Chapter 3.2

Viral Infections of Laboratory Mice

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Introduction

In interpreting the microbiological status of laboratory animals, it must be understood that infection and disease are not synonymous. *Infection* refers to the invasion and multiplication of microorganisms in body tissues and may occur with or without apparent disease. *Disease* refers to interruption or deviation from normal structure and function of any tissue, organ or system. Many of the infections with which we are concerned may not cause discernable disease in many strains of mice. However, they may cause inapparent or subclinical changes that can interfere with research. Such interference often remains undetected, and therefore modified results may be obtained and published.

The types of interference of an agent with experimental results may be diverse. There is no doubt that research complications due to overt infectious disease are significant and that animals with clinical signs of disease should not be used for scientific experiments. But clinically inapparent infections may also have severe effects on animal experiments. There are numerous examples of influences of microorganisms on host physiology and hence of the interference of inapparent infections with the results of animal experiments. Many microorganisms have the potential to induce activation or suppression of the immune system, or both at the same time but on different parts of the

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immune system, regardless of the level of pathogenicity. All infections, apparent or inapparent, are likely to increase interindividual variability and hence result in increased numbers of animals necessary to obtain reliable results. Microorganisms, in particular viruses, present in an animal may contaminate biological materials such as sera, cells or tumours [1, 2]. This may interfere with *in vitro* experiments conducted with such materials and may also lead to contamination of animals [3]. Mouse antibody production (MAP) testing or polymerase chain reaction (PCR) testing of biologics to be inoculated into mice is an important component of a disease prevention programme. Finally, latent infections may be activated by environmental factors, by experimental procedures, or by the combination and interaction between various microorganisms. For all these reasons, prevention of infection, not merely prevention of clinical disease, is essential.

Unfortunately, research complications due to infectious agents are usually considered artefacts and published only exceptionally. Information on influences of microorganisms on experiments is scattered in diverse scientific journals, and many articles are difficult to find. To address this problem, several meetings have been held on viral complications on research. The knowledge available is summarized in conference proceedings [4, 5] and has later repeatedly been reviewed [6–8].

Viral infections of mice have been studied in detail, and comprehensive information on their pathogenic potential, their impact on research, and the influence of host factors such as age, genotype, and immune status on the response to infection is available. The nomenclature and taxonomy of viruses is described based on recent nomenclature rules by the International Union of Microbiological Societies [9] and the Universal Virus Database of the International Committee on the Taxonomy of Viruses (http://www.ictvdb.org). Retroviruses are not covered in this chapter because they are not included in routine health surveillance programmes and cannot be eradicated with the methods presently available. This is because most of them are incorporated in the mouse genome as proviruses and thus are transmitted via the germline.

The ability to accurately determine whether or not laboratory animals or animal populations have been infected with a virus depends on the specificity and sensitivity of the detection methods used. Most viral infections in immunocompetent mice are acute or short term, and lesions are often subtle or subclinical. The absence of clinical disease and pathological changes has therefore only limited diagnostic value. However, clinical signs, altered behaviour or lesions may be the first indicator of an infection and often provide clues for further investigations.

Serology is the primary means of testing mouse colonies for exposure to viruses, largely because serological tests are sensitive and specific, are relatively inexpensive and allow screening for a multitude of agents with one serum sample. They are also employed to monitor biological materials for viral contamination using the MAP test. Serological tests detect specific antibodies, usually immunoglobulin G (IgG), produced by the host against the virus and do not actually test for the presence of the virus. An animal may have been infected, mounted an effective antibody response and cleared the virus, but remains seropositive for weeks or months or for ever, even though it is no longer infected or shedding the agent. Active infection can only be detected by using direct detection methods such as virus isolation, electron microscopy or PCR. Meanwhile, PCR assays have been established for the detection of almost every agent of interest. They are highly sensitive and, depending on the demands, they can be designed to broadly detect all members of a genus or only one species. However, good timing and selection of the appropriate specimen is critical for establishing the diagnosis. In practice, combinations of diagnostic tests are often necessary, including the use of sentinel animals or immunosuppression to get clear aetiological results or to avoid consequences from false-positive results.

Reports on the prevalence of viral infections in laboratory mice throughout the world have been published frequently. In general, the microbiological quality of laboratory mice has constantly improved during the last decades, and several agents (e.g. herpesviruses and polyomaviruses) have been essentially eliminated from contemporary colonies due to advances in diagnostic methodologies and modern husbandry and rederivation practices [10–15]. They may, however, reappear, since most have been retained or are still being used experimentally. Furthermore, the general trend towards better microbiological quality is challenged by the increasing reliance of biomedical research on genetically modified and immunodeficient mice, whose responses to infection and disease can be unpredictable. Increasing numbers of scientists are creating genetically modified mice, with minimal or no awareness of infectious disease issues. As a consequence, these animals are more frequently infected than 'standard' strains of mice coming from commercial breeders, and available information on their health status is often insufficient. Frequently they are exchanged between laboratories, which amplifies the risk of introducing infections from a range of animal facilities. Breeding cessation strategies that have been reported to eliminate viruses from immunocompetent mouse colonies may prove to be costly and ineffective in genetically modified colonies of uncertain or incompetent immune status. It must also be expected that new agents will be detected, although only occasionally. Infections therefore remain a threat to biomedical research, and users of laboratory mice must be cognizant of infectious agents and the complications they can cause.

DNA viruses

Herpesviruses

Two members of the family Herpesviridae can cause natural infections in mice (*Mus musculus*). Mouse cytomegalovirus 1 (MCMV-1) or murid herpesvirus 1 (MuHV-1) belongs to the subfamily Betaherpesvirinae, genus *Muromegalovirus*. Murid herpesvirus 3 (MuHV-3) or mouse thymic virus (MTV) has not yet been assigned to a genus within the family Herpesviridae. Both are enveloped, double-stranded DNA viruses that are highly host-specific and relatively unstable to environmental conditions such as heat and acidic pH. Both agents are antigenically distinct and do not cross-react in serological tests, but their epidemiology is similar [16].

MCMV-1 is very uncommon in European and American colonies of laboratory mice and is found at a very low rate [11] or reported as not found [14, 15]. Seropositivity has, however, been reported from Asian countries [17, 18]. Testing for MTV is not frequently reported, and no sample tested positive in recent studies [11]. The data available suggest that the prevalence of both viruses in contemporary colonies and thus their importance for laboratory mice is negligible. However, both MCMV-1 and MTV are frequently found in wild mice, which may be coinfected with both viruses [8, 19–21].

Mouse cytomegalovirus 1 (MCMV-1) or murid herpesvirus 1 (MuHV-1)

Natural infection with MCMV-1 causes subclinical salivary gland infection in mice. Like other cytomegaloviruses, MCMV-1 is strictly host-specific. It persists in the salivary glands (particularly in the submaxillary glands) and also in other organs [22–24]. The virus can be cultured in mouse fibroblast lines like 3T3 cells, but primary mouse embryo fibroblasts are more sensitive to infection and produce higher virus titres. However, passage in cell culture results in its attenuation. To maintain virulence, the virus is best propagated by salivary gland passages of sublethal virus doses in weanling mice of a susceptible strain (e.g. BALB/c) [25].

Most information concerning the pathogenesis of MCMV-1 infection is based on experimental infection studies. These results are very difficult to summarize because the outcome of experimental infection in laboratory mice depends on various factors such as mouse strain and age, virus strain and passage history [26], virus dose and route of inoculation [24]. In general, newborn mice are most susceptible to clinical disease and to lethal infection and develop higher levels of resistance with increasing age. Infection of neonates leads to abnormal brain development [27, 28]. Virus replication is observed in newborn mice in many tissues and appears in the salivary glands towards the end of the first week of infection when virus concentrations in liver and spleen have already declined. Resistance develops rapidly after weaning between days 21 and 28 of age. Experimental infection of adult mice results in mortality only in susceptible strains and only if high doses are administered. Not even intravenous or intraperitoneal injections of adult mice usually produce signs of illness in resistant strains [29]. Mice of the *H-2^b^* (e.g. C57BL/6) and *H-2^d^* (e.g. BALB/c) haplotype are more sensitive to experimental infection than are mice of the *H-2^k^* haplotype (e.g. C3H), which are approximately 10-fold more resistant to mortality than those of the *b* or *d* haplotype [24].

Subclinical or latent infections can be activated by immunosuppression (e.g. with cyclophosphamide or cortisone) or critical illness such as sepsis [30]. Reactivation of MCMV-1 also occurs after implantation of latently infected salivary glands into $Prkdc^{scid}$ mice [31]. Immunodeficient mice lacking functional T cells or natural killer (NK) cells, such as $Foxn1^{nu}$ and $Lyst^{bg}$ mice are more susceptible than are immunocompetent animals. Experimental infection in $Prkdc^{scid}$ mice causes severe disease or is lethal, with necrosis in spleen, liver and other organs, and multinucleate syncythia with inclusion bodies in the liver [32]. Similarly to AIDS patients infected with human cytomegalovirus, athymic $Foxn1^{nu}$ mice experimentally infected with MCMV-1 also develop adrenal necrosis [33]. The virus also replicates in the lungs, leading to pneumonitis, whereas replication and disease are not seen in heterozygous ($Foxn1^{nu/+}$) littermates [34]. The pathogenesis of MCMV-1 infection in immunocompetent and in immunocompromised mice, as well as the role of the immune system, have been reviewed by Krmpotic et al. [35].

The most prominent histological finding of cytomegaloviruses is enlarged cells (cytomegaly) of salivary gland epithelium with eosinophilic nuclear and cytoplasmic inclusion bodies. The inclusion bodies contain viral material and are found also in other organs such as liver, spleen, ovary and pancreas [24]. Depending on inoculation route, dose, strain, and age of mice, experimental infections may result in inflammation or cytomegaly with inclusion bodies in a variety of tissues, pneumonitis, myocarditis, meningoencephalitis or splenic necrosis in susceptible strains [8, 24, 36].

Virus is transmitted by the oronasal route, by direct contact and is excreted in saliva, tears and urine for several months. The virus is ubiquitous in wild house mice worldwide. They serve as a natural reservoir for infection and can even be infected with different virus strains [37]. The virus is most frequently transmitted horizontally through mouse-to-mouse contact but does not easily spread between cages. Sexual transmission and transmission with tissues or organs is also possible. The virus does not cross the placenta in immunocompetent mice, although infection of pregnant females results in fetal death or resorption and wasting of borne pups. However, fetal infection is possible by direct injection of MCMV-1 into the placenta [38] and also occurs by transplacental transmission in mice with severe immunodeficiency [39]. Vertical transmission is also possible by milk during lactation [40].

It is generally assumed that MCMV-1 has a very low prevalence in contemporary colonies of laboratory mice. The risk of introduction into facilities housing laboratory mice is very low if wild mice are strictly excluded. Monitoring is necessary if populations of laboratory mice may have been contaminated by contact with wild mice. As for other viruses, different serological tests, including multiplex fluorescent immunoassay (MFIA) [41], are used for health surveillance of rodent colonies. As the virus persists, direct demonstration of MCMV-1 in infected mice is possible by PCR [42–44] or by virus isolation using mouse embryo fibroblasts (3T3 cells).

Although MCMV-1 does not play a significant role as a natural pathogen of laboratory mice, it is frequently used as a model for human cytomegalovirus infection [45]. These aspects have been discussed in detail by Shellam et al. [24]. MCMV-1 has also been used as a vaccine vector aiming at a disseminating mouse control agent by inducing immunocontraception in mice [46]. The virus is known to influence immune reactions in infected mice [47, 48] and may therefore have an impact on immunological research [6, 8].

Mouse thymic virus (MTV) or murid herpesvirus 3 (MuHV-3)

MTV was detected during studies in which samples from mice were passaged in newborn mice. Unlike other herpesviruses, MTV is difficult to culture *in vitro* and is usually propagated by intraperitoneal infection of newborn mice. The thymus is removed 7–10 days later, and thymus suspensions serve as virus material for further studies. The prevalence of MTV is believed to be low in laboratory mice, and for this reason, and also due to the difficulties in virus production for serological assays, it is not included in many standard diagnostic or surveillance testing protocols. Limited data are available indicating that it is common in wild mice [8, 49]. Further, MTV obviously represents a significant source of contamination of MCMV-1 (and vice versa) if virus is prepared from salivary glands, since both viruses cause chronic or persistent salivary gland infections and can coinfect the same host.

All mouse strains are susceptible to infection, but natural or experimental infection of adult mice is subclinical. Gross lesions appear only in the thymus and only if experimental infection occurs at an age of less than about 5 days. Infection results in nuclear inclusions in thymocytes and their almost complete destruction within 2 weeks. Virus is present in the thymus but may also be found in the blood and in salivary glands of surviving animals. Salivary glands are the only site yielding positive virus isolations if animals are infected as adults. The virus persists here and is shed via saliva for months. MTV also establishes a persistent infection in athymic *Foxn1*^{nu} mice, but virus shedding is reduced compared to euthymic mice, with virus recovery possible only in a lower percentage of mice [50].

Pathological changes caused by MTV occur in the thymus, and reduced thymus mass due to necrosis in suckling mice is the most characteristic gross lesion [36]. Lymphoid necrosis may also occur in lymph nodes and spleen [51], with necrosis and recovery similar to that in the thymus. In mice infected during the first 3 days after birth, necrosis of thymus becomes evident within 3–5 days, and the animals' size and weight are markedly reduced at day 12–14. Intranuclear inclusions may be present in thymocytes between days 10–14 after infection. The thymus and the affected peripheral tissues regenerate within 8 weeks after infection. Regardless of the age of mice at infection, a persistent infection is established in the salivary glands, and infected animals shed virus for life.

Several alterations of immune responses are associated with neonatal MTV infection. There is transient immunosuppression, attributable to lytic infection of T lymphocytes, but activity (e.g. response of spleen cells to T-cell mitogens) returns to normal as the histological repair progresses [51]. Selective depletion of CD4+ T cells by MTV results in autoimmune disease [52, 53]. Information about additional influences on the immune system is given in textbooks [6, 8].

In experimentally infected newborn mice, oral and intraperitoneal infections similarly result in thymus necrosis, seroconversion and virus shedding, suggesting that the oral–nasal route is likely to be involved in natural transmission [54]. The virus spreads to cagemates after long periods of contact. It is transmitted between mice kept in close contact, and transmissibility from cage to cage seems to be low. MTV is not transmitted to fetuses by the transplacental route, and intravenous infection of pregnant mice does not lead to congenital damage, impairment in size or development, or abortion [55].

MTV and MCMV-1 do not cross-react serologically [16]. Serological monitoring of mouse populations for antibodies to MTV is possible by indirect immunofluorescent assay (IFA) testing, which is commercially available; enzyme-linked immunosorbent assays (ELISA) tests have also been established [41, 56]. ELISA and complement fixation yield similar results [57]. Serological tests based on recombinant proteins and direct detection of virus by PCR are currently not possible because the genome of the virus has not yet been sequenced. It must be noted that the immune response depends on the age at infection. Antibody responses are not detectable in mice infected as newborns, whereas adult mice develop high titres that are detectable by serological testing. If neonatal infection is suspected, homogenates of salivary glands or other materials can be inoculated into pathogen-free newborn mice followed by gross and histological examination of thymus, lymph nodes and spleens for lymphoid necrosis [49]. Alternatives to the *in vivo* infectivity assay for detecting MTV in infected tissues include a competition ELISA [58] and MAP testing, although this is slightly less sensitive than infectivity assays [59].

There is very little experience of eradication methods for MTV because of its low prevalence in contemporary mouse colonies. Methods that eliminate other herpesviruses will likely eliminate MTV. Procurement of animals of known negative MTV status is an appropriate strategy to prevent infection. Strict separation of laboratory mice from wild rodents is essential to avoid introduction of the virus into laboratory animal facilities.

Other murid herpesviruses

Murid herpesvirus 2 (MuHV-2) or rat cytomegalovirus infects rats and is also a member of the genus *Muromegalovirus*. Murid herpesvirus 4 (MuHV-4) is a member of the genus *Rhadinovirus* in the subfamily Gammaherpesvirinae and is also known as mouse herpesvirus strain 68 (MHV-68). Other murid herpesviruses are not yet assigned to a subfamily within the family Herpesviridae. Among these is murid herpesvirus 3 (mouse thymic herpesvirus), but also murid herpesvirus 5 (field mouse herpesvirus) which infects voles (*Microtus pennsylvanicus*), murid herpesvirus 6 (sand rat nuclear inclusion agent), and murid herpesvirus 7 [60]. Furthermore, a gammaherpesvirus of house mice (*Mus musculus*) has been described recently which is clearly distinct from MHV-68 [61].

Experimental infection of laboratory mice with MHV-68 is a frequently used model system for the study of human gammaherpesvirus pathogenesis, e.g. of Kaposi's sarcoma-associated herpesvirus or Epstein–Barr virus (EBV) [62, 63] which are members of the same subfamily. They are also important models to study viral latency and immune mechanisms controlling latency [64–66]. *Mus musculus* is not the natural host for this virus; it was first isolated in Slovakia from bank voles (*Myodes glareolus*). Additional closely related strains (MHV-60, MHV-72) exist from the same host species, and similar strains (MHV-76, MHV-78) were isolated from wood mice (*Apodemus flavicollis* and *Apodemus sylvaticus*). *Apodemus* sp. seem to be the major hosts for MHV-68 in Great Britain [67]. Different virus strains exhibit different genetic and biological properties and also differ in their pathogenicity, e.g. for *Prkdc*^{scid} mice [68].

Infections in laboratory mice take the same course as in their natural hosts [69]. There are, however, some differences as, e.g. higher virus levels are reached in the lungs of BALB/c mice, and wood mice develop higher titres of neutralizing antibodies [70]. House mice develop an acute infection in the lungs after intranasal infection. A latent infection develops within 2 weeks and the virus persists lifelong in epithelial cells in the lungs and also spreads to the spleen and other organs (e.g. bone marrow, peritoneal cells) where it persists in different cells of the immune system. It behaves like a natural pathogen in inbred strains of mice and persists without causing disease.

Mousepox (ectromelia) virus

The mousepox (ectromelia) virus (ECTV) is a member of the genus *Orthopoxvirus* belonging to the family Poxviridae. It is antigenically and morphologically very similar to vaccinia virus and other orthopoxviruses. Poxviruses are the largest and most complex of all viruses, with a diameter of 200 nm and a length of 250–300 nm. Mousepox (ectromelia) virus contains one molecule of double-stranded DNA with a total genome length of nearly 210 000 nucleotides [71]. It is the causative agent of mousepox, a generalized disease in mice. Experimental transmission to young rats (up to 30 days of age) is possible [72]. Unlike various other orthopoxviruses, ectromelia virus does not infect humans [73].

The virus is resistant to desiccation, dry heat and many disinfectants. It is not consistently inactivated in serum heated for 30 min at 56 °C [3, 74] and remains active for months when maintained at 4 °C in fetal bovine serum [75]. Effective disinfectants include vapour-phase formaldehyde, sodium hypochlorite and iodophores [8, 76].

