patent infections may be egg-free. A 24-hour cumulative sample is more definitive (Bunte and Nolan 2006). Tape tests may yield false negatives due to cyclic egg excretion in *S. muris* (Van der Gulden 1967) or a mature infection in *S. obvelata* (Clarke and Perdue 2004). As noted above, egg excretion decreases with increasing age and duration of infection (Behnke 1976, 2007; Chan 1952). For example, Baker (2007) refers to a study in which only 15% of *S. obvelata*-infected mice were positive by tape test at 7 weeks of age, compared with 100% at 4 weeks. Given that sentinels are typically exposed to soiled bedding for several months, they may no longer be regularly shedding eggs by the time they are tested. In *A. tetraptera*, male mice harbor more worms than females (Behnke 1976) and worm count also varies by mouse strain (Derothe et al. 1997).

The most dependable diagnostic method is also the most time consuming: demonstration of adult worms in the cecum (for *Syphacia* sp.) or rugal folds of the proximal colon (for *A. tetraptera*) (Anya 1966b). Although worms are relatively easy to see in the colon, cecal contents are somewhat murky; opening the cecum in a petri dish with a small amount of warm water causes the adults to migrate away from the fecal mass into the liquid where they are easier to see.

**Effects on Research**

The potential research effects of pinworms and treatment regimens may inform decisions about whether to treat or
ignore facility infections. There have been a number of reports of pinworm effects on research, although many are in the older literature and open to question. Because most are retrospective evaluations of data after a change in research results, it is difficult to be confident that pinworms were the actual or sole cause of the altered results. In addition, many of the same studies failed to provide health monitoring data, thus results could have been due to concomitant infections.

For example, one study reported that infection with S. muris slowed the growth of male germ-free Lobund-Wistar rats (Wagner 1988), but there was no health monitoring and other studies have not confirmed this finding. Another study reported infection with S. muris impaired intestinal electrolyte transport in spontaneously hypertensive rats (SHR) and in Wistar-Kyoto (WKY) rats (Lubcke et al. 1992), but it was not a controlled prospective study, the WKY rats had a higher worm burden (but milder changes) than the SHR, and again there was no health monitoring. A n oft-cited study (Mohn and Phillipp 1981) of the deleterious effects of pinworms in mice reported that two strains of mice with heavy infections of S. muris, the rat pinworm, grew slowly. Again this was a retrospective study without health monitoring information, and the data did not support the assertion of slow growth. An earlier, prospective study (McNair and Timmons 1977) that used experimental challenge with 200 to 300 eggs reported that infection with S. obvelata, but not A. tetraptera, depressed exploratory activity in mice. However, again there was no health screening and the result has not been duplicated in subsequent literature.

More recent reports have convincingly demonstrated the impact of pinworms on immunological research. S. obvelata infection induces a protective T helper cell type 2 (Th2) cytokine response, with elevated interleukins and circulating IgG (Agersborg et al. 2001; Michels et al. 2006), and these changes in host defense mechanisms can affect research unrelated to parasites. For example, S. obvelata-infected AKR mice mounted an increased antibody response to sheep red blood cells (Sato et al. 1995), and S. obvelata-infected BALB/c mice immunized against ovalbumin showed an increased allergic response to antigenic challenge (Michels et al. 2006). It has also been suggested that early infection with helminths may reduce subsequent atopy by increasing the proportion of Th2 to Th1 responses, although this is unproven (Mao et al. 2000; Weiss 2000). Studies have also demonstrated reduced incidence of insulin-dependent diabetes mellitus in nonobese diabetic mice with other helminths (Cooke et al. 1999; Imai et al. 2001) due to a switch to Th2 dominant responses, and a similar effect can be predicted with pinworms for the same reason (Franke and Shirwan 2006; Michels et al. 2006).

As with other pathogens, variations in exposure to pinworm infection, stage of infection, and host resistance are likely to increase variability in affected research parameters. The broadest impact, however, may be felt in the inhibition of interinstitutional transfers of mice for research collaboration.

Treatments and Their Effects

Treatments intended to eradicate pinworms can also affect research. Pritchett and Johnston (2002) published an excellent review of a range of available treatments. The two most commonly used are ivermectin and fenbendazole because of their relative safety, but both can affect research.

Ivermectin

Ivermectin is a macroline antibiotic in the avermectin group originally derived from a Japanese soil fungus, Streptomyces avermitilis. Administered orally or topically (Klement et al. 1996; LeBlanc et al. 1993), it is an agonist for the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). Its usefulness as an anthelmintic results from differences in the distribution of GABA receptors between mammals and helminths: GABA receptors in mammals are mostly in the central nervous system (CNS) protected by the blood brain barrier, whereas in arthropods and nematodes they are found in the peripheral nervous system at the neuromuscular junction.