Historically, ECTV has been an extremely important natural pathogen of laboratory mice. The virus was widespread in mouse colonies worldwide and can still be found in several countries. Between 1950 and 1980 almost 40 individual ectromelia outbreaks were reported in the USA. The last major epizootic in the USA occurred in 1979–80 and has been described in great detail [77]. Severe outbreaks were also described in various European countries [78–80]. A more recent outbreak in the USA, which resulted in the eradication of almost 5000 mice in one institution, was described by Dick et al. [81]. Another recent and well-documented case of mousepox was published by Lipman et al. [3]. Few additional but unpublished cases of ectromelia have been observed since then; the latest report of an outbreak was published in 2009 [74]. In general, positive serological reactions are occasionally reported from routine health surveillance studies [17] but the virus is extremely rare in European and American colonies of laboratory mice [13–15].

Natural infections manifest differently, depending on many factors. Mousepox may occur as a rapidly spreading outbreak with acute disease and deaths, or may be inconspicuous with slow spreading and mild clinical signs and may therefore be very difficult to diagnose [81]. The mortality rate can be very low in populations in which the virus has been present for long periods. The infection usually takes one of three clinical courses: acute asymptomatic infection, acute lethal infection (systemic form) or subacute to chronic infection (cutaneous form) [8, 81–83]. The systemic or visceral form is characterized clinically by facial oedema, conjunctivitis, multisystemic necrosis and usually high mortality. This form is less contagious than the cutaneous form because the animals die before there is virus shedding. The cutaneous form is characterized by typical dermal lesions and variable mortality. The outcome of infection depends on many factors including strain and dose of virus; route of viral entry; strain, age, and sex of mouse; husbandry methods and duration of infection in the colony. While all mouse strains seem to be susceptible to infection with ECTV, clinical signs and mortality are strain-dependent [84–86]. Acute lethal (systemic) infection occurs in highly susceptible inbred strains such as DBA/1, DBA/2, BALB/c, A and C3H/HeJ. Immunodeficient mice may also be very susceptible [87]. Outbreaks among susceptible mice can be explosive, with variable morbidity and high mortality (>80%). Clinical disease may not be evident in resistant strains such as C57BL/6 and AKR, and the virus can be endemic in a population for long periods before being recognized. Furthermore, females seem to be more resistant to disease than males, at least in certain strains of mice [84, 85]. Killer cells are necessary to control mousepox infections [88]. Mice that are resistant to mousepox may lose their resistance with increasing age, most likely due to the decreased number and activity of NK cells [89].

The mechanisms determining resistance versus susceptibility are not fully understood but appear to reflect the action of multiple genes. The genetic loci considered to be important include $H2D^b$ (termed *Rmp3,* resistance to mousepox), on chromosome 17 [90]; the *C5* genes (*Rmp2,* on chromosome 2); *Rmp1,* localized to a region on chromosome 6 encoding the NK cell receptor NKR-P1 alloantigens [91]; the *nitric oxide synthase 2* locus on chromosome 11 [92]; and the *signal transducer and activator of transcription 6* locus on chromosome 10 [93]. Mousepox infections are controlled for several days during the initial course of infection by the complement system until the adaptive immune system can react. Loss of the complement system results in lethal infection [94]. Clearance of the virus by the immune system is dependent upon the effector functions of CD8+ T cells while NK cells, CD4+ T cells and macrophages are necessary for the generation of an optimal response [95, 96]. T- and B-cell interactions and antibodies play a central role during recovery from a secondary infection [97].

Mousepox (ectromelia) virus usually enters the host through the skin with local replication and extension to regional lymph nodes [8, 82, 86]. It escapes into the blood (primary viraemia) and infects splenic and hepatic macrophages, resulting in necrosis of these organs and a massive secondary viraemia. This sequence takes approximately 1 week. Many animals die at the end of this stage without premonitory signs of illness; others develop varying clinical signs including ruffled fur, hunched posture, swelling of the face or extremities, conjunctivitis and skin lesions (papules, erosions or encrustations mainly on ears, feet and tail; Figure 3.2.1). Necrotic amputation of limbs and tails can sometimes be seen in mice that survive the acute phase, hence the

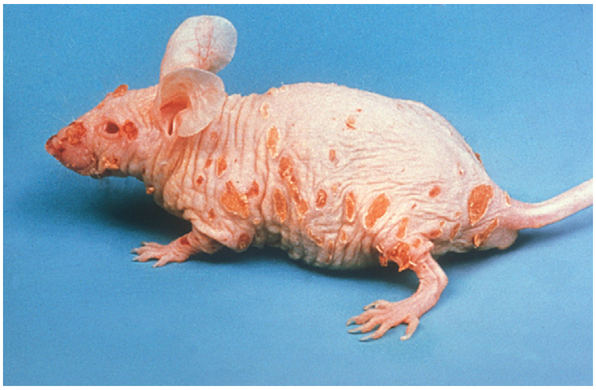

Figure 3.2.1 The rash of mousepox in a hairless (hr) mutant mouse. *From Deerberg* et al. *[78]; with permission from Schlütersche Verlagsgesellschaft.*

Figure 3.2.2 Dry gangrene of the left hind foot of a mouse infected with ECTV.

original name of the disease: 'ectromelia' means absent or short limbs (Figure 3.2.2).

Common gross lesions of acute mousepox include enlarged lymph nodes, Peyer's patches, spleen and liver; multifocal to semiconfluent white foci of necrosis in the spleen and liver; and haemorrhage into the small intestinal lumen [36, 81, 86, 87]. In animals that survive, necrosis and scarring of the spleen can produce a mosaic pattern of white and red-brown areas that is a striking gross finding.

The most consistent histological lesions of acute mousepox are necroses of the spleen (Figure 3.2.3), lymph nodes, Peyer's patches, thymus and liver [3, 36, 81, 86, 87]. Occasionally, necrosis may also be observed in other organs such as ovaries, uterus, vagina, intestine and lungs. The primary skin lesion, which occurs about a week after exposure at the site of inoculation (frequently on the head), is a localized swelling that enlarges from inflammatory oedema. Necrosis of dermal epithelium provokes a surface scab and heals as a deep, hairless scar. Secondary skin lesions (rash) develop 2–3 days later as the result of viraemia (Figure 3.2.1). They are often multiple and widespread and can be associated with conjunctivitis. The skin lesions also can ulcerate and scab before scarring. Mucosal and dermal epithelial cells may have characteristic intracytoplasmic eosinophilic (Cowdry type A) inclusion bodies (Figure 3.2.4). Basophilic (Cowdry type B) inclusions may be found in the cytoplasm of all infected cells, especially in hepatocytes.

Natural transmission of ECTV mainly occurs by direct contact and fomites [8, 82, 98, 99]. The primary route of infection is through skin abrasions. Faecal–oral and aerosol routes may also be involved [98]. In addition, the common practice of cannibalism by mice may contribute to the oral route of infection [99]. Intrauterine transmission is possible at least under experimental conditions [100]. Virus particles are shed from infected mice (mainly via scabs and/or faeces) for about 3–4 weeks, even though the virus can persist for months in the spleen of an occasional mouse [8, 99]. Cage-to-cage transmission of ECTV and transmission between rooms or units is usually low and largely depends on husbandry practices (e.g. mixing mice from

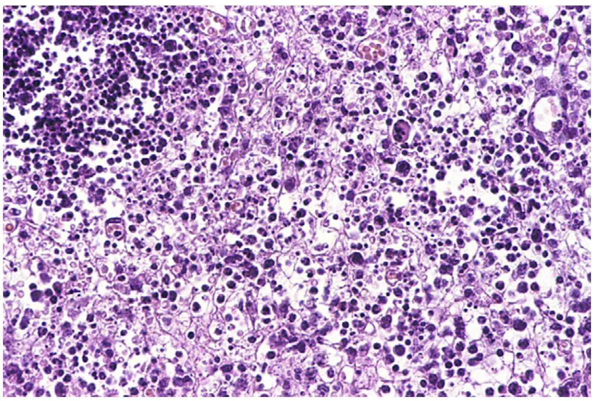

Figure 3.2.3 Section of the spleen of a mouse infected with ectromelia virus. There is marked parenchymal necrosis with extensive cellular debris and only few lymphoid cells left (H&E stain, magnification 200×). *Courtesy of Professor A. D. Gruber.*

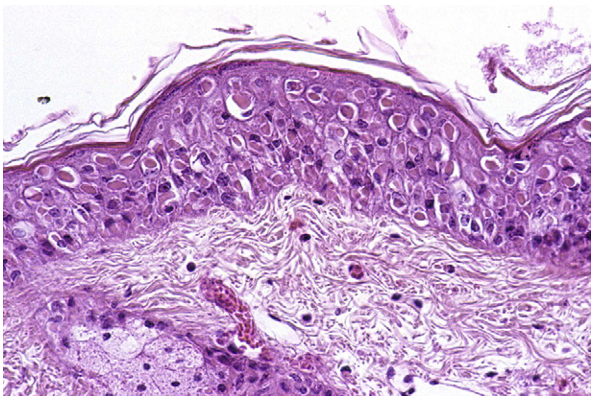

Figure 3.2.4 Section of the skin of a mouse infected with ECTV. Cutaneous hyperplasia with epithelial cell degeneration and numerous large intra-epithelial cytoplasmic viral inclusion bodies (Cowdry type A) are seen (H&E stain, magnification 400×). *Courtesy of Professor A. D. Gruber.*

different cages). Importantly, the virus may not be transmitted effectively to sentinel mice exposed to dirty bedding [3].

Various tests have been applied for the diagnosis of ectromelia. Previous epidemics were difficult to deal with because of limited published data and information on the biology of the virus and the lack of specific and sensitive assays [101]. In the 1950s, diagnosis relied on clinical signs, histopathology and animal passages of tissues from moribund and dead animals. Culture of the virus on the chorioallantoic membrane of embryonated eggs was also used. Serology is currently the primary means of routine health surveillance for testing mouse colonies for exposure to ECTV. The methods of choice are MFIA, ELISA and IFA; they are more sensitive and specific than the previously used haemagglutination inhibition (HI) assay [41, 102, 103]. Serological tests based on virus particles detect antibodies to orthopoxviruses and do not distinguish between ECTV and vaccinia virus or other orthopoxviruses, respectively. Vaccinia virus is commonly used as an antigen for serological testing to avoid the risk of infection for mice. Thus, false-positive serological reactions may be found after experimental administration of replication-competent vaccinia virus. It has been shown that even cage contact sentinels may develop antibodies, and vaccinia virus leading to seroconversion may even be transmitted by dirty bedding [104]. Confirmation of positive serological results is important before action is taken because vaccinia virus is increasingly prevalent in animal facilities as a research tool (e.g. for vaccination or gene therapy). As observed in different outbreaks, serological testing is of little value in the initial stages of the disease. For example, in the outbreak described by Dick et al. [81] depopulation was nearly completed before serological confirmation was possible. For this reason, negative serological results should be confirmed by direct detection methods (PCR, immunohistochemistry, virus isolation) or by histopathology, especially when clinical signs suggestive of mousepox are observed. PCR assays to detect different genes of poxviruses in infected tissues have been used [3, 81, 105]. Other PCR tests which were developed to detect smallpox virus have also been shown to detect ectromelia virus and can be used as well [106, 107].

The key to prevention and control of mousepox is early detection of infected mice and contaminated biological materials. All institutions that must introduce mice from other than commercial barrier facilities should have a health surveillance programme and test incoming mice. Perhaps even more important than living animals are samples from mice (tumours, sera, tissues). The virus replicates in lymphoma and hybridoma cell lines [108], and such cells or material derived from them may therefore be a vehicle for inadvertent transfer between laboratories. The last three published outbreaks of ectromelia were introduced into the facilities by mouse serum [3, 74, 81]. Lipman et al. [3] found that the contaminated serum originated from a pooled lot of 43 L that had been imported from China, but in both other cases, serum was obtained from animals in the USA. Because mouse serum is commonly sold to the end user in small aliquots (a few millilitres), it has to be expected that aliquots of the contaminated lot may still be stored in freezers. These published cases of ectromelia outbreaks provide excellent examples of why testing should be performed on all biological materials to be inoculated into mice. In the case of ectromelia virus it was shown that PCR is more sensitive, and MAP testing failed to detect contamination [74].

Eradication of mousepox has usually been accomplished by elimination of the affected colonies, disinfection of rooms and equipment, and disposal of all infected tissues and sera. While culling of entire mouse colonies is the safest method for eradication of mousepox, it is not a satisfactory method because of the uniqueness of numerous lines of genetically modified animals housed in many facilities. Several studies indicate that mousepox is not highly contagious [75, 84, 99] and that it may be self-limiting when adequate husbandry methods are applied. Therefore, strict quarantine procedures along with cessation of breeding (to permit resolution of infection) and frequent monitoring, with removal of clinically sick and seropositive animals, are a potential alternative. The period from the last births until the first matings after cessation of breeding should be at least 6 weeks [99]. Sequential testing of immunocompetent contact sentinels for seroconversion should be employed with this option.

In the past, immunization with live vaccinia virus was used to suppress clinical expression of mousepox. Vaccination may substantially reduce the mortality rate, but it does not prevent virus transmission or eradicate the agent from a population [109, 110]. After vaccination, typical pocks develop at the vaccination site, and infectious vaccinia virus is detectable in spleen, liver, lungs and thymus [111]. Vaccination also causes seroconversion so that serological tests are not applicable for health surveillance in vaccinated populations. It is therefore more prudent to control mousepox by quarantine and serological surveillance than by relying on vaccination.

Mortality and clinical disease are the major factors by which ECTV interferes with research. Severe disruption of research can also occur when drastic measures are taken to control the infection. The loss of time, animals and financial resources can be substantial.

Experimental mousepox infections are frequently used as a model to study various aspects of smallpox infections of humans [112–114]. Mousepox shares many aspects of virus biology and pathology, and models the course of human smallpox. Experimental mousepox infections are used to study vaccination procedures [115, 116] or anti-poxvirus therapies [117].

Murine adenoviruses

Murine adenoviruses (MAdV) are non-enveloped, double-stranded DNA viruses of the family Adenoviridae. Two distinct strains have been isolated from mice. The FL strain (MAdV-1) was first isolated in the USA as a contaminant of a Friend leukaemia [118] and has been classified as a member of the genus *Mastadenovirus*. The K87 strain (MAdV-2) was isolated in Japan from the faeces of a healthy mouse [119] and has not yet been assigned to a genus. Both strains are considered to represent different species [120–122]. They are host species specific and are not infectious for infant rats [123]. MAdV-1 can be cultured *in vitro* in mouse fibroblasts (e.g. 3T6 or L929 cells), MAdV-2 is usually cultured *in vitro* in a mouse rectum carcinoma cell line (CMT-93). In laboratory mice, seropositivity to adenoviruses was reported to be very low [11, 14, 15, 17, 18, 124] or negative [12, 13]. Antibodies to MAdV are also found in wild mice [21, 125] and in rats [21, 126].

Neither virus is known to cause clinical disease in naturally infected, immunocompetent mice. However, MAdV-1 can cause a fatal systemic disease in suckling mice after experimental inoculation [118, 127, 128]. Disease is characterized by scruffiness, lethargy, stunted growth and often death within 10 days. Experimental infection of adult mice with MAdV-1 is most often subclinical and persistent but can cause fatal haemorrhagic encephalomyelitis with neurological symptoms, including tremors, seizures, ataxia and paralysis in susceptible C57BL/6 and DBA/2J mice [129]. BALB/c mice are relatively resistant to this condition. Athymic *Foxn1*^{nu} mice experimentally infected with MAdV-1 develop a lethal wasting disease [130]. Similarly, *Prkdc*^{scid} mice succumb to experimental infection with MAdV-1 [131].

Gross lesions in response to natural MAdV infections are not detectable. Occasional lesions observed after experimental infection with MAdV-1 include small surface haemorrhages in the brain and spinal cord of C57BL/6 and DBA/2J mice [129], duodenal haemorrhage in *Foxn1*^{nu} mice [130] and pale yellow livers in *Prkdc*^{scid} mice [131].

Histologically, experimental MAdV-1 infection of suckling mice is characterized by multifocal necrosis and large basophilic intranuclear inclusion bodies in liver, adrenal gland, heart, kidney, salivary glands, spleen, brain, pancreas and brown fat [8, 36, 127, 132]. In experimentally induced haemorrhagic encephalomyelitis, multifocal petechial haemorrhages occur throughout the brain and spinal cord, predominantly in the white matter, and are attributed to infection and damage to the vascular epithelium of the central nervous system (CNS) [129]. Histopathological manifestations in MAdV-1-infected *Prkdc*^{scid} mice are marked by microvesicular fatty degeneration of hepatocytes [131]. In contrast to MAdV-1, the tissue tropism of MAdV-2 is limited to the intestinal epithelium. Naturally or experimentally infected mice develop intranuclear inclusions in enterocytes, especially in the ileum and caecum [8, 36, 133].

Transmission of MAdV primarily occurs by ingestion. MAdV-1 is excreted in the urine and may be shed for up to 2 years [134]. MAdV-2 infects the intestinal tract and is shed in faeces for only a few weeks in immunocompetent mice [135]; immunodeficient mice may shed the virus for longer periods [136].

Murine adenovirus infections are routinely diagnosed by serological tests. However, there is a one-sided cross-reactivity of MAdV-1 with MAdV-2 [137]. Serum from mice experimentally infected with MAdV-1 yielded positive reactions in serological tests with both viruses, while serum from mice infected with MAdV-2 reacted only with the homologous antigen [138]. Smith et al. [126] reported that sera might react with MAdV-1 or MAdV-2 or both antigens. Occasional reports of mice with lesions suggestive of adenovirus infections and negative serology (with MAdV-1) indicate that the infection may not be detected if only one virus is used as an antigen [139]. It is therefore usual to test sera for antibodies to both MAdV-1 and MAdV-2. The commonly used methods are IFA, ELISA and MFIA.

The low prevalence in colonies of laboratory mice indicates that MAdV can easily be eliminated (e.g. by hysterectomy derivation or embryo transfer) and that barrier maintenance has been very effective in preventing infection.

The low pathogenicity and the low prevalence in contemporary mouse populations are the main reasons why adenoviruses are considered to be of little importance, which is also indicated by the fact that recent publications about murine adenoviruses are very rare. However, the viruses might easily be spread by the exchange of genetically modified mice and therefore re-emerge. Only a few influences on research attributable to MAdV have been published. For example, it has been shown that MAdV-1 significantly aggravates the clinical course of scrapie disease in mice [140]. Natural infections with MAdV could also interfere with studies using adenovirus as a gene vector.

Other murine adenoviruses

A novel murine adenovirus classified as a Mastadenovirus has recently been isolated from a striped field mouse (*Apodemus agrarius*) [141]. It was cultured in Vero E6 cells and named MAdV type 3 (MAdV-3). It revealed the highest similarity to MAdV-1 but it represents a separate serotype. However, there is some cross-reactivity between MAdV-3 and both other mouse viruses [142]. In addition to serological and antigenic differences it also shows a unique organotropism and infects predominantly the heart tissue of C57BL/6N mice after experimental infection. Experimentally infected mice show no clinical signs. The virus is not easily transmitted from experimentally infected mice to contact sentinels [142].

Polyomaviruses

Polyomaviridae are enveloped, double-stranded DNA viruses. Two different agents of this family exclusively infect mice (*Mus musculus*), and both belong to the genus *Polyomavirus*. Murine pneumotropic virus (MPtV) was formerly known as 'newborn mouse pneumonitis virus' or 'K virus' (named after L. Kilham who first described the virus). The second is murine polyomavirus (MPyV). Both are related, but antigenically distinct, from each other [143], and also virus-like particles from the major capsid protein (VP1) do not cross-react [144]. They are enzootic in many populations of wild mice but are very uncommon in laboratory mice. Even older reports indicate that both have been eradicated from the vast majority of contemporary mouse colonies, and their importance is negligible [8]. Seropositivity to these viruses was not reported in a recent survey conducted in the USA [13], and other publications also indicate that these viruses do not presently play a significant role in laboratory mice [11, 14, 15]. Because of their low prevalence, neither virus is included in the list of agents for which testing is recommended on a regular basis by FELASA [145].

Although polyomavirus genes, especially those of SV40, are widely used in gene constructs for insertional mutagenesis, very few reports have been published on spontaneous or experimental disease due to MPyV or MPtV in the last 10–15 years. The reader is therefore referred to previous review articles for details [8, 146].

Murine pneumotropic virus (MPtV)

Natural infections with MPtV are subclinical. The prevalence of infection is usually low in an infected population. The virus may persist in infected animals for months and perhaps for life depending on the age at infection and is reactivated under conditions of immunosuppression.

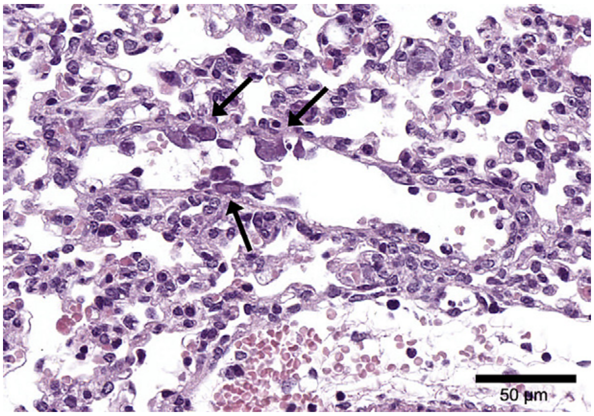

Figure 3.2.5 Section of the lung of a mouse infected with MPtV (K virus). Mild lymphohistiocytic interstitial pneumonia and large amphophilic to basophilic intranuclear inclusion bodies are visible (H&E stain). *Courtesy of Professor A. D. Gruber.*

Virus replicates primarily in endothelial cells, but renal tubular epithelial cells are the major site of viral persistence [147, 148].

Clinical signs are observed only after infection of infant mice less than 6–8 days of age. Infected pups suddenly develop respiratory symptoms after an incubation period of approximately 1 week, and many die within a few hours of onset of symptoms with an interstitial pneumonia caused by productive infection of and damage to pulmonary endothelium (Figure 3.2.5). Endothelial cells in other organs are also involved in virus replication [148, 149] (Figure 3.2.6). In older suckling mice, MPtV produces a more protracted infection, and the virus or viral antigen can be detected for as long as 4 months. In adult animals, the virus produces a transient asymptomatic infection. Even in immunodeficient *Foxn1*^{nu} mice, experimental infection of adults is clinically asymptomatic, although virus is detectable for a period of several months [150].

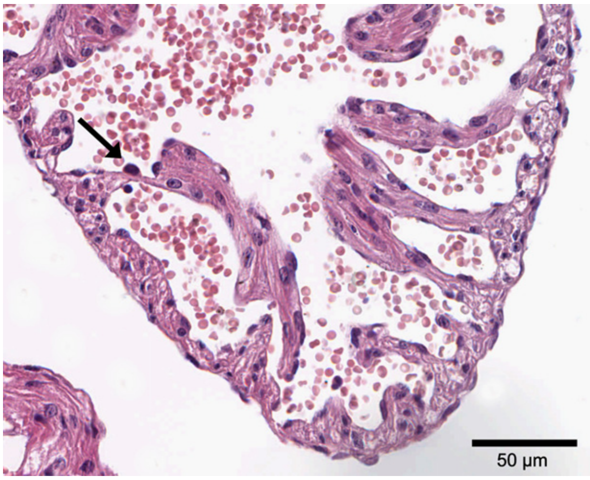

Figure 3.2.6 Section of the right auricle of a mouse infected with MPtV (K virus). Endothelial cell containing large amphophilic to basophilic intranuclear inclusion bodies (H&E stain). *Courtesy of Professor A. D. Gruber.*

In vitro cultivation of MPtV is difficult. No susceptible permanent cell line is known to support growth. It can be cultured in primary mouse embryonic cells, but viral titres are not sufficient for use in serological assays [151]. For this reason, the HI test using homogenates of livers and lungs of infected newborn mice is still frequently used, but IFA and ELISA tests are also available [152]. Furthermore, a PCR test for demonstration of MPtV in biological samples has also been published [153].

Murine polyomavirus (MPyV)

MPyV was first detected as a contaminant of murine leukaemia virus (MuLV) when sarcomas developed in mice after experimental inoculation of contaminated samples. It has later been shown to be a frequent contaminant of transplantable tumours [1]. Natural infection of mice is subclinical, and gross lesions including tumours are usually not found. Tumour formation occurs when mice are experimentally infected at a young age or when inoculated with high virus doses. Development of tumours may be preceded by multifocal necrosis and mortality during the viraemic stage [36]. Parotid, salivary gland and mammary tumours are common, and sarcomas or carcinomas of kidney, subcutis, adrenal glands, bone, cartilage, teeth, blood vessels and thyroid also occur. Virus strains vary with regard to the tumour types or lesions that they induce, and mouse strains vary in their susceptibility to different tumour types. Those of C57BL and C57BR/cd lineage are considered to be the most resistant strains; athymic *Foxn1*^{nu} mice are considered to be most susceptible; C3H mice are particularly susceptible to adrenal tumours and A mice tend to develop bone tumours. Immunosuppression or inoculation into immunodeficient strains (e.g. *Foxn1*^{nu}) also supports the growth of tumours. On the other hand, experimental infection of adult immunocompetent mice does not result in tumour formation because the immune response suppresses tumour growth, and newborn immunocompetent mice develop runting only if inoculated with high virus doses [154].

After experimental intranasal infection, MPyV initially infects the respiratory tract followed by a systemic phase in which liver, spleen, kidney and colon become infected [155]. The virus is shed in faeces and in all body fluids, and transmission occurs rapidly by direct contact between animals, but also between cages in a room. Further, intrauterine transmission has been documented after experimental infection [156]. MPyV persists in all organs in *Prkdc*^{scid} mice while viral DNA is detectable in immunocompetent mice after experimental infection for only a limited period of about 4 weeks [157]. However, virus may persist and can be reactivated by prolonged immunosuppression [158] or during pregnancy, at least in young mice [159]. It has been shown that interferon-gamma is an important factor of the host defence against tumour formation and MPyV infection [160]. Biological materials of mouse origin are likely to be the most common source of contamination of laboratory mice, emphasizing the importance of MAP or PCR screening of biological materials to be inoculated into mice.

The most frequently used tests for health surveillance of mouse colonies are ELISA, MFIA and IFA; in addition, the HI test is still used. Latent infections can be detected by intracerebral inoculation of neonate mice or by MAP testing, but direct demonstration of virus in biological samples is also possible by PCR testing [153].

While MPyV infections are of low importance for laboratory animal medicine, the virus is used in models of persistent virus infection [161, 162]. Virus-like particles from both murine polyomaviruses have been used as a vector for gene therapy or vaccines [163, 164].

Parvoviruses

Parvoviruses are non-enveloped small viruses (approximately 20 nm in diameter) with a single-stranded DNA genome of approximately 5000 nucleotides. Murine parvoviruses are members of the family Parvoviridae, genus *Parvovirus*. They are remarkably resistant to environmental conditions like heat, desiccation, acidic and basic pH-values. Up to date, two distinct species that infect laboratory mice are officially listed: the minute virus of mice (MVM), previously named mice minute virus (MMV), and the mouse parvovirus (MPV). Non-structural proteins (NS-1 and NS-2) are highly conserved among both viruses whereas the capsid proteins (VP-1, VP-2, VP-3) are more divergent and determine the serogroup [165]. Both viruses require mitotically active cells for replication. Severe clinical signs are therefore not found in mature animals because of the lack of a sufficient number of susceptible cells in tissues. General aspects of rodent parvovirus infections and their potential effects on research results have been reviewed [6, 8, 166–170].

Mouse parvovirus (MPV)

Already in the mid-1980s mouse colonies were identified that gave positive reactions for MVM by IFA but not by HI tests. It was subsequently shown that these colonies were infected with a novel parvovirus, initially referred to as ‘mouse orphan parvovirus’. The first isolate of MPV was detected as a contaminant of cultivated T-cell clones interfering with *in vitro* immune responses [171] and was named ‘mouse parvovirus’. It does not replicate well in currently available cell cultures, and sufficient quantities of virus for serological tests are difficult to generate. Hitherto, only very few isolates of MPV have been cultured and subsequently characterized on a molecular basis [165, 172]. On the basis of epidemiological analyses, further parvoviruses were recently identified in mice, sequenced, and tentatively named serially MPV-2 and MPV-3 [173], MPV-4 (GenBank FJ440683) and MPV-5 (GenBank FJ441297). In addition, several variants are published for MPV-1 [172, 174, 175].

At present, MPV is among the most common viruses found in colonies of laboratory mice. The prevalence of sera positive for parvoviruses ranged from 1% to nearly 10% in Western Europe and North America, with the majority of sera being positive for MPV in studies differentiating between the two parvovirus species [12, 14, 15, 176]. These prevalence data are based on testing at commercial laboratories and do not reflect that, despite highly specific and sensitive test methods, enzootic parvovirus infections are difficult to detect due to virus-associated characteristics [169, 170]. A recent survey conducted in the USA showed that during a 24–36 month period mouse parvoviruses were detected at almost all facilities that responded to a questionnaire, with MPV being more often diagnosed than MVM [13].

Clinical disease and gross or histological lesions have not been reported for mice naturally or experimentally infected with MPV. Infections are subclinical even in newborn and immunocompromised animals [177, 178]. In contrast to many other viruses infecting mice, viral replication and excretion is not terminated by the onset of host immunity. Tissue necrosis has not been observed at any stage of infection in infected infant or adult mice [177, 178]. Humoral immunity to MPV does not protect against MVM infections, and vice versa [179].

Serological surveys have indicated that MPV naturally infects only mice, with the exception that MPV-3 shows genetic similarity to hamster parvovirus, suggesting that a cross-species transmission has occurred, where the mouse probably served as the natural host [173, 180]. Differences in mouse strain susceptibility to clinical MPV infection do not exist. However, seroconversion seems to be strain-dependent. After experimental infection with MPV-1b, seroconversion occurred in all C3H/HeN mice, fewer BALB/c, DBA/2 and ICR mice, and seroconversion could not be detected in C57BL/6 mice [181]. Upon MPV-1f inoculation, antibody response was absent in BALB/cArc mice [182]. Diagnosis of MPV infection by PCR testing of small intestine and mesenteric lymph nodes also depended on the mouse strain. MPV DNA was detected in all mouse strains evaluated except DBA/2 even though seroconversion was detected in these mice.

After oral infection, the intestine is the primary site of viral entry and replication. The virus spreads to the mesenteric lymph nodes and other lymphoid tissues, where it persists for more than 2 months [178], and seems to be excreted via the intestinal and the urinary tract. After experimental inoculation of weanling mice, MPV is transmitted to cagemates by direct contact for 2–6 weeks [177], and transmission by dirty bedding is also possible. These results implicate a role for urinary, faecal, and perhaps respiratory excretion of virus. Another study showed that naturally infected mice might not transmit the virus under similar experimental conditions [183].

Serology is a useful tool to identify MPV infections in immunocompetent hosts, but reaching a diagnosis based on serological assays may be difficult and requires a good knowledge of the available techniques. Neither the virion ELISA nor HI is a practical screening test for MPV because they require large quantities of purified MPV, which is difficult to obtain. Diagnosis of MPV infections has long been made on the basis of an MVM HI-negative result coupled with an MVM IFA-positive result. IFA provides the opportunity to detect both serogroup-specific VP proteins as well as NS proteins that are conserved among mouse parvoviruses. A generic rodent parvovirus ELISA using a recombinant NS-1 protein as antigen has been developed [184], but MPV IFA and MPV HI assays are more sensitive techniques than the NS-1 ELISA and the MVM IFA [181]. In contrast, ELISA tests that use recombinant VP-2 provide sensitive and serogroup-specific assays for the diagnosis of MPV infections in mice [176, 185], although considerable cross-reactivity with heterologous capsid antigens exists [173]. Nevertheless, when using the ELISA technique, one needs to consider that MPV-2 may not consistently be detected by MPV-1 VP-2 ELISA [168, 173], especially when antibody titres are low (own observations). Therefore, ELISAs using MPV-2 VP-2 and MPV-3 VP-2 antigens are also used for diagnostics. As parvovirus diagnostics using recombinant assays should be based on a combination of antigens, bead-based mulitplex assays are a convenient extension of traditional ELISA, allowing the use of multiple antigens simultaneously.

In immunodeficient mice that do not generate a humoral immune response, PCR assays can be used to detect MPV [186, 187] and other parvoviruses. MPV has been shown to persist for at least 9 weeks in the mesenteric lymph nodes [178]. This tissue is considered the best suited for PCR analysis, but spleen and small intestine can also be used with good success [181]. For antemortem detection, shedding of parvoviruses can also be detected by PCR of faecal samples [188]. The virus persists sufficiently long in mesenteric lymph nodes so that PCR assays may also be used as a primary screening tool for laboratories that do not have access to specific MPV antigen-based serological assays. The PCR is further a good confirmatory method for serological assays and has also been described for the detection of parvoviruses in cell lines and tumours [189]. In addition, the MAP test has been reported as a sensitive tool to detect MPV [183].

Given the high environmental stability of the virus and the potential fomite transmission, together with the long virus persistence in infected animals, spontaneous disappearance from a mouse population (e.g. by cessation of breeding) is unlikely. Eradication of infection is possible by elimination of infected animals and subsequent replacement with uninfected mice, and the agent can be eliminated from breeding populations by embryo transfer or by hysterectomy. It should be noted that recent studies suggest a risk of virus transmission by embryo transfer, though successful sanitation of immunodeficient mice was achieved despite antibody response in recipients and progeny after embryo transfer [190, 191].

Although there are few published reports of confounding effects of MPV on research, it is lymphocytotropic and may perturb immune responses *in vitro* and *in vivo*. Infections with MPV have been shown to influence rejection of skin and tumour grafts [192].

Minute virus of mice (MVM)

MVM is the type species of the genus *Parvovirus*. The virus was intermediately named mice minute virus (MMV). It was originally isolated by Crawford [193] from a stock of mouse adenovirus, and this prototype isolate was later designated MVMp. Its allotropic variant was detected as a contaminant of a transplantable mouse lymphoma [194] and designated MVMi because it exhibits immunosuppressive properties *in vitro*. Both variants have distinct cell tropisms *in vivo* and *in vitro*. MVMp infects fibroblast cell lines and does not cause clinical disease [195, 196]. MVMi grows lytically in T cells and inhibits various functions mediated by these cells *in vitro*. Both strains are apathogenic for adult mice, but the immunosuppressive variant is more pathogenic for neonatal mice than is MVMp. A third strain, the Cutter strain MVMc, was isolated from BHK-21 cells [172]. In contrast to these three strains detected as cell culture contaminants, an isolate was obtained from naturally infected mice with a B-cell maturational defect maintained at the University of Missouri and therefore denominated MVMm [173].

Serological surveys show that the mouse is the primary natural host [19, 125, 197], but the virus is also infective for rats, hamsters [168, 198], and *Mastomys* [199] during fetal development or after parenteral inoculation.

Natural infections are usually asymptomatic in adults and infants, and the most common sign of infection is seroconversion. Kilham and Margolis [200] observed mild growth retardation a few days after experimental infection of neonatal mice with MVMp. Studies of transplacental infection yielded no pathological findings in mice [201]. The immunosuppressive variant, but not the prototype strain, is able to produce a runting syndrome after experimental infection of newborn mice [195]. Depending on the host genotype, experimental infections of fetal and neonatal mice with MVMi produce various clinical presentations and lesions. Infection in C57BL/6 mice is asymptomatic, but the virus causes lethal infections with intestinal haemorrhage in DBA/2 mice. Infection of strains such as BALB/c, CBA, C3H/He and SJL is also lethal and mice have renal papillary haemorrhage [196]. The MVMi also infects haematopoietic stem cells and mediates an acute myelosuppression [202, 203]. Because of its dependence on mitotically active tissues, the fetus is at particular risk for damage by parvoviruses. MVM and other parvoviruses may have severe teratogenic effects and cause fetal and neonatal abnormalities by destroying rapidly dividing cell populations, often resulting in fetal death. Adult *Prkdc^{scid}* mice develop an acute leukopenia 1 month after experimental infection with MVMi and die within 3 months. The virus persists lifelong in the bone marrow of these mice [204]. During a natural concurrent outbreak of MVMm and MPV, a runting syndrome with lymphohistiocytic renal inflammation and inclusion bodies in cells resembling splenic haematopoietic progenitor cells was reported in B-cell (*Ighm*)-deficient mice [205].

MMV is shed in faeces and urine. In faecal samples, MVM was detected for up to 4–6 weeks by PCR [206, 207], although shorter periods (9–12 days) have been observed [208]. Notably, shedding re-occurred after immunosuppression by irradiation [207]. Contaminated food and bedding are important factors in viral transmission because the virus is very resistant to environmental conditions. Direct contact is also important and the virus does not easily spread between cages.

Routine health surveillance is usually conducted by serological methods. Unlike MPV, MVM can easily be cultured in cell lines so that antigen production for HI and ELISA (using whole purified virions) is easy. HI is a highly specific diagnostic test whereas IFA always exhibits some degree of cross-reactivity with MPV and other closely related parvoviruses. ELISA is probably the most frequently used test, but depending on the purity of the antigen preparation, cross-reactions with MPV may occur due to contamination with non-structural proteins that are common to both viruses. This problem can be avoided by the use of recombinant VP-2 antigen [176]. By using serological methods, one needs to consider that the mouse strain has a considerable effect on seroconversion so that an antibody response might not be detectable despite infection; while C57BL/6J mice showed good antibody response, seroconversion was observed only in some BALB/c, AKR/N, DBA/2J, FVB/N and C3H/HeN, but not in NMRI and ICR mice upon contact exposure to MVMi-inoculated mice [206]. Viral detection is also possible by PCR in biological materials, organs (intestine, mesenteric lymph node, kidney, spleen) and faeces from infected animals [187, 189, 206, 207, 209]. Although MVM was not thought to cause persistent infection in immunocompetent mice, recent data show that it can be detected in spleens for up to 16 weeks after exposure in some mouse strains [207]. Therefore, PCR may be considered as a confirmatory method for serology.