Stimulation of GABA receptors in nematodes causes flaccid paralysis and subsequent expulsion (Roder and Stair 1998). Ivermectin toxicity (depression, coma, and death) has been reported in adult mice due to P-glycoprotein deficiencies in nervous system capillary endothelium. P-glycoprotein plays a role in the blood brain barrier, acting as an efflux pump to prevent the entry of specific drugs into the nervous system (Saio et al. 2001). Mice with abnormal P-glycoprotein, and thus ivermectin sensitivity, include a subpopulation of CF-1 mice (Lankas et al. 1997) and mice homozygous for disruption of the Abcb1a (previously known as mdr1a) P-glycoprotein gene (Didier and Loor 1996; Saito et al. 2001). Reports have described ivermectin toxicity in neonatal rodents (Lankas et al. 1997; Skopets et al. 1996), perhaps because P-glycoprotein protein expression in brain capillary cells is incomplete until postnatal day 21 (Tsai et al. 2002). Ivermectin treatment has also been reported to cause subtle effects on behavioral testing (Davis et al. 1999).

Fenbendazole

Fenbendazole treatment, administered in food at between 150 ppm and 450 ppm, has proven safe and effective for pinworm eradication in rodents (Coghlan et al. 1993; Huerkamp et al. 2000, 2004). Although it was originally thought to have limited systemic absorption, data from biliary excretion studies suggest >50% absorption and subsequent liver metabolism, primarily to the active form, fenbendazole sulfoxide (Short et al. 1988). Fenbendazole acts as an inhibitor of microtubule polymerization and is
most active against developing stages (eggs and larvae) (Lacey 1988).

Fenbendazole is very safe; the oral LD<sub>50</sub> for rats and mice is in excess of 10,000 mg/kg (Duwel 1977) and there are no reports of toxicity in research rodents. Its safety margin results from a much greater affinity for nematode tubulin than mammalian tubulin at 37°C due to slower dissociation (Lacey 1990); interestingly, benzimidazole-resistant helminths have a greater proportion of low-affinity tubulin (Lacey and Gill 1994). Although apparently safe, treatment with oral fenbendazole was reported to reduce litter size in Sprague-Dawley (SD) but not GEPR-9 rats (Johston et al. 2006). Unfortunately, feed consumption and body weight were not measured, both of which could affect litter size. In addition, statistical significance was found only with continuous treatment of SD rats, in which there was dramatic difference in ages between groups: treated rats averaging 297 days of age produced smaller litters, whereas untreated rats averaging 142 days of age produced larger litters. Studies have found no effect (Barlow et al. 2005; Keen et al. 2005) or only minor effects of fenbendazole treatment on behavioral testing (Barron et al. 2000).

Environmental Decontamination

Environmental decontamination may not be necessary if all at-risk mice are treated for a period exceeding the maximum time that eggs persist in the environment plus the time required for anthelminthics to eradicate worms from the mice. However, in the absence of data on the longevity of eggs in the environment, environmental decontamination seems prudent. The lack of an ovicidal disinfectant remains a major problem, as does the challenge of locating and decontaminating equipment that may harbor pinworm eggs. It is particularly important to decontaminate biosafety cabinets and change stations to prevent them from serving as sources for widespread dissemination. For A. tetraptera outbreaks, steam cleaning or scrubbing surfaces with detergent at frequencies of less than 5 days (i.e., twice weekly) should prevent the transfer of embryonated eggs from one user to another via fomites. The rapid embryonation of Syphacia sp. (within hours) necessitates decontamination after each user to prevent spread.

Conclusions

We have considered some of the reasons for the persistence of adventitious infection by several “old enemies,” agents that have been known for a long time—paroviruses, rotavirus of mice, TMEV, MHV, and pinworms. Although other infections have dramatically declined in prevalence, these continue to plague laboratory animal managers and researchers alike.

Evaluation of the reasons for the ongoing presence of these infections is useful if it can inform future eradication attempts; the reasons presented here draw on the scientific literature but are also, necessarily and admittedly, somewhat speculative.

- All of these old enemies cause subclinical infection, requiring laboratory testing for detection.
- The increased use of animals with known or unrecognized immune deficits probably contributes to the continued existence of these agents.
- The sharing between institutions of rodent lines with incompletely characterized health status likely allows the geographic spread of these infections.
- Most of the agents are shed for prolonged times (MHV and rotavirus infection of immunocompetent mice are the exceptions).
- Enterotropic MHV may persist, despite its environmental fragility relative to the other agents, because of its capacity, enabled by massive viral shedding, for explosive spread in a facility.
- Parovirus, rotavirus, TMEV, and pinworms remain active in the environment for relatively long periods, complicating both eradication and exclusion.
- The use of individual cage-level bioexclusion such as individually ventilated caging, which can keep the prevalence low, may actually hamper detection of paroviruses, pinworms, and other infections.

It is possible to facilitate prevention by requiring strict adherence to long-established control measures—for incoming animals and materials, potential fomites in a facility, and personnel practices—rather than merely detecting contamination after it occurs. Improved diagnostic technologies will enable more confident determination of the health status of each rack of cages or even each individual animal, where necessary, as well as confirmation of the disinfection status of a wide variety of supplies and equipment.

Once scientists can sufficiently and confidently pinpoint contaminated animals and materials in a facility, and exercise effective control of all incoming animals and materials, it may finally be possible to successfully eradicate these old enemies.

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