The virus can be eliminated from infected breeding populations by caesarean derivation or by embryo transfer. However, certain precautions such as careful washing and accompanying testing need to be minded, as MVM has been detected in reproductive organs and gametes and this virus firmly attaches to the zona pellucida or might even cross it [210, 211]. In experimental colonies, elimination of infected animals and subsequent replacement with uninfected mice is practical if careful environmental sanitation is conducted by appropriate disinfection procedures. It is important that reintroduction is avoided by exclusion of wild mice and by strict separation from other infected populations and potentially contaminated materials in the same facility. Admission of biological materials must be restricted to samples that have been tested and found to be free from viral contamination.

Both allotropic variants of MVM have been used as models for molecular virology, and their small size and simple structure have facilitated examination of their molecular biology and expedited understanding of cell tropism, viral genetics and structure. The significance for laboratory mouse populations was considered low or uncertain because natural infections are inapparent. However, various effects on mouse-based research have been published [6, 7, 166, 167, 170]. Because of their predilection for replicating in mitotically active cells, they are frequently associated with tumour cells and have a marked oncosuppressive effect [212]. Special attention is also necessary for immunological research and other studies involving rapidly dividing cells (embryology, teratology). In addition, MVM is a common contaminant of transplantable tumours, murine leukaemias and other cell lines [1, 2, 213].

RNA viruses

Lactate dehydrogenase-elevating virus

Lactate dehydrogenase-elevating virus (LDV) is a single-stranded RNA virus of the genus *Arterivirus* belonging to the family Arteriviridae. The genome organization and replication of LDV and other arteriviruses, their cell biology and other molecular aspects have been reviewed by Snijder and Meulenberg [214]. LDV has repeatedly been detected in wild mice (*Mus musculus*), which are considered to be a virus reservoir [215, 216]. After infection of mice, virus titres of 10^{10}–10^{11} particles per ml serum are found within 12–14 h after infection. The virus titre drops to 10^5 particles per ml within 2–3 weeks and remains constant at this level for life. It persists in infected mice for the whole lifetime although it stimulates various immune mechanisms [216–219]. The virus can be stored in undiluted mouse plasma at −70 °C without loss of infectivity, but it is not stable at room temperature and is very sensitive to environmental conditions. Only mice and primary mouse cells are susceptible to infection with LDV. It replicates in a subpopulation of macrophages in almost all tissues and persists in lymph nodes, spleen, liver, and testes tissues [220]. As suitable cell systems have not been available for virus production, routine serology has not been easily possible so that testing for LDV was not included in serological health monitoring programs. The prevalence of LDV in contemporary colonies of laboratory rodents is likely to be very low but detailed information about its prevalence comparable to most other agents is not available.

LDV was first detected during a study of methods that could be used in the early diagnosis of tumours [221]. It produces a persistent infection with continuous virus production and a lifelong viraemia despite LDV-specific immune reactions of the host [217]. LDV has been found in numerous biological materials that are serially passaged in mice such as transplantable tumours including human tumours or matrigel prepared from such materials [1, 2, 222, 223], monoclonal antibodies or ascitic fluids [224], or infectious agents (e.g. haemoprotozoans, K virus, *Clostridium piliforme*). These materials are contaminated after serial passage in an infected and viraemic mouse. Contamination with LDV leads to the infection of each sequential host and to transmission of the virus by the next passage and remains associated with the specimen. It is therefore the most frequently detected contaminant in biological materials [1, 2].

Infection with LDV is usually asymptomatic, and there are no gross lesions in immunocompetent as well as in immunodeficient mice. The only exception is poliomyelitis with flaccid paralysis of hindlimbs developing in C58 and AKR mice when they are immunosuppressed either naturally with ageing or experimentally. It has been shown that only mice harbouring cells in the CNS that express a specific endogenous MuLV are susceptible to poliomyelitis [225].

The characteristic feature of LDV infection is the increased activity of lactate dehydrogenase (LDH) and other plasma enzymes [8, 226], which is due to the continuous destruction of permissive macrophages that are responsible for the clearance of LDH from the circulation. As a consequence, the activity of plasma LDH begins to rise by only 24 h after infection and peaks 3–4 days after infection at 5–10-fold normal levels, or can even be up to 20-fold in SJL/J mice. The enzyme activity declines during the next 2 weeks but remains elevated throughout life.

Antigen-antibody complexes produced during infection circulate in the blood and are deposited in the glomeruli [226]. In contrast to other persistent virus infections (e.g. lymphocytic choriomeningitis virus), these complexes do not lead to immune complex disease and produce only a very mild glomerulopathy. The only gross finding associated with LDV infection is mild splenomegaly. Microscopically, necrosis of lymphoid tissues is visible during the first days of infection. In mouse strains that are susceptible to poliomyelitis, LDV induces lesions in the grey matter of the spinal cord and the brainstem.

LDV is not easily transmitted between mice, even in animals housed in the same cage. Fighting and cannibalism increase transmission between cagemates, most likely via blood and saliva. Infected females transmit the virus to their fetuses if they have been infected few days prior to birth and before IgG anti-LDV antibodies are produced, but developmental and immunological factors (e.g. gestational age, timing of maternal infection with LDV, placental barrier) are important in the regulation of transplacental LDV infection [227, 228]. Maternal immunity protects fetuses from intrauterine infection. Immunodeficient *Prkdc*^{scid} mice also transmit virus to their offspring during chronic infection [229]. An important means of transmission is provided by experimental procedures such as mouse-to-mouse passage of contaminated biological materials or the use of the same needle for sequential inoculation of multiple mice.

In principle, serological methods such as IFA may be used for detecting LDV infection [230] but they are not of practical importance. Circulating virus-antibody complexes interfere with serological tests, and sufficient quantities of virus for serological tests are difficult to generate because LDV replicates only in specific subpopulations of primary cultures of murine macrophages and monocytes for one cell cycle [226]. However, it is meanwhile possible to use recombinant viral proteins of LDV as antigens [231] in ELISA and MFIA tests so that routine testing by serology is possible. In the past, diagnosis of LDV infection has primarily been based on increased LDH activity in serum or plasma of mice. LDV activity in serum or plasma can be measured directly, or samples (e.g. plasma, cell or organ homogenates) are inoculated into pathogen-free mice and the increase in LDH activity within 3–4 days is measured. An 8–10-fold increase is indicative of LDV infection. Detection of infectivity of a plasma sample by the induction of increased LDH activity in the recipient animal is the most reliable means of identifying an infected animal. However, it is important to use clear non-haemolysed samples because haemolysis will (falsely) elevate activities of multiple serum or plasma enzymes, including LDH. This assay was usually included in a 'MAP test', but antibody detection similar to other viruses was not involved for reasons mentioned earlier. Persistent infection makes LDV an ideal candidate for PCR detection in plasma or in organ homogenates [232, 233]. However, reports exist that PCR may produce false-negative results and should be used cautiously [234]. Just as important as detecting LDV in animals is its detection in biological materials. This may be done by assay for increased LDH activity after inoculation of suspect material into pathogen-free mice [1, 2] or by PCR [232, 233, 235–237].

LDV spreads slowly in a population because direct contact is necessary. Therefore, LDV-negative breeding populations can easily be established by selecting animals with normal plasma LDH activity. Embryo transfer and hysterectomy derivation are also efficient. The presence of LDV in experimental populations may be indicative of contaminated biological materials. In such cases, it is essential that the virus is also eliminated from these samples. This is easily achieved by maintenance of cells by *in vitro* culture instead of by animal-to-animal passages [238]. Due to the extreme host specificity of the virus, contaminated tumour samples can also be sanitized by passages in nude rats [223] or other animal species. Another method to remove LDV from contaminated cells, which is based on cell sorting, has recently been described [239].

LDV is a potential confounder of any research using biological materials that are passaged in mice. Once present in an animal, the virus persists lifelong. The most obvious signs are increased levels of plasma LDH and several other enzymes. LDV may also exhibit numerous effects on the immune system (thymus involution, depression of cellular immunity, enhanced or diminished humoral responses, NK cell activation, development of autoimmunity, and suppression of development of diabetes in NOD mice); [218, 219, 224, 240–244] and enhance or suppress tumour growth [6, 7, 226]. Interaction with other viruses has also been described [245].

Lymphocytic choriomeningitis virus (LCMV)

Lymphocytic choriomeningitis virus (LCMV) is an enveloped, segmented single-stranded RNA virus of the genus *Arenavirus* family, Arenaviridae. It can easily be propagated in several commonly used cell lines like BHK-21 cells. However, cells are not lysed and a cytopathic effect (CPE) is not visible. The virus name refers to the condition that results from experimental intracerebral inoculation of the virus into adult mice and is not considered to be a feature of natural infections. Mice (*Mus musculus*) serve as the natural virus reservoir [246], but Syrian hamsters are also important hosts [247]. Additional species such as rabbits, guinea-pigs, squirrels, monkeys and humans are susceptible to natural or experimental infection [248]. Natural infection of callitrichid primates (marmosets and tamarins) leads to a progressive hepatic disease that is known as 'callitrichid hepatitis' [249, 250]. Antibodies to LCMV have been found in wild mice in Europe [251, 252], Africa [253], Asia [254], Australia [125] and America [255]. Thus, it is the only arenavirus with worldwide distribution. Infection with LCMV is rarely found in laboratory mice [248]. Seropositivity to LCMV in laboratory mice was reported to be low during the last decade [11, 15, 17, 124] or negative [12–14]. In addition to laboratory mice and other vertebrate hosts, the virus has frequently been found in transplantable tumours and tissue culture cell lines from mice and hamsters [2, 256].

Despite the low prevalence in laboratory mice, seropositivity to this zoonotic agent should raise serious concern for human health. LCMV is frequently transmitted to humans from wild mice and is also endemic to a varying degree in the human population [257–261] due to contact with wild mice. It has also been transmitted to humans by infected laboratory mice [262] and by pet and laboratory Syrian hamsters [263–266]. In addition, contaminated biological materials are important sources of infections for humans, and several outbreaks of LCM among laboratory personnel have been traced to transplantable tumours [267, 268]. Transmission of LCMV to humans also occurred repeatedly by organ transplantation and was most likely transmitted to organ donors by close contact with infected pets [266, 269]. LCMV can cause mild-to-serious or fatal disease in humans [262, 270, 271]. Congenital infection in humans may result in hydrocephalus, or fetal or neonatal death [272].

In mice, clinical signs of LCMV infection vary with strain and age of mouse, strain and dose of virus, and route of inoculation [8, 248, 251]. Two forms of natural LCMV infection are generally recognized: a persistent tolerant and an (acute) non-tolerant form. The persistent form results from infection of mice that are immunotolerant. This is the case if mice are infected *in utero* or during the first days after birth. This form is characterized by lifelong viraemia and viral shedding. Mice may show growth retardation, especially during the first 3–4 weeks, but they appear otherwise normal. Infectious virus is bound to specific antibodies and complement, and these complexes accumulate in the renal glomeruli, the choroid plexus, and sometimes also in synovial membranes and blood vessel walls. At 7–10 months of age, immune complex nephritis develops with ruffled fur, hunched posture, ascites and occasional deaths. This immunopathologic phenomenon is called 'late onset disease' or 'chronic immune complex disease'. The incidence of this type of disease varies between mouse strains. Gross lesions include enlarged spleen and lymph nodes due to lymphoid hyperplasia. Kidneys affected with glomerulonephritis may be enlarged with a granular surface texture or may be shrunken in later stages of the disease process. Microscopically, there is generalized lymphoid hyperplasia and immune complex deposition in glomeruli and vessel walls, resulting in glomerulonephritis and plasmacytic, lymphocytic perivascular cuffs in all visceral organs [36].

The non-tolerant acute form occurs when infection is acquired after the development of immunocompetence (in mice older than 1 week). These animals become viraemic but do not shed virus and may die within a few days or weeks. Natural infections of adults are usually asymptomatic. Surviving mice are seropositive and in most cases clear the virus to below detection levels of conventional methods. However, virus may persist at low levels in tissues (particularly spleen, lung and kidney) of mice for at least 12 weeks after infection as determined by sensitive assays such as nested reverse transcriptase (RT)-PCR or immunohistochemistry [273]. Such non-lethal infection leads to protection against otherwise lethal intracerebral challenge. Protection from lethal challenge is also achieved by maternally derived anti-LCMV antibodies through nursing or by the administration of anti-LDV monoclonal IgG2a antibodies [274].

In experimentally infected mice, the route of inoculation (subcutaneous, intraperitoneal, intravenous, intracerebral) also influences the type and degree of disease [248]. Intracerebral inoculation of adult immunocompetent mice typically results in tremors, convulsions and death due to meningoencephalitis and hepatitis. Neurological signs usually appear on day 6 after inoculation, and animals die within 1–3 days after the onset of symptoms, or recover within several days. The classic histological picture is of dense perivascular accumulations of lymphocytes and plasma cells in meninges and choroid plexus. While infection following subcutaneous inoculation usually remains inapparent, reaction of mice to intraperitoneal or intravenous inoculation depends on the virus strain and on the mouse strain. Infection by these routes primarily causes multifocal hepatic necrosis and necrosis of lymphoid cells. Athymic *Foxn1*^{nu} mice and other immunodeficient mice do not develop disease but become persistently viraemic and shed virus.

As a general rule, all pathological alterations following LCMV infection are immune-mediated; and mice can be protected from LCMV-induced disease by immunosuppression [275]. LCMV disease is a prototype for virus-induced

T-lymphocyte-mediated immune injury and for immune complex disease. For detailed information on the pathogenesis, clinical and pathological features of LCMV infection, the reader is referred to review articles [248, 276, 277].

In nature, carrier mice with persistent infection serve as the principal source of virus. Intrauterine transmission is very efficient, and with few exceptions all pups born from carrier mice are infected. Furthermore, persistently infected mice and hamsters can shed large numbers of infectious virions primarily in urine, but also in saliva and milk. The virus can replicate in the gastric mucosa after intragastric infection [278, 279]. Gastric inoculation elicits antibody responses of comparable magnitudes as intravenous inoculation and leads to active infection with LCMV, indicating that oral infection is possible, e.g. by ingestion of contaminated food or by cannibalism. A self-limiting infection frequently results from infection of adult mice. The virus does not spread rapidly after introduction in populations of adult mice, and the infectious chain usually ends. However, if the virus infects a pregnant dam or a newborn mouse, a lifelong infection results, and soon a whole breeding colony of mice may become infected if the mice live in close proximity (which is the case under laboratory conditions). The virus is not easily transmitted to dirty-bedding sentinels, and it is important that colony animals or animals having had direct contact with a population are tested to exclude LCMV infection [280].

LCMV is most commonly diagnosed by serological methods such as MFIA, IFA and ELISA [281]. All strains show a broad cross-reactivity and are serologically uniform. However, subclinical persistent infections may be difficult to detect because they may be associated with minimal or undetectable levels of circulating antibody. It is important that bleeding of mice is done carefully because of a potential risk due to viraemic animals. Historically, direct viral detection was performed by inoculating body fluids or tissue homogenates into the brain of LCMV-free mice or by subcutaneous injection into mice and subsequent serological testing (MAP test). More recently, PCR assays have been developed for the direct detection of viral RNA in clinical samples or animals [282–284]. Both MAP test and PCR can also be used to detect contamination of biological materials [235, 237]. Specifically for exclusion of contamination by LCMV, it was requested by different authorities that virus is inoculated intracerebrally at a lethal dose 3–4 weeks after administration of the material to be tested. In case of contamination by LCMV and subsequent seroconversion, animals survive the challenge infection.

Vertical transmission of LCMV by transuterine infection is efficient so that this virus cannot reliably be eliminated by caesarean rederivation [280]. Caesarean derivation may be effective if dams acquired infection after the development of immunocompetence (non-tolerant acute infection) and subsequently eliminated the virus, but such a strategy is difficult to justify in light of LCMV's zoonotic potential. In breeding colonies of great value, virus elimination might be possible soon after introduction into the colony by selecting non-viraemic breeders. This procedure is expensive and time consuming and requires special safety precautions.

Fortunately, infections of laboratory mice with LCMV are very uncommon. However, once LCMV has been detected in animals, or in biological materials, immediate destruction of all contaminated animals and materials is advisable to avoid risk of human infection. *Foxn1*^{nu} and *Prkdc*^{scid} mice may pose a special risk because infections are silent and chronic [268]. Cages and equipment should be autoclaved, and animal rooms should be fumigated with disinfectants such as formaldehyde, vaporized paraformaldehyde, hydrogen peroxide or other effective disinfectants. Prevention of introduction into an animal facility requires that wild mice cannot get access to the facility. Similarly important is screening of biological materials originating from mice and hamsters because these can be contaminated by LCMV. Finally, it has been shown that the virus can also be introduced into a population by mice with an undetected infection [280].

Appropriate precautions are necessary for experiments involving LCMV, or LCMV-infected animals or materials. Biological safety level (BSL) 2 will be considered to be sufficient in most cases. BSL 3 practices may be considered when working with infected animals owing to the increased risk of virus transmission by bite wounds, scratching or aerosol formation from the bedding. Animal

Biosafety Level (ABSL) 3 practices and facilities are generally recommended for work with infected hamsters. Appropriate precautions have been defined for different BSLs or ABSLs by CDC [285].

LCMV is frequently utilized as a model organism to study virus–host interactions, immunological tolerance, virus-induced immune complex disease, and a number of immunological mechanisms *in vivo* and *in vitro* [286–288]. Accidental transmission may have a severe impact on various kinds of experiments [6, 7, 248, 251] and also affect infection with other agents [289].

Mammalian orthoreovirus serotype 3 (MRV-3)

Mammalian orthoreoviruses (MRV) are non-enveloped, segmented double-stranded RNA viruses of the family Reoviridae, genus *Orthoreovirus*. They have a wide host range and are ubiquitous throughout the world. The designation *reo* stands for respiratory enteric orphan and reflects the original isolation of these viruses from human respiratory and intestinal tract without apparent disease. The term 'orphan' virus refers to a virus in search of a disease. Mammalian orthoreovirus can be grouped into three serotypes, numbered 1–3. Mammalian orthoreovirus-3 (synonyms: hepatoencephalomyelitis virus; ECHO 10 virus) infection remains prevalent in contemporary mouse colonies and has been reported in wild mice [20, 125, 290]. A study in France reported antibodies to MRV-3 in 9% of mouse colonies examined [10]. In more recent studies in North America and western Europe, such antibodies were detected in 0.01–0.2% of mice monitored [11, 14, 15]. Schoondermark-van de Ven et al. [12] found antibodies to MRV-3 in 0.6% of mouse samplings from western European institutions; and in a survey conducted by Carty [13], about 6% of responding institutions in the USA reported MRV-3 infection in their mouse colonies. In addition, contamination of mouse origin tumours and cell lines by MRV-3 has been reported many times [2, 8, 290]. Experimentally, MRV-3 infection of infant mice has been used to model human hepatobiliary disease, pancreatitis, diabetes mellitus and lymphoma [8, 291].

The literature on MRV-3 infections in mice is dominated by studies on experimentally infected animals. The virus can cause severe pantropic infection in infant mice [290–292]. After parenteral inoculation, virus can be recovered from the liver, brain, heart, pancreas, spleen, lymph nodes and blood vessels. Following oral inoculation, reoviruses gain entry by infecting specialized epithelial cells (M cells) that overlie Peyer's patches. The virus then becomes accessible to leukocytes and spreads to other organs by way of the lymphatic system and the bloodstream. Neural spread to the CNS has also been well documented [293, 294]. The mechanisms of viral pathogenesis and their interactions with the host cell as well as the host's immune response are reviewed in detail by Tyler et al. [295], Schiff et al. [296] and Ward et al. [291].

Natural infection by MRV-3 in a mouse colony is usually subclinical, although diarrhoea or steatorrhoea and oily hair effect in suckling mice may be noted [8, 36, 290–292]. The latter term has been used to describe the matted, unkempt appearance of the hair coat that results from steatorrhoea due to pancreatitis, maldigestion and biliary atresia. In addition, runting (attributed to immune-mediated destruction of cells in the pituitary gland that produce growth hormone), transient alopecia, jaundice (due to excessive bilirubin in the blood, which is attributed to the liver pathology, especially biliary atresia) and neurological signs such as incoordination, tremors or paralysis may develop. When present in natural infections, clinical signs and lesions are similar to but milder than in experimental neonatal infections. Early descriptions of naturally occurring disease may have been complicated by concurrent infections such as MHV (murine hepatitis virus) or murine rotavirus A (MuRV-A)/epizootic diarrhoea of infant mice (EDIM) virus that contributed to the severity of the lesions especially in liver, pancreas, CNS and intestine. The outcome of MRV-3 infection depends on age and immunological status of mouse, dose of virus and route of inoculation. Adult immunocompetent mice typically show no clinical signs and have no discernible lesions even in experimental infections. Mucosal and maternally conferred immunity are considered to be important in protection from or resolution of disease [297, 298]. Experimental infection of adult $Prkdc^{scid}$ mice is lethal [299]. Depending on the route of inoculation, experimental infection of adult $Foxn1^{nu}$ mice is subclinical or results in liver disease [299, 300].

Histological findings reported to occur after experimental MRV-3 infection of neonatal mice include inflammation and necrosis in liver, pancreas, heart, adrenal, brain, and spinal cord; lymphoid depletion in thymus, spleen, and lymph nodes; and hepatic fibrosis with biliary atresia [36, 290–292, 298].

Transmission of reoviruses probably involves the aerosol as well as the faecal–oral route [8, 291]. Fomites may play an important role as passive vectors because reoviruses resist environmental conditions moderately well.

Serological screening with MFIA, ELISA or IFA is in widespread use for detection of antibodies to MRV-3 in diagnostic and health surveillance programmes. Both ELISA and IFA detect cross-reacting antibodies to heterologous MRV serotypes that can infect mice [301], although a recent report indicates that some IFA-positive MRV infections in mice may not be detected by commonly used ELISAs [302]. The HI test does not detect such cross-reacting antibodies but is prone to give false-positive results due to non-specific inhibitors of haemagglutination [301, 303]. RT-PCR methods for the detection of MRV-3 RNA [304, 305] or MRV RNA [302, 306] are also available. Reports on contamination of mouse origin tumours and cell lines by MRV-3 and its interference with transplantable tumour studies [307, 308] emphasize the importance of screening of biological materials to be inoculated into mice by MAP test or PCR. Natural seroconversion to MRV-3 without clinical disease is also observed in laboratory rats, hamsters and guinea-pigs [8, 290].

Caesarean derivation and barrier maintenance have proven effective in the control and prevention of MRV-3 infection [8, 291].

The virus may interfere with research involving transplantable tumours and cell lines of mouse origin. It has the potential to alter intestinal studies and multiple immune response functions in mice. In enzootically infected colonies, protection of neonates by maternal antibody could complicate or prevent experimental infections with reoviruses. It could further complicate experiments that require evaluation of liver, pancreas, CNS, heart, lymphoid organs and other tissues affected by the virus.

Murine hepatitis virus (MHV)

The term murine hepatitis virus (MHV; commonly referred to as 'mouse hepatitis virus') designates a large group of antigenically and genetically related, single-stranded RNA viruses belonging to the family Coronaviridae, genus *Coronavirus*. They are surrounded by an envelope with a corona of surface projections (spikes). MHV is antigenically related to rat coronaviruses and other coronaviruses of pigs, cattle and humans. Numerous different strains or isolates of MHV have been described. They can be distinguished by neutralization tests that detect strain-specific spike (S) antigens, by use of monoclonal antibodies, or by sequencing [309]. The best-studied strains are the prototype strains MHV-1, MHV-2, MHV-3, JHM (MHV-4), A59, and S, of which MHV-3 is regarded as the most virulent. Like other coronaviruses MHV mutates rapidly, and strains readily form recombinants, so that new (sub)strains are constantly evolving. Strains vary in their virulence, organotropism and cell tropism [310]. Based on their primary organotropism, MHV strains can be grouped into two biotypes: respiratory (or polytropic) and enterotropic. However, intermediate forms (enterotropic strains with tropism to other organs) also exist. Murine hepatitis virus is relatively resistant to repeated freezing and thawing, heating (56 °C for 30 min) and acid pH but is sensitive to drying and disinfectants, especially those with detergent activity [8]. Given the environmental conditions present in mouse rooms, MHV might remain infective for several days, at low humidity (20% relative humidity) or low temperatures (4 °C) even for weeks on surfaces [311].

Mus musculus is the natural host of MHV. It can be found in wild and laboratory mice throughout the world and is one of the most common viral pathogens in contemporary mouse colonies. While polytropic strains have historically been considered more common, this situation is thought to have reversed. Monitoring results for research institutions across North America and Europe indicate that the prevalence of MHV has decreased in the past, though it seems to have remained quite stable since the

1990s [11, 12]. Recently 1.57% of North American laboratory mouse serum samples tested positive [15]. In Europe, prevalence rates ranged from 3.25% to 12% [12, 14, 15]. A retrospective study in France covering the period from 1988 to 1997 reported antibodies to MHV in 67% of mouse colonies examined [10], and a survey performed in 2006 revealed that almost half of North American research institutions detected MHV in their mouse populations [13]. Suckling rats inoculated experimentally with MHV had transient virus replication in the nasal mucosa and seroconversion but no clinical disease [312]. Similarly, deer mice seroconverted but showed no clinical disease after experimental infection [313]. MHV is also a common contaminant of transplantable tumours [1, 2] and cell lines [314, 315].

The pathogenesis and outcome of MHV infections depend on interactions between numerous factors related to the virus (e.g. virulence and organotropism) and the host (e.g. age, genotype, immune status, and microbiological status) [8, 36, 309, 310, 316, 317]. MHV strains appear to possess a primary tropism for the upper respiratory or enteric mucosa. Those strains with respiratory tropism initiate infection in the nasal mucosa and then may disseminate via blood and lymphatics to a variety of other organs because of their polytropic nature. Respiratory (polytropic) strains include MHV-1, MHV-2, MHV-3, A59, S and JHM. Infection of mice with virulent polytropic MHV strains, infection of mice less than 2 weeks of age, infection of genetically susceptible strains of mice or infection of immunocompromised mice favour virus dissemination. Virus then secondarily replicates in vascular endothelium and parenchymal tissues, causing disease of the brain, liver, lymphoid organs, bone marrow and other sites. Infection of the brain by viraemic dissemination occurs primarily in immunocompromised or neonatal mice. Additionally, infection of adult mouse brain can occur by extension of virus along olfactory neural pathways, even in the absence of dissemination to other organs. In contrast, enterotropic MHV strains (e.g. LIVIM, MHV-D, MHV-Y) tend to selectively infect intestinal mucosal epithelium, with no or minimal dissemination to other organs such as mesenteric lymph nodes or liver.

All ages and strains are susceptible to active infection, but disease is largely age related. Infection of neonatal mice results in severe necrotizing enterocolitis with high mortality within 48 h. Mortality and lesion severity diminish rapidly with advancing age at infection. Adult mice develop minimal lesions although replication of equal or higher titres of virus occurs compared with neonates. The age-dependent decrease in severity of enterotropic MHV disease is probably related to the higher mucosal epithelium turnover in older mice, allowing more rapid replacement of damaged mucosa. Another factor that is of considerable importance to the outcome of MHV infections is host genotype. For example, BALB/c mice are highly susceptible to enterotropic MHV disease while SJL mice, at the other end of the spectrum, are highly resistant [318]. Unlike in polytropic MHV infection where resistance is correlated with reduced virus replication in target cells [319], enterotropic MHV grows to comparable titres in SJL and BALB/c mice at all ages [318]. Therefore, the resistance of the SJL mouse to disease caused by enterotropic MHV seems to be mediated through an entirely different mechanism than resistance to polytropic MHV. Furthermore, mouse genotypes that are susceptible to disease caused by one MHV strain may be resistant to disease caused by another strain [316]. It is therefore not possible to strictly categorize mouse strains as susceptible or resistant. The genetic factors determining susceptibility versus resistance in MHV infections are as yet poorly understood. Both polytropic and enterotropic MHV infections are self-limiting in immunocompetent mice. Immune-mediated clearance of virus usually begins about a week after infection, and most mice eliminate the virus within 3–4 weeks [316, 318, 320]. Humoral and cellular immunity appear to participate in host defences to infection, and functional T cells are an absolute requirement [321–324]. Therefore, immunodeficient mice such as *Foxn1*^{nu} and *Prkdc*^{scid} mice cannot clear the virus [317, 325]. Similarly, some genetically modified strains of mice may have deficits in antiviral responses or other alterations that allow the development of persistent MHV infection [326]. Recovered immune mice are resistant to reinfection with the same MHV strain but remain susceptible to repeated infections with different strains of MHV [327–329]. Similarly, maternal immunity protects suckling mice against homologous MHV strains but not necessarily against other strains [329, 330]. However, maternal immunity, even to homologous strains, depends on the presence of maternally acquired antibody in the lumen of the intestine [330]. Therefore, the susceptibility of young mice to infection significantly increases at weaning.

Most MHV infections are subclinical and follow one of two epidemiological patterns in immunocompetent mice [8, 310]. Enzootic (subclinical) infection, commonly seen in breeding colonies, occurs when a population has been in contact with the virus for a longer period (e.g. several weeks). Adults are immune (due to prior infection), sucklings are passively protected, and infection is perpetuated in weanlings. Epizootic (clinical) infection occurs when the virus is introduced into a naive population (housed in open cages). The infection rapidly spreads through the entire colony. Clinical signs depend upon the virus and mouse strains and are most evident in infant mice. Typically, they include diarrhoea, poor growth, lassitude, and death. In infections due to virulent enterotropic strains, mortality can reach 100% in infant mice. Some strains may also cause neurological signs such as flaccid paralysis of hindlimbs, convulsions and circling. Adult infections are again usually asymptomatic. As the infection becomes established in the colony, the epizootic pattern is replaced by the enzootic pattern. In immunodeficient (e.g. *Foxn1^{nu}* and *Prkdc^{scid}*) mice, infection with virulent polytropic MHV strains is often rapidly fatal while less virulent strains cause chronic wasting disease [317]. In contrast, adult immunodeficient mice can tolerate chronic infection by enterotropic MHV, with slow emaciation and diarrhoea, or minimal clinical disease [316, 325]. Subclinical MHV infections can be activated by a variety of experimental procedures (e.g. thymectomy, whole body irradiation, treatment with chemotherapeutic agents, halothane anaesthesia) or by coinfections with other pathogens (e.g. *Eperythrozoon coccoides*, K virus; reviewed in [8, 309]).

In most natural infections, gross lesions are not present or are transient and not observed. Gross findings in neonates with clinical signs include dehydration, emaciation, and in contrast to EDIM, an empty stomach [309, 331, 332]. The intestine is distended and filled with watery to mucoid yellowish, sometimes gaseous contents. Haemorrhage or rupture of the intestine can occur. Depending on the virus strain, necrotic foci on the liver [36, 309, 332] and thymus involution [331, 333] may also be seen in susceptible mice. Liver involvement may be accompanied by jaundice and haemorrhagic peritoneal exudate. Splenomegaly may occur as a result of compensatory haematopoiesis [334].

Histopathological changes in susceptible mice infected with polytropic MHV strains include acute necrosis with syncytia in liver, spleen, lymph nodes, gut-associated lymphoid tissue, and bone marrow [8, 36, 309, 316] (Figure 3.2.7). Recently, pulmonary inflammation has been observed in susceptible mouse strains (C3H/HeJ and A/J) after intranasal inoculation with polytropic MHV-1 [335, 336]. Neonatally infected mice can have vascular-oriented necrotizing (meningo)encephalitis with demyelination in the brainstem and periependymal areas. Lesions in peritoneum, bone marrow, thymus and other tissues can be variably present. Mice can develop nasoencephalitis due to extension of infection from the nasal mucosa along olfactory pathways to the brain, with meningoencephalitis and demyelination, the latter of which is thought to be largely T-cell mediated [324]. This pattern of infection regularly occurs after intranasal inoculation of many MHV strains but is a relatively rare event after natural exposure. Syncytium arising from endothelium, parenchyma or leukocytes is a hallmark of infection in many tissues including intestine, lung, liver, lymph nodes, spleen, thymus, brain and bone marrow. Lesions are transient and seldom fully developed in adult immunocompetent mice, but they are manifest in immunocompromised mice. Highly unusual presentations can occur in mice with specific gene defects. For example, granulomatous peritonitis and pleuritis were found in interferon-gamma-deficient mice infected with MHV [337].

Histopathological changes caused by enterotropic strains of MHV are mainly confined to the intestinal tract and associated lymphoid tissues [8, 36, 309, 316]. The most common sites are terminal ileum, caecum and proximal colon. The severity of disease is primarily age-dependent, with neonatal mice being most severely affected. These mice show segmentally

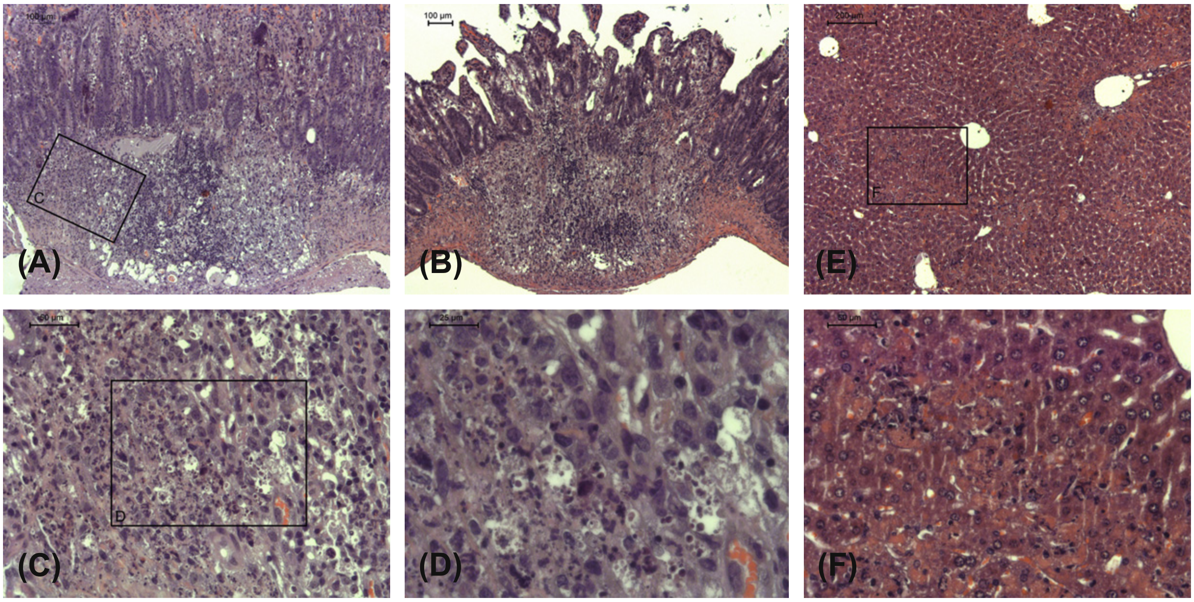

Figure 3.2.7 Mouse infected with a polytropic necrosis in intestine (A-D) and liver (E, F).

distributed areas of villus attenuation, enterocytic syncytia (balloon cells) and mucosal necrosis accompanied by leukocytic infiltration. Intracytoplasmic inclusions are present in enterocytes. Erosions, ulceration, and haemorrhage may be seen in more severe cases. Lesions can be fully developed within 24–48 h, but are usually more severe at 3–5 days after infection. Surviving mice may develop compensatory mucosal hyperplasia. Mesenteric lymph nodes usually contain lymphocytic syncytia, and mesenteric vessels may contain endothelial syncytia. Pathological changes in older mice are generally much more subtle and may only consist of transient syncytia. An occasional exception seems to occur in immunodeficient animals such as *Foxn1*^{nu} mice, which can develop chronic hyperplastic typhlocolitis of varying severity [325], but other agents such as *Helicobacter spp.* may have been involved. In general, enterotropic MHV strains do not disseminate, but hepatitis and encephalitis can occur with some virus strains in certain mouse genotypes. In T-cell deficient mice, multisystemic lethal infection was observed after experimental infection with the enterotropic strain MHV-Y [338].

MHV is highly contagious. It is shed in faeces and nasopharyngeal secretions and appears to be transmitted via direct contact, aerosol and fomites [8,309]. Vertical (*in utero*) transmission has been demonstrated in experimental infections [339] but does not seem to be of practical importance under natural conditions. MHV was transmitted by ovarian transplantation after reproductive organs became infected [340]. However, risk of MHV transmission by sperm or oocytes (IVF) or by embryo transfer seems to be low, though thorough washing of gametes and embryos is required [211, 340–342].

Diagnosis during the acute stage of infection can be made by histological demonstration of characteristic lesions with syncytia in target tissues, but clinical signs and lesions can be highly variable and may not be prominent. Suckling, genetically susceptible or immunocompromised mice are the best candidates for evaluation. Active infection can be confirmed by immunohistochemistry [343] or by virus isolation. Virus recovery from infected tissues is difficult but can be accomplished using primary macrophage cultures or a number of established cell lines such as NCTC 1469 or DBT [301]. These cells, however, may not be successful substrates for some enterotropic MHV strains. Virus in suspect tissue can also be confirmed by bioassays such as MAP testing or infant or *Foxn1*^{nu} mouse inoculation [301, 344]. Amplification by passage in these mice increases the likelihood of detection of lesions and antigen, or virus recovery. Other direct diagnostic methods that have been successfully utilized to detect MHV in faeces or tissue of infected mice include monoclonal antibody solution hybridization assay [345] and a number of RT-PCR assays [346–349]. Because of the transient nature of MHV infection in immunocompetent mice, serology is the most appropriate diagnostic tool for routine monitoring. Multiplex fluorescent immunoassay, ELISA and IFA are well established and sensitive, and all known MHV strains cross-react in these tests [301, 350, 351]. The magnitude of antibody response depends on MHV strain and mouse genotype [319, 352]. DBA/2 mice are poor antibody responders whereas C57BL/6 mice produce a high antibody titre and are therefore good sentinels. Antibody titres remain high over a period of at least 6 months [327, 329]. Infected mice may not develop detectable antibodies for up to 14 days after initial exposure [350]. In such cases, a direct diagnostic method, as discussed above, may be useful. Another drawback of serology is that mice weaned from immune dams can have maternal antibodies until they are 10 weeks of age [353]. This may impact serological monitoring because the possibility must be considered that low positive results are due to maternally derived passive immunity. Because the virus can be transmitted by transplantable tumours and other biological materials from mice, including hybridomas [354] and embryonic stem cells [355, 356] these materials should also be routinely screened for MHV contamination. Mouse inoculation bioassay, MAP test and RT-PCR can be used for this purpose. Therefore, surveillance programmes should combine careful evaluation of clinically ill animals, testing of biological materials and routine health monitoring. Soiled-bedding sentinel mice, which are frequently used for routine monitoring, are likely well suited for detecting enterotropic strains of MHV, but might not indicate the presence of less contagious respiratory strains of MHV [309] equally well. The mouse strain used as sentinel should be considered as a critical factor. Furthermore, duration of MHV shedding and stability of the virus, which seems to be lower in static microisolator cages than in IVC cages, might interfere with detection. The amount of bedding transferred seems not to be as critical as for, e.g. parvoviruses, at least for enterotropic strains [357]. Use of contact and exhaust air sentinels and testing of exhaust filters by PCR was also shown to be effective at detecting MHV [358].

The best means of MHV control is to prevent its entry into a facility. This can be accomplished by purchasing mice from virus-free sources and maintenance under effective barrier conditions monitored by a well-designed quality assurance programme. Control of wild mouse populations, proper husbandry and sanitation, and strict monitoring of biological materials that may harbour virus are also important measures to prevent infection. If infection occurs, the most effective elimination strategy is to cull the affected colony and obtain clean replacement stock. However, this is not always a feasible option when working with valuable mice (e.g. genetically modified lines, breeding stocks). Caesarean derivation or embryo transfer can be used to produce virus-free offspring, and foster-nursing has also been reported to be effective [359]. Quarantine of an affected colony with no breeding and no introduction of new animals for approximately 2 months has been effective in immunocompetent mice [360]. The infection is likely to be terminated because MHV requires a constant supply of susceptible animals. This method works best when working with small numbers of mice. Large populations favour the development of new MHV strains that may result in repeated infections with slightly different strains [361]. It may be practical to select a few future breeders from the infected population and quarantine them for approximately 3 weeks [317]. This can be achieved in isolators, or in individually ventilated cages if proper handling is guaranteed. After this interval, breeding can resume. The 3-week interval should permit recovery from active infection, and the additional 3-week gestation period effectively extends the total quarantine to 6 weeks. It is advisable to select seropositive breeders because the possibility of active infection is lower in such animals. The breeding cessation strategy may not be successful if immunodeficient mice are used because they are susceptible to chronic infection and viral excretion [325]. Genetically engineered mice of unclear, unknown or deficient immune status pose a special challenge because they may develop unusual manifestations of infection or may be unable to clear virus. Rederivation is likely to be the most cost-effective strategy in such situations. Along with the measures described, proper sanitation and disinfection of caging and animal quarters, as well as stringent personal sanitation, are essential to eliminate infection. Careful testing with sentinel mice should be applied to evaluate the effectiveness of rederivation. If transplantable tumours are contaminated with MHV, virus elimination can be achieved by passage of tumours in athymic *Foxn1*^{rnu} rats [362].

MHV is one of the most important viral pathogens of laboratory mice and has been intensively studied from a number of research perspectives (e.g. as a model organism for studying coronavirus molecular biology or the pathogenesis of viral-induced demyelinating disease). Numerous reports document the effects of natural and experimental infections with MHV on host physiology and research, especially in the fields of immunology and tumour biology (reviewed in [6–8, 310, 316, 317]).

Murine norovirus (MNV)

Noroviruses are non-enveloped, single-stranded RNA viruses with high environmental resistance and belong to the family Caliciviridae, genus *Norovirus*. They were first identified after an outbreak of acute gastroenteritis at a school in Norwalk (Ohio, USA) in 1968 and cause about 90% of non-bacterial epidemic gastroenteritis in humans. Noroviruses found in animals include bovine, porcine and murine noroviruses. Noroviruses are not known to cross species. Murine norovirus (MNV) is endemic in many research mouse colonies and currently the most commonly detected viral agent in laboratory mice [14, 15, 363]. In the hitherto largest survey [15], about 32% of mouse serum samples examined had antibodies against MNV.

The first norovirus to infect mice was described in 2003 [364]. Experimental inoculation studies with this murine norovirus (MNV-1) show that duration of infection and disease manifestation vary depending on the mouse strain [363–365]. In immunocompetent strains, MNV infection is variable in length (e.g. ≥7–14 days in 129S6 mice, ≥5 weeks in Hsd:ICR mice) and does not induce clinical signs. Infection is associated with mild histopathological alterations in the small intestine (increase in inflammatory cells) and spleen (red pulp hypertrophy and white pulp activation) of 129S6 mice. In certain immunodeficient strains, however, infection can cause lethal systemic disease (encephalitis, vasculitis, meningitis, hepatitis and pneumonia in interferon-alpha-beta-gamma-receptor-deficient and *Stat1*^{tm1} mice) or persist without symptoms (≥90 days in *Rag1*$^{-/-}$ and *Rag2*$^{-/-}$ mice). These findings indicate that components of the innate immune system are critical for resistance to MNV-1 induced disease. Consistent with this hypothesis, it was demonstrated that MNV-1 replicates in macrophages and dendritic cells [366]. Meanwhile, many additional strains of MNV with diverse biological properties were isolated [367, 368]. An analysis of 26 MNV isolates revealed 15 distinct MNV strains that comprise a single genogroup and serotype [368]. Experimental inoculation studies show that several MNV strains are able to persist in various tissues (small intestine, caecum, mesenteric lymph node, spleen) of immunocompetent (C57BL/6J, Hsd:ICR, Jcl:ICR) and immunodeficient (CB17-*Prkdc*^{scid}) mice with viral shedding in faeces for the duration of at least 35–60 days [367–369]. Murine norovirus is transmitted via the faecal-oral route and is efficiently transferred to sentinel mice by soiled bedding [370, 371].

MNV infection can be detected directly by RT-PCR on faecal pellets or tissue specimens (see above) and indirectly by serology (MFIA, ELISA, IFA) [363, 367, 369]. Detection is facilitated by high stability of MNV RNA in faeces (at least 2 weeks at room temperature) [371] and by broad serological cross-reactivity among different strains of MNV [367, 368].

Embryo transfer [370] and hysterectomy [369] are most likely effective means of eliminating MNV from mouse colonies. Since 1- to 3-day-old pups are resistant to infection, elimination of MNV may also be achieved by transferring neonates from infected dams to uninfected foster dams ('cross-fostering') [372]. This transfer should ideally be performed within 24 h after birth.

MNV is used as a surrogate to evaluate resistance of human noroviruses to disinfectants. The impact of MNV on animal experiments remains to be evaluated. Recent studies show that MNV is immunmodulatory and may alter disease phenotypes in mouse models of inflammatory bowel disease [373–375] and other experimental mouse models [376, 377].

Murine pneumonia virus (PVM)

Murine pneumonia virus, commonly referred to as 'pneumonia virus of mice' (PVM), is an enveloped, single-stranded RNA virus of the family Paramyxoviridae, genus *Pneumovirus*. It is closely related to human respiratory syncytial virus (HRSV). The virus name is officially abbreviated as 'MPV' according to the International Union of Microbiological Societies [9]; however, the former designation 'PVM' will be used in this chapter to avoid confusion with the official abbreviation of mouse parvovirus (MPV). PVM infection remains prevalent in contemporary colonies of mice and rats throughout the world. A serological survey in France demonstrated antibodies to PVM in 16% of mouse colonies examined [10]. In more recent studies in North America and western Europe, the prevalence of PVM-specific antibodies in mice ranged between 0% and 0.1% [11, 14, 15]. Schoondermark-van de Ven et al. [12] found antibodies to PVM in 0.2% of mouse samplings from western European institutions. Antibodies to PVM have also been detected in hamsters, gerbils, cotton rats, guinea-pigs and rabbits [8, 378, 379]. Experimentally, PVM infection of mice is used as a model for HRSV infection and has therefore been extensively studied (reviewed by Rosenberg and Domachowske [380]).

In immunocompetent mice, natural infection with PVM is transient and usually not associated with clinical disease or pathological findings [8, 379, 381]. However, natural disease and persistent infection may occur in immunodeficient mice [382–384]. In particular, athymic *Foxn1*^{nu} mice seem to be susceptible to PVM infection, which can result in dyspnoea, cyanosis, emaciation and death due to pneumonia [383, 384]. Similar clinical signs have been reported for experimentally infected immunocompetent mice [385].

Necropsy findings in naturally infected *Foxn1*^{nu} mice include cachexia and diffuse pulmonary oedema or lobar consolidation [384]. Pulmonary consolidation (dark red or grey in color) has also been found after experimental infection of immunocompetent mice [381].

Histologically, natural infection of *Foxn1*^{nu} mice with PVM presents as interstitial pneumonia [383, 384]. Experimental intranasal inoculation of immunocompetent mice can result in rhinitis, erosive bronchiolitis and interstitial pneumonia with prominent early pulmonary eosinophilia and neutrophilia [381, 386]. Hydrocephalus may result from intracerebral inoculation of neonatal mice [387]. Susceptibility to infection is influenced by age and strain of mouse, dose of virus, and a variety of local and systemic stressors [8, 379, 386]. In terms of the extent of the alveolar inflammatory response, 129/Sv and DBA/2 mice are susceptible to PVM infection, while BALB/c and C57BL/6 mice are relatively resistant [386]. In terms of the control of viral replication, mice of strains 129/Sv, DBA/2, BALB/c and C57BL/6 are susceptible to PVM infection, while SJL mice are relatively resistant.

PVM is labile in the environment and rapidly inactivated at room temperature [8, 379]. The virus is tropic for the respiratory epithelium [382, 385], and transmission is exclusively horizontal via the respiratory tract, mainly by direct contact and aerosol [8, 379]. Therefore, transmissibility in mouse colonies is low, and infections tend to be focal enzootics.

Serology (MFIA, ELISA, IFA or HI) is the primary means of testing mouse colonies for exposure to PVM. Immunohistochemistry has been applied to detect viral antigen in lung sections [382, 384]; however, proper sampling (see Chapter 4.4, 'Health Management and Monitoring') is critical for establishing the diagnosis due to the focal nature of the infection. An RT-PCR assay to detect viral RNA in respiratory tract tissues has also been reported [388]. However, the use of direct methods requires good timing because the virus is present for only up to about 10 days in immunocompetent mice [381].

Embryo transfer or caesarean derivation followed by barrier maintenance can be used to rear mice that are free of PVM. Because active infection is present in the individual immunocompetent mouse for only a short period, strict isolation of a few (preferably seropositive) mice with the temporary cessation of breeding might also be successful in eliminating the virus [8, 378].

PVM could interfere with studies involving the respiratory tract or immunological measurements in mice. In addition, PVM can have devastating effects on research using immunodeficient mice because they are particularly prone to develop fatal disease [383, 384] or become more susceptible to the deleterious effects of other agents such as *P. murina* [389].

Murine rotavirus A or epizootic diarrhoea of infant mice virus (MuRV-A/EDIM)

MuRV-A/EDIM (commonly referred to as 'mouse rotavirus' or 'epizootic diarrhoea of infant mice virus') is a non-enveloped, segmented double-stranded RNA virus of the family Reoviridae, genus *Rotavirus*. It is antigenically classified as a group A rotavirus, similar to rotaviruses of many other species that cause neonatal and infantile gastroenteritis [291]. MuRV-A/EDIM infection remains prevalent in contemporary mouse colonies and appears to occur worldwide. Large commercial laboratories found 0.6% to 9% of mouse sera from North American and European facilities to be positive for antibodies against MuRV-A/EDIM [11, 12, 14, 15], and up to 30% of mouse colonies in the USA were identified as affected in a survey performed in 2006 [13]. Experimentally, MuRV-A/EDIM infection in mice is used as a model for human rotavirus infection, especially in investigations on the mechanisms of rotavirus immunity and in the development of vaccination strategies [390].

Clinical symptoms following MuRV-A/EDIM infection range from inapparent or mild to severe, sometimes fatal, diarrhoea. 'Epizootic diarrhoea of infant mice' describes the clinical syndrome associated with natural or experimental infection by MuRV-A/EDIM during the first 2 weeks of life [8, 36, 291, 391, 392]. Diarrhoea usually begins around 48 h after infection and persists for about 1 week. Affected suckling mice have soft, yellow faeces that wet and stain the perianal region (Figure 3.2.8). In severe instances, the mice may be stunted, have dry scaly skin, or are virtually covered with faecal material. Morbidity is very high but mortality is usually low.

Gross lesions in affected mice are confined to the intestinal tract. The caecum and colon may be distended with gas and watery to paste-like contents that are frequently bright yellow. The stomach of diarrhoeic mice is almost always filled with milk, and this feature has been reported to be a reliable means to differentiate diarrhoea caused by rotavirus from the diarrhoea caused by MHV infection.

Histopathological changes may be subtle even in animals with significant diarrhoea (Figure 3.2.8). They are most prominent at the apices of villi, where rotaviruses infect and replicate within epithelial cells; the large intestinal surface mucosa may also be affected. Though inflammation is minimal, the lamina propria may be oedematous, lymphatics may be dilated and mild leukocytic infiltration in the large intestinal mucosa and submucosa has been observed in a recent outbreak of disease [36, 393]. Hydropic change of villous epithelial cells is the hallmark finding of acute disease. The villi become

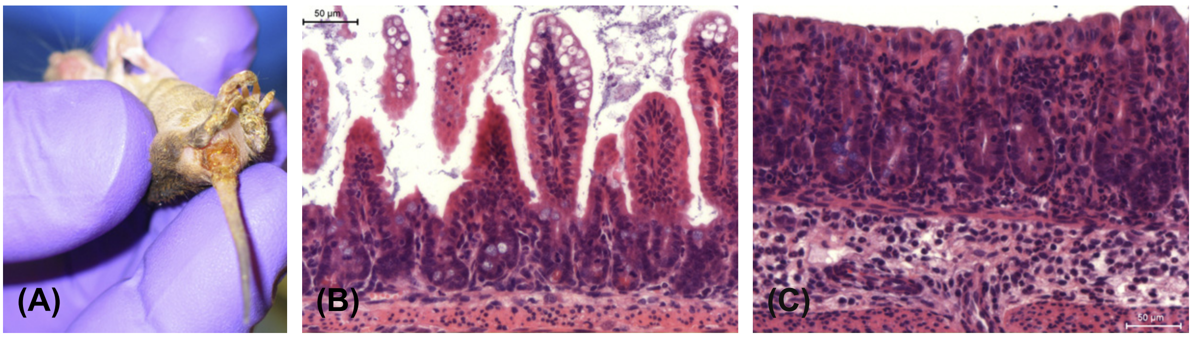

Figure 3.2.8 Clinical and histological presentation of EDIM in an affected suckling during an outbreak of disease. Watery to oily and yellow faeces and inflamed perianal region that appears wet and stained (A). Vacuolation and cytoplasmatic swelling of villar epithelial cells in the small intestine (B) and mixed infiltration of leucocytes in mucosa and submucosa (C) of the colon. *From Held* et al. *[393], used with permission from RSM Press.*

shortened, and the cells that initially replace the damaged cells are less differentiated, typically cuboidal instead of columnar, and lack a full complement of enzymes for digestion and absorption, resulting in diarrhoea due to maldigestion and malabsorption. Undigested milk in the small intestine promotes bacterial growth and exerts an osmotic effect, exacerbating damage to the villi. Intestinal fluid and electrolyte secretion is further enhanced by activation of the enteric nervous system [394] and through the effects of a viral enterotoxin called NSP4 (for non-structural protein 4) [395]. It is hypothesized that NSP4 is released from virus-infected cells and then triggers a signal transduction pathway that alters epithelial cell permeability and chloride secretion.

Susceptibility to EDIM depends on the age of the host and peaks between 4 and 14 days of age [8, 36, 291, 391, 392]. Mice older than about 2 weeks can still be infected with MuRV-A/EDIM, but small numbers of enterocytes become infected, there is little replication of virus and diarrhoea does not occur. The exact reason for this age-related resistance to disease is unknown. Pups suckling from immune dams are protected against EDIM during their period of disease susceptibility [396]. In general, the infection is self-limiting and resolves within days. Successful viral control and clearance is promoted by an intact immune response [396–399], and some immunodeficient mice (e.g. $Prkdc^{scid}$ and $Rag2^{tm1Fwa}$ mice) may shed virus for extended periods or become persistently infected [400, 401]. Protection against MuRV-A/EDIM reinfection is primarily mediated by antibodies [396, 397].

Murine rotavirus-A/EDIM is highly contagious and transmitted by the faecal–oral route [8, 291, 391]. Dissemination of the virus occurs through direct contact or contaminated fomites and aerosols and is facilitated by the general property of rotaviruses that they remain infectious outside the body, show resistance to inactivation (e.g. low pH, non-ionic detergents, hydrophobic organic liquids, proteolytic enzymes), and are shed in high quantities (>10^{11} particles/g faeces) [291]. MuRV-A/EDIM is stable at −70 °C but otherwise tends to be susceptible to extreme environmental conditions, detergents and disinfectants containing phenols, chlorine or ethanol [291].

MFIA, ELISA and IFA are in widespread use for detection of serum antibodies to MuRV-A/EDIM in diagnostic and health surveillance programmes; other assay systems such as those using latex agglutination are also used [402]. As MuRV-A/EDIM shares the VP6 protein determined group A antigen, for example, with human, simian or bovine rotavirus strains, commercially available ELISA assays utilizing polyclonal or monoclonal antibodies have been used to detect rotavirus antigen in mice; however, great care must be taken in interpreting the results because some feeds have been reported to cause false-positive reactions with certain ELISA kits [403]. Electron microscopy of faeces of diarrhoeic pups should reveal typical wheel-shaped rotavirus particles, 60–80 nm in diameter. RT-PCR also can be used to detect rotavirus RNA in faecal samples [404]. Good timing is critical for establishing the diagnosis from faeces because virus is shed for only a few days in immunocompetent mice.

Embryo transfer or caesarean derivation followed by barrier maintenance is recommended for rederivation of breeding stocks [8]. In immunocompetent mice in which infection is effectively cleared, a breeding suspension strategy for 8–10 weeks combined with excellent sanitation, filter tops and conscientious serological testing of offspring and sentinel mice has also been reported to be effective, and prolongation of breeding cessation up to 12 weeks resolved infection even in immunocompromised mice [393].

MuRV-A/EDIM has the potential to interfere with any research using suckling mice. It may have a significant impact on studies where the intestinal tract of neonatal or infant mice is the target organ. The infection also poses a problem for infectious disease and immune response studies, particularly those involving enteropathogens in infant mice [405]. A disease-induced stress-related thymic necrosis may occur and alter immunology experiments [36]. In addition, runting could be interpreted erroneously as the effect of genetic manipulation or other experimental manipulation.

Sendai virus (SeV)

Sendai virus (SeV) is an enveloped, single-stranded RNA virus of the family Paramyxoviridae, genus *Respirovirus*. It is antigenically related to human parainfluenza virus 1. The virus was named after Sendai, Japan, where it was first isolated from mice. Historically, infections were relatively common in mouse and rat colonies worldwide. In addition, there is evidence that hamsters, guinea-pigs and rabbits are susceptible to infection with SeV [8, 301, 406, 407]; however, some apparently seropositive guinea-pigs may in fact be seropositive to other parainfluenza viruses instead of SeV. A study in France reported antibodies to SeV in 17% of mouse colonies examined [10]. A low rate of seropositive mice (0.2%) was found in a survey in North America [11]. Schoondermark-van de Ven et al. [12] also found antibodies to SeV in 0.2% of mouse samplings from western European institutions. In more recent surveys in North America and western Europe, SeV infection was not detected [13–15], indicating that SeV, like most viruses, has meanwhile been eliminated from the majority of mouse colonies. SeV can contaminate biological materials [1].

SeV is pneumotropic and can cause significant respiratory disease in mice. The pneumotropism is partially a consequence of the action of respiratory serine proteases such as tryptase Clara, which activate viral infectivity by specific cleavage of the viral fusion glycoprotein [408]. In addition, the apical budding behaviour of SeV may hinder the spread of virus into subepithelial tissues and subsequently to distant organs via the blood.

Two epidemiologic patterns of SeV infection have been recognized, an enzootic (subclinical) and epizootic (clinically apparent) type [8, 379, 409]. Enzootic infections commonly occur in breeding or open colonies, where the constant supply of susceptible animals perpetuates the infection. In breeding colonies, mice are infected shortly after weaning as maternal antibody levels wane. Normally, the infection is subclinical, with virus persisting for approximately 2 weeks, accompanied by seroconversion that persists for a year or longer. Epizootic infections occur upon first introduction of the virus to a colony and either die out (self-cure) after 2–7 months or become enzootic depending on colony conditions. The epizootic form is generally acute, and morbidity is very high, resulting in nearly all susceptible animals becoming infected within a short time. Clinical signs vary and include rough hair coat, hunched posture, chattering, respiratory distress, prolonged gestation, death of neonates and sucklings and runting in young mice. Breeding colonies may return to normal productivity within 2 months and thereafter maintain the enzootic pattern of infection. Factors such as strain susceptibility, age, husbandry, transport and copathogens are important in precipitating overt disease. DBA and 129 strains of mice are very susceptible to SeV pneumonia, whereas SJL/J and C57BL/6/J and several outbred stocks are relatively resistant. Resistance to SeV infection is under multigenic control with epistatic involvement [410]. There is no evidence for persistent infection in immunocompetent mice, but persistent or prolonged infection may occur in immunodeficient mice and can result in wasting and death due to progressive pneumonia [411, 412]. Clearance of a primary SeV infection is mediated by CD8+ and CD4+ T-cell mechanisms [413, 414].

Heavier than normal, consolidated, plum-colored or grey lungs are a characteristic gross finding in severe SeV pneumonia [8, 36, 379, 409]. Lymphadenopathy and splenomegaly reflect the vigorous immune response to infection.

Histologically, three phases of disease can be recognized in susceptible immunocompetent mice: acute, reparative and resolution phases [36, 409]. Lesions of the acute phase, which lasts 8–12 days, are primarily attributed to the cell-mediated immune response that destroys infected respiratory epithelial cells and include necrotizing rhinitis, tracheitis, bronch(iol)itis and alveolitis. Epithelial syncytiae and cytoplasmic inclusion bodies in infected cells may be seen early in this phase. Alveoli contain sloughed necrotic epithelium, fibrin, neutrophils and mononuclear cells. Atelectasis, bronchiectasis and emphysema may occur as a result of damage and obstruction of airways. The reparative phase, which may overlap the acute phase but continues through about the third week after infection, is indicated by regeneration of airway lining epithelium. Adenomatous hyperplasia and squamous metaplasia (with multilayered flat epithelial cells instead of normal columnar cells) in the terminal bronchioles and alveoli are considered to be a hallmark of SeV pneumonia. Mixed inflammatory cell infiltrates in this phase tend to be primarily interstitial, rather than alveolar, as they are in the acute phase. The resolution phase may be complete by the fourth week after infection and lesions may be difficult to subsequently identify. Residual, persistent lesions that may occur include organizing alveolitis and bronchiolitis fibrosa obliterans. Alveoli and bronchioles are replaced by collagen and fibroblasts, foamy macrophages and lymphoid infiltrates, often with foci of emphysema, cholesterol crystals and other debris, which represent attempts to organize and wall off residual necrotic debris and fibrin. Lesions are more severe and variable when additional pathogens such as *Mycoplasma pulmonis* are present [8]. Otitis media has also been reported in natural infections with SeV although some of these studies have been complicated by the presence of other pathogens [415]. SeV has been detected in the inner ear after experimental intracerebral inoculation of neonatal mice [416].

SeV is extremely contagious. Infectious virus is shed during the first 2 weeks of infection and appears to be transmitted by direct contact, contaminated fomites and respiratory aerosol [8, 379].

Serology (MFIA, ELISA, IFA, or HI) is the approach of choice for routine monitoring because serum antibodies to SeV are detectable soon after infection and persist at high levels for many months, although active infection lasts only 1–2 weeks in immunocompetent mice. The short period of active infection limits the utility of direct methods such as immunohistochemistry [382] and RT-PCR [388, 417]. Although SeV is considered to be highly contagious, studies have shown that dirty bedding sentinel systems do not reliably detect the infection and that outbred stocks may not seroconvert consistently [418, 419]. MAP testing and RT-PCR can be used to detect SeV in contaminated biological materials.

SeV infection in mouse colonies has proved to be one of the most difficult virus infections to control because the virus is highly infectious and easily disseminated. Depopulation of infected colonies is probably the most appropriate means of eliminating the virus in most situations. Embryo transfer, or caesarean derivation, followed by barrier maintenance, can also be used to eliminate the virus [8, 379]. A less effective alternative is to place the infected animals under strict quarantine, remove all young and pregnant mice, suspend all breeding and prevent addition of other susceptible animals for approximately 2 months until the infection is extinguished, and then breeding and other normal activities are resumed. Vaccines against the virus have been developed [8, 379, 409], but these probably do not represent a practical means to achieve or maintain the seronegative status of colonies that is in demand today.

SeV has the potential to interfere with a wide variety of research involving mice. Reported effects include interference with early embryonic development and fetal growth; alterations of macrophage, NK-cell, and T- and B-cell function; altered responses to transplantable tumours and respiratory carcinogens; altered isograft rejection; and delayed wound healing (reviewed in [6–8]). Pulmonary changes during SeV infection can compromise interpretation of experimentally induced lesions and may lead to opportunistic infections by other agents. They could also affect the response to anaesthetics. In addition, natural SeV infection would interfere with studies using SeV as a gene vector.

Theiler's murine encephalomyelitis virus (TMEV)

Theiler's murine encephalomyelitis virus (TMEV), or murine poliovirus, is a member of the genus *Cardiovirus* in the family Picornaviridae. Members of this genus are non-enveloped viruses with single-stranded RNA. The virus is rapidly destroyed at temperatures above 50 °C. It is considered to be a primary pathogen of the CNS of mice and can cause clinical disease resembling that due to poliomyelitis virus infections in humans. Antibodies to TMEV have been identified in mouse colonies and feral populations worldwide, and *Mus musculus* is considered to be the natural host of TMEV [420]. The best-known and most frequently mentioned TMEV strain is GDVII, which is virulent for mice. Infant or young hamsters and laboratory rats are also susceptible to intracerebral infection. The original isolate is designated TO (Theiler's original) and represents a group of TMEV strains with low virulence for mice. Many additional virus strains have been isolated and studied, and they all fall in the broad grouping of TO and GDVII. A similar virus strain has also been isolated from rats, but in contrast to mouse isolates, this virus is not pathogenic for rats and mice after intracerebral inoculation [421]. Recently, another rat isolate has been characterized and shown to be most closely related to, but quite distinct from, other TMEV viruses [422]. Antibodies to TMEV (strain GDVII) have been detected in guinea-pigs and are considered to indicate infection with another closely related cardiovirus [423].

Seropositivity to TMEV was reported in approximately 48% of French mouse colonies in a retrospective study [10]. In more recent studies, the prevalence of TMEV infections was found to be lower. Schoondermark-van de Ven et al. [12] detected antibodies to TMEV in 2.2% of mouse samplings from western European institutions. In a survey conducted by Carty [13], about 9% of responding institutions in the USA reported TMEV infection in their mouse colonies. Further surveys in North America and western Europe revealed antibodies in 0.09–0.26% of mice monitored [11, 14, 15].

TMEV is primarily an enteric pathogen, and virus strains are enterotropic. In natural infections, virus can be detected in intestinal mucosa and faecal matter, and in some cases it is also found in the mesenteric lymph nodes. However, histological lesions in the intestine are not discerned. Virus may be shed via intestinal contents for up to 22 weeks, sometimes intermittently [424], and transmission under natural conditions is via the faecal–oral route, by direct contact between mice, as well as by indirect contact (e.g. dirty bedding). The host immune response limits virus spread, but it does not immediately terminate virus replication in the intestines. Virus is cleared from extraneural tissues, but persists in the CNS for at least a year.

Clinical disease due to natural TMEV infection is rare, with a rate of only 1 in 1000–10 000 infected immunocompetent animals [36]. In immunodeficient mice, especially in weanlings, clinical signs may be more common and mortality may be higher [425]. This group of viruses usually causes asymptomatic infections of the intestinal tract. They may spread to the CNS as a rare event where they cause different neurological disease manifestations. The most typical clinical sign of TMEV infection is flaccid paralysis of hindlimbs. The animals appear otherwise healthy, and there is no mortality.

Experimental infection in mice provides models of poliomyelitis-like infection and virus-induced demyelinating disease including multiple sclerosis [426]. After experimental infection, TMEV causes a biphasic disease in susceptible strains of mice. The acute phase is characterized by early infection of neurons in the grey matter. Encephalomyelitis may develop during this phase and may be fatal, but most animals survive and enter the second phase of the disease at 1–3 months after the acute phase. This phase is characterized by viral persistence in the spinal cord white matter, mainly in macrophages, and leads to white matter demyelination. Persistence and demyelination occur only in genetically susceptible mouse strains, while resistant strains clear the infection after early grey matter encephalomyelitis through a cytotoxic T lymphocyte response.

The severity and nature of disease depend on virus strain, route of inoculation, host genotype and age [8, 36, 427]. In general, virus isolates with low virulence produce persistent CNS infection in mice whereas virulent strains are unable to cause persistent infection. Intracerebral inoculation results in the most severe infections, but the intranasal route is also effective. Experimental intracerebral infections with virulent FA and GDVII strains of TMEV are more likely to cause acute encephalomyelitis and death in weanling mice 4–5 days after inoculation ('early disease'). Death may be preceded by neurological manifestations of encephalitis such as hyperexcitability, convulsions, tremors, circling, rolling and weakness. Animals may develop typical flaccid paralysis of hindlimbs, and locomotion is possible only by use of the forelimbs. Interestingly, the tail is not paralyzed. Experimental infections with low-virulence virus strains (e.g. TO, DA, WW) are more likely to cause persistent infection with development of mild encephalomyelitis followed by a chronic demyelinating disease after a few months ('late disease'). These virus strains infect neurons in the grey matter of the brain and spinal cord during the acute phase of viral growth, followed by virus persistence in macrophages and glial cells in the spinal cord white matter. SJL, SWR and DBA/2 strains are most susceptible to this chronic demyelinating disease. CBA and C3H/He are less susceptible strains, and strains A, C57BL/6, C57BL/10 and DBA/1 are relatively resistant [428]. Differences in humoral immune responses play a role in resistance to TMEV infection [429], but genetic factors are also important. Several genetic loci implicated in susceptibility to virus persistence, demyelination, or clinical disease have been identified, including the H-2D region of the major histocompatibility complex [430]. Furthermore, the age at infection influences the severity of clinical disease. In infant mice, intracerebral infection with low-virulence virus strains (e.g. TO) is often lethal. Young mice develop paralysis after an incubation period of 1–4 weeks while adult mice often show no clinical signs of infection.

The only gross lesions are secondary to the posterior paralysis and may include urine scald or dermatitis due to incontinence of urine and trauma to paralyzed limbs, or wasting or atrophy of the hindlimbs in long-term survivors.

TMEV infects neurons and glial cells, and histological changes in the CNS include non-suppurative meningitis, perivasculitis and poliomyelitis with neuronolysis, neuronophagia and microgliosis in the brainstem and ventral horns of the spinal cord [36]. Demyelination in immunocompetent mice is considered to be immune-mediated. Susceptible strains develop a specific delayed-type hypersensitivity response which is the basis for inflammation and demyelination. This reaction is mediated by T cells that release cytokines leading to recruitment of monocytes and macrophages as a consequence of infection of macrophages and other CNS-resident cells [431–433]. Protection from chronic demyelinating disease is possible by vaccination with live virus given previously by subcutaneous or intraperitoneal inoculation [434, 435]. Early immunosuppression at the time of infection, e.g. by treatment with cyclophosphamide or antithymocyte serum, inhibits or diminishes demyelination. Immunosuppression in mice chronically infected with TMEV leads to remyelination of oligodendrocytes [436]. Further details related to the pathogenesis of TMEV infections and the role of immune mechanisms have been reviewed by Yamada et al. [437], Kim et al. [432] and Lipton et al. [433].

Experimental infection of *Foxn1^{nu}* mice results in acute encephalitis and demyelination. Demyelination associated with minimal inflammation and neurological signs, including the typical hindlimb paresis, develop 2 weeks after inoculation, and most animals die within 4 weeks. In *Foxn1^{nu}* mice, demyelination is caused by a direct lytic effect of the virus on oligodendrocytes [438]. Demyelination and lethality are reduced after administration of neutralizing antibodies [439]. Histopathological changes in *Prkdc^{scid}* mice are very similar to those in *Foxn1^{nu}* mice [440].

Young mice born in infected populations usually acquire infection shortly after weaning and are almost all infected by 30 days of age. Intrauterine transmission to fetuses is possible during the early gestation period, but a placental barrier develops during gestation and later prevents intrauterine infection [441].

All TMEV isolates are closely related antigenically and form a single serogroup, as determined by complement fixation and HI [427]. Hemelt et al. [421] demonstrated cross-reactions among four strains used in experimental infections, but differences were evident in homologous and heterologous titres. The viral strain most commonly used as antigen for serological testing is GDVII. This strain agglutinates human type O erythrocytes at 4 °C, and HI has been the standard test for routine screening of mouse populations. Meanwhile, HI has been replaced by MFIA, ELISA or IFA, all of which are more sensitive and specific. Virus isolation is possible from brains or spinal cords of mice with clinical disease or from the intestinal contents of asymptomatic mice. PCR techniques are also available to test for virus-specific nucleotide sequences in biological samples [442].

Mice that have been shown to be free from TMEV by serological testing can be selected for breeding populations. If the virus is introduced into a mouse population, depopulation of infected colonies may be the most appropriate means to eliminate TMEV. Embryo transfer or caesarean derivation is the method of choice for eliminating virus from valuable breeding populations. Foster-nursing has been reported to be effective in generating virus-free offspring [359], although transplacental transmission has been demonstrated with experimental infection early in gestation.

Lesions of demyelination in CNS of mice with clinically inapparent chronic infection may interfere with investigations that require evaluation of the CNS [443]. Conceivably, such lesions could also affect neuromuscular responses or coordination, and affect neurological and behavioural evaluations.

References

[1] Collins MJ, Parker JC. Murine virus contaminants of leukemia viruses and transplantable tumors. J Natl Cancer Inst 1972;49:1139–43.

[2] Nicklas W, Kraft V, Meyer B. Contamination of transplantable tumors, cell lines, and monoclonal antibodies with rodent viruses. Lab Anim Sci 1993;43:296–300.

[3] Lipman NS, Perkins S, Nguyen H, Pfeffer M, Meyer H. Mousepox resulting from use of ectromelia virus-contaminated, imported mouse serum. Comp Med 2000;50:426–35.

[4] Bhatt PN, Jacoby RO, Morse III HC, New AE, editors. Viral and Mycoplasmal Infections of Laboratory Rodents: Effect on Biomedical Research. New York: Elsevier Academic Press; 1986.

[5] Hamm TE, editor. Complications of Viral and Mycoplasmal Infections in Rodents to Toxicology Research and Testing. Washington, DC: Hemisphere Publishing; 1986.

[6] Baker DG. Natural Pathogens of Laboratory Animals: Their Effects on Research. Washington, DC: ASM Press; 2003.

[7] Nicklas W, Homberger FR, Illgen-Wilcke B, Jacobi K, Kraft V, Kunstyr I, et al. Implications of infectious agents on results of animal experiments. Lab Anim 1999;33:S1: 39–S31:87.

[8] National Research Council, Committee on Infectious Diseases of Mice and Rats. Infectious Diseases of Mice and Rats. Washington, DC: National Academy Press; 1991.

[9] Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, editors. Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses. New York: Elsevier Academic Press; 2005.

[10] Zenner L, Regnault JP. Ten-year long monitoring of laboratory mouse and rat colonies in French facilities: a retrospective study. Lab Anim 2000;34:76–83.

[11] Livingston RS, Riley LK. Diagnostic testing of mouse and rat colonies for infectious agents. Lab Anim (NY) 2003;32:44–51.

[12] Schoondermark-van de Ven EM, Philipse-Bergmann IM, van der Logt JT. Prevalence of naturally occurring viral infections, *Mycoplasma pulmonis* and *Clostridium piliforme* in laboratory rodents in Western Europe screened from 2000 to 2003. Lab Anim 2006;40:137–43.

[13] Carty AJ. Opportunistic infections of mice and rats: Jacoby and Lindsey revisited. ILAR J 2008;49:272–6.

[14] Mähler M, Köhl W. A serological survey to evaluate contemporary prevalence of viral agents and *Mycoplasma pulmonis* in laboratory mice and rats in western Europe. Lab Anim (NY) 2009;38:161–5.

[15] Pritchett-Corning KR, Cosentino J, Clifford CB. Contemporary prevalence of infectious agents in laboratory mice and rats. Lab Anim 2009;43:165–73.

[16] Cross SS, Parker JC, Rowe WP, Robbins ML. Biology of mouse thymic virus, a herpesvirus of mice, and the antigenic relationship to mouse cytomegalovirus. Infect Immun 1979;26:1186–95.

[17] Liang CT, Shih A, Chang YH, Liu CW, Lee YT, Hsieh WC, et al. Microbial contaminations of laboratory mice and rats in Taiwan from 2004 to 2007. J Am Assoc Lab Anim Sci 2009;48:381–6.

[18] Na YR, Seok SH, Lee HY, Baek MW, Kim DJ, Park SH, et al. Microbiological quality assessment of laboratory mice in Korea and recommendations for quality improvement. Exp Anim 2010;59:25–33.

[19] Singleton GR, Smith AL, Krebs CJ. The prevalence of viral antibodies during a large population fluctuation of house mice in Australia. Epidemiol Infect 2000; 125:719–27.

[20] Becker SD, Bennett M, Stewart JP, Hurst JL. Serological survey of virus infection among wild house mice (*Mus domesticus*) in the UK. Lab Anim 2007;41:229–38.

[21] Parker SE, Malone S, Bunte RM, Smith AL. Infectious diseases in wild mice (*Mus musculus*) collected on and around the University of Pennsylvania (Philadelphia) Campus. Comp Med 2009;59:424–30.

[22] Kercher L, Mitchell BM. Persisting murine cytomegalovirus can reactivate and has unique transcriptional activity in ocular tissue. J Virol 2002;76:9165–75.

[23] Lenzo JC, Fairweather D, Cull V, Shellam GR, James (Lawson) CM. Characterisation of murine cytomegalovirus myocarditis: cellular infiltration of the heart and virus persistence. J Mol Cell Cardiol 2002;34:629–40.
[24] Shellam GR, Redwood AJ, Smith LM, Gorman S. Murine cytomegalovirus and other herpesviruses. In: Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, Smith AL, editors. The Mouse in Biomedical Research. Diseases. 2nd ed. vol. 2. New York: Elsevier Academic Press; 2007. pp. 1–48.
[25] Brune W, Hengel H, Koszinowski UH. A mouse model for cytomegalovirus infection. In: Coligan JE, Bierer B, Margulies DH, Shevach EM, Strober W, Coico R, editors. Current Protocols in Immunology. Hoboken, NJ: John Wiley & Sons; 2001. Chapter 19, Unit 19.7 pp. 1-13.
[26] Smith LM, McWhorter AR, Masters LL, Shellam GR, Redwood AJ. Laboratory strains of murine cytomegalovirus are genetically similar to but phenotypically distinct from wild strains of virus. J Virol 2008;82:6689–96.
[27] Mutnal MB, Cheeran MC, Hu S, Lokensgard JR. Murine cytomegalovirus infection of neural stem cells alters neurogenesis in the developing brain. PLoS ONE 2011;6:e16211.
[28] Tsutsui Y. Effects of cytomegalovirus infection on embryogenesis and brain development. Congenit Anom (Kyoto) 2009;49:47–55.
[29] Shanley JD, Biczak L, Forman SJ. Acute murine cytomegalovirus infection induces lethal hepatitis. J Infect Dis 1993;167:264–9.
[30] Forster MR, Trgovcich J, Zimmerman P, Chang A, Miller C, Klenerman P, et al. Antiviral prevention of sepsis induced cytomegalovirus reactivation in immunocompetent mice. Antiviral Res 2010;85:496–503.
[31] Schmader K, Henry SC, Rahija RJ, Yu Y, Daley GG, Hamilton JD. Mouse cytomegalovirus reactivation in severe combined immune deficient mice after implantation of latently infected salivary gland. J Infect Dis 1995;172:531–4.
[32] Reynolds RP, Rahija RJ, Schenkman DI, Richter CB. Experimental murine cytomegalovirus infection in severe combined immunodeficient mice. Lab Anim Sci 1993;43:291–5.
[33] Shanley JD, Pesanti EL. Murine cytomegalovirus adrenalitis in athymic nude mice. Arch Virol 1986;88:27–35.
[34] Shanley JD, Thrall RS, Forman SJ. Murine cytomegalovirus replication in the lungs of athymic BALB/c nude mice. J Infect Dis 1997;175:309–15.
[35] Krmpotic A, Bubic I, Polic B, Lucin P, Jonjic S. Pathogenesis of murine cytomegalovirus infection. Microbes Infect 2003;5: 1263–77.
[36] Percy DH, Barthold SW. Pathology of Laboratory Rodents & Rabbits. 3rd ed. Ames, Iowa: Wiley-Blackwell; 2007.
[37] Gorman S, Harvey NL, Moro D, Lloyd ML, Voigt V, Smith LM, et al. Mixed infection with multiple strains of murine cytomegalovirus occurs following simultaneous or sequential infection of immunocompetent mice. J Gen Virol 2006;87:1123–32.
[38] Chen J, Feng Y, Chen L, Xiao J, Liu T, Yin Z, et al. Long-term impact of intrauterine MCMV infection on development of offspring nervous system. J Huazhong Univ Sci Technolog Med Sci 2011;31:371–5.
[39] Woolf NK, Jaquish DV, Koehrn FJ. Transplacental murine cytomegalovirus infection in the brain of SCID mice. Virol J 2007;4:26.
[40] Wu CA, Paveglio SA, Lingenheld EG, Zhu L, Lefrancois L, Puddington L. Transmission of murine cytomegalovirus in breast milk: a model of natural infection in neonates. J Virol 2011;85:5115–24.
[41] Khan IH, Kendall LV, Ziman M, Wong S, Mendoza S, Fahey J, et al. Simultaneous serodetection of 10 highly prevalent mouse infectious pathogens in a single reaction by multiplex analysis. Clin Diagn Lab Immunol 2005;12:513–9.
[42] Palmon A, Tel-or S, Shai E, Rager-Zisman B, Burstein Y. Development of a highly sensitive quantitative competitive PCR assay for the detection of murine cytomegalovirus DNA. J Virol Methods 2000;86:107–14.
[43] Wheat RL, Clark PY, Brown MG. Quantitative measurement of infectious murine cytomegalovirus genomes in real-time PCR. J Virol Methods 2003;112:107–13.
[44] Vliegen I, Herngreen S, Grauls G, Bruggeman C, Stassen F. Improved detection and quantification of mouse cytomegalovirus by real-time PCR. Virus Res 2003;98:17–25.

[45] Bolger G, Lapeyre N, Rheaume M, Kibler P, Bousquet C, Garneau M, et al. Acute murine cytomegalovirus infection: a model for determining antiviral activity against CMV induced hepatitis. Antiviral Res 1999;44:155-65.
[46] Redwood AJ, Harvey NL, Lloyd M, Lawson MA, Hardy CM, Shellam GR. Viral vectored immunocontraception: screening of multiple fertility antigens using murine cytomegalovirus as a vaccine vector. Vaccine 2007;25:698-708.
[47] Onyeagocha C, Hossain MS, Kumar A, Jones RM, Roback J, Gewirtz AT. Latent cytomegalovirus infection exacerbates experimental colitis. Am J Pathol 2009;175: 2034-42.
[48] Thomas AC, Forster MR, Bickerstaff AA, Zimmerman PD, Wing BA, Trgovcich J, et al. Occult cytomegalovirus in vivarium-housed mice may influence transplant allograft acceptance. Transpl Immunol 2010;23:86-91.
[49] Morse SS. Mouse thymic necrosis virus: a novel murine lymphotropic agent. Lab Anim Sci 1987;37:717-25.
[50] Morse SS. Mouse thymic virus (MTLV; murid herpesvirus 3) infection in athymic nude mice: evidence for a T lymphocyte requirement. Virology 1988;163:255-8.
[51] Wood BA, Dutz W, Cross SS. Neonatal infection with mouse thymic virus: spleen and lymph node necrosis. J Gen Virol 1981;57:139-47.
[52] Morse SS, Valinsky JE. Mouse thymic virus (MTLV). A mammalian herpesvirus cytolytic for CD4+ (L3T4+) T lymphocytes. J Exp Med 1989;169:591-6.
[53] Morse SS, Sakaguchi N, Sakaguchi S. Virus and autoimmunity: induction of autoimmune disease in mice by mouse T lymphotropic virus (MTLV) destroying CD4+ T cells. J Immunol 1999;162:5309-16.
[54] Morse SS. Thymic necrosis following oral inoculation of mouse thymic virus. Lab Anim Sci 1989;39:571-4.
[55] St-Pierre Y, Potworowski EF, Lussier G. Transmission of mouse thymic virus. J Gen Virol 1987;68:1173-6.
[56] Morse SS. Critical factors in an enzyme immunoassay (ELISA) for antibodies to mouse thymic virus (MTLV). Lab Anim 1990;24:313-20.
[57] Lussier G, Guenette D, Shek WR, Descoteaux JP. Evaluation of mouse thymic virus antibody detection techniques. Lab Anim Sci 1988;38:577-9.
[58] Prattis SM, Morse SS. Detection of mouse thymic virus (MTLV) antigens in infected thymus by competition immunoassay. Lab Anim Sci 1990;40:33-6.
[59] Morse SS. Comparative sensitivity of infectivity assay and mouse antibody production (MAP) test for detection of mouse thymic virus (MTLV). J Virol Methods 1990;28:15-23.
[60] Davison AJ, Eberle R, Ehlers B, Hayward GS, McGeoch DJ, Minson AC, et al. The order Herpesvirales. Arch Virol 2009;154:171-7.
[61] Ehlers B, Kuchler J, Yasmum N, Dural G, Voigt S, Schmidt-Chanasit J, et al. Identification of novel rodent herpesviruses, including the first gammaherpesvirus of *Mus musculus*. J Virol 2007;81:8091-100.
[62] Flano E, Woodland DL, Blackman MA. A mouse model for infectious mononucleosis. Immunol Res 2002;25:201-17.
[63] Simas JP, Efstathiou S. Murine gammaherpesvirus 68: a model for the study of gammaherpesvirus pathogenesis. Trends Microbiol 1998;6:276-82.
[64] Speck SH, Ganem D. Viral latency and its regulation: lessons from the gammaherpesviruses. Cell Host Microbe 2010;8: 100-15.
[65] Stevenson PG, Efstathiou S. Immune mechanisms in murine gammaherpesvirus-68 infection. Viral Immunol 2005;18:445-56.
[66] Stevenson PG, Simas JP, Efstathiou S. Immune control of mammalian gammaherpesviruses: lessons from murid herpesvirus-4. J Gen Virol 2009;90:2317-30.
[67] Blasdell K, McCracken C, Morris A, Nash AA, Begon M, Bennett M, et al. The wood mouse is a natural host for murid herpesvirus 4. J Gen Virol 2003;84:111-3.
[68] Oda W, Mistrikova J, Stancekova M, Dutia BM, Nash AA, Takahata H, et al. Analysis of genomic homology of murine gammaherpesvirus (MHV)-72 to MHV-68 and impact of MHV-72 on the survival and tumorigenesis in the MHV-72-infected CB17 scid/scid and CB17+/+ mice. Pathol Int 2005;55:558-68.
[69] Francois S, Vidick S, Sarlet M, Michaux J, Koteja P, Desmecht D, et al. Comparative study of murid gammaherpesvirus 4 infection in mice and in a natural host, bank voles. J Gen Virol 2010;91:2553-63.

[70] Hughes DJ, Kipar A, Sample JT, Stewart JP. Pathogenesis of a model gammaherpesvirus in a natural host. J Virol 2010;84:3949–61.
[71] Chen N, Danila MI, Feng Z, Buller RM, Wang C, Han X, et al. The genomic sequence of ectromelia virus, the causative agent of mousepox. Virology 2003; 317:165–86.
[72] Buller RM, Potter M, Wallace GD. Variable resistance to ectromelia (mousepox) virus among genera of Mus. Curr Top Microbiol Immunol 1986;127:319–22.
[73] Essbauer S, Pfeffer M, Meyer H. Zoonotic poxviruses. Vet Microbiol 2010;140:229–36.
[74] Labelle P, Hahn NE, Fraser JK, Kendall LV, Ziman M, James E, et al. Mousepox detected in a research facility: case report and failure of mouse antibody production testing to identify ectromelia virus in contaminated mouse serum. Comp Med 2009;59:180–6.
[75] Bhatt PN, Jacoby RO. Mousepox in inbred mice innately resistant or susceptible to lethal infection with ectromelia virus. I. Clinical responses. Lab Anim Sci 1987;37: 11–5.
[76] Small JD, New AE. Prevention and control of mousepox. Lab Anim Sci 1981;31:616–29.
[77] Wagner JE, Daynes RA. Observations of an outbreak of mousepox in laboratory mice in 1979 at the University of Utah Medical Center, USA. Lab Anim Sci 1981;31:565–9.
[78] Deerberg F, Kastner W, Pittermann W, Schwanzer V. Demonstration of an ectromelia enzootic in hairless mice. Dtsch Tierarztl Wochenschr 1973;80:78–81.
[79] Owen D, Hill A, Argent S. Reaction of mouse strains to skin test for ectromelia using an allied virus as inoculum. Nature 1975;254:598–9.
[80] Osterhaus AD, Teppema JS, Wirahadiredja RM, van Steenis G. Mousepox in the Netherlands. Lab Anim Sci 1981;31:704–6.
[81] Dick EJ, Kittell CL, Meyer H, Farrar PL, Ropp SL, Esposito JJ, et al. Mousepox outbreak in a laboratory mouse colony. Lab Anim Sci 1996;46:602–11.
[82] Fenner F. Mousepox (infectious ectromelia): past, present, and future. Lab Anim Sci 1981;31:553–9.
[83] Manning PJ, Frisk CS. Clinical, pathologic, and serologic features of an epizootic of mousepox in Minnesota. Lab Anim Sci 1981;31:574–7.
[84] Wallace GD, Buller RM. Kinetics of ectromelia virus (mousepox) transmission and clinical response in C57BL/6J, BALB/cByJ and AKR/J inbred mice. Lab Anim Sci 1985;35:41–6.
[85] Brownstein D, Bhatt PN, Jacoby RO. Mousepox in inbred mice innately resistant or susceptible to lethal infection with ectromelia virus. V. Genetics of resistance to the Moscow strain. Arch Virol 1989; 107:35–41.
[86] Mark R, Buller L, Fenner F. Mousepox. In: Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, Smith AL, editors. The Mouse in Biomedical Research. Diseases. 2nd ed., vol. 2. New York: Elsevier Academic Press; 2007. pp. 67–92.
[87] Allen AM, Clarke GL, Ganaway JR, Lock A, Werner RM. Pathology and diagnosis of mousepox. Lab Anim Sci 1981;31:599–608.
[88] Parker AK, Parker S, Yokoyama WM, Corbett JA, Buller RM. Induction of natural killer cell responses by ectromelia virus controls infection. J Virol 2007;81: 4070–9.
[89] Fang M, Roscoe F, Sigal LJ. Age-dependent susceptibility to a viral disease due to decreased natural killer cell numbers and trafficking. J Exp Med 2010;207:2369–81.
[90] O'Neill HC, Blanden RV, O'Neill TJ. H-2-linked control of resistance to ectromelia virus infection in B10 congenic mice. Immunogenetics 1983;18:255–65.
[91] Brownstein DG, Gras L. Differential pathogenesis of lethal mousepox in congenic DBA/2 mice implicates natural killer cell receptor NKR-P1 in necrotizing hepatitis and the fifth component of complement in recruitment of circulating leukocytes to spleen. Am J Pathol 1997;150:1407–20.
[92] Karupiah G, Chen JH, Nathan CF, Mahalingam S, MacMicking JD. Identification of nitric oxide synthase 2 as an innate resistance locus against ectromelia virus infection. J Virol 1998;72:7703–6.
[93] Mahalingam S, Karupiah G, Takeda K, Akira S, Matthaei KI, Foster PS. Enhanced resistance in STAT6-deficient mice to infection with ectromelia virus. Proc Natl Acad Sci U S A 2001;98:6812–7.
[94] Moulton EA, Atkinson JP, Buller RM. Surviving mousepox infection requires the complement system. PLoS Pathog 2008;4: e1000249.

[95] Delano ML, Brownstein DG. Innate resistance to lethal mousepox is genetically linked to the NK gene complex on chromosome 6 and correlates with early restriction of virus replication by cells with an NK phenotype. J Virol 1995;69:5875–7.
[96] Karupiah G, Buller RM, Van Rooijen N, Duarte CJ, Chen J. Different roles for CD4+ and CD8+ T lymphocytes and macrophage subsets in the control of a generalized virus infection. J Virol 1996;70:8301–9.
[97] Panchanathan V, Chaudhri G, Karupiah G. Correlates of protective immunity in poxvirus infection: where does antibody stand? Immunol Cell Biol 2008;86:80–6.
[98] Werner GT. Transmission of mouse-pox in colonies of mice. Zentralbl Veterinarmed B 1982;29:401–4.
[99] Bhatt PN, Jacoby RO. Mousepox in inbred mice innately resistant or susceptible to lethal infection with ectromelia virus. III. Experimental transmission of infection and derivation of virus-free progeny from previously infected dams. Lab Anim Sci 1987;37:23–7.
[100] Schwanzer V, Deerberg F, Frost J, Liess B, Schwanzerova I, Pittermann W. Intrauterine infection of mice with ectromelia virus. Z Versuchstierkd 1975;17:110–20.
[101] Wallace GD. Mouse pox threat. Science 1981;211:438.
[102] Collins MJ, Peters RL, Parker JC. Serological detection of ectromelia virus antibody. Lab Anim Sci 1981;31:595–8.
[103] Buller RM, Bhatt PN, Wallace GD. Evaluation of an enzyme-linked immunosorbent assay for the detection of ectromelia (mousepox) antibody. J Clin Microbiol 1983;18:1220–5.
[104] Gaertner DJ, Batchelder M, Herbst LH, Kaufman HL. Administration of vaccinia virus to mice may cause contact or bedding sentinel mice to test positive for orthopoxvirus antibodies: case report and follow-up investigation. Comp Med 2003; 53:85–8.
[105] Neubauer H, Pfeffer M, Meyer H. Specific detection of mousepox virus by polymerase chain reaction. Lab Anim 1997;31: 201–5.
[106] Olson VA, Laue T, Laker MT, Babkin IV, Drosten C, Shchelkunov SN, et al. Real-time PCR system for detection of orthopoxviruses and simultaneous identification of smallpox virus. J Clin Microbiol 2004;42: 1940–6.
[107] Putkuri N, Piiparinen H, Vaheri A, Vapalahti O. Detection of human orthopoxvirus infections and differentiation of smallpox virus with real-time PCR. J Med Virol 2009;81:146–52.
[108] Buller RM, Weinblatt AC, Hamburger AW, Wallace GD. Observations on the replication of ectromelia virus in mouse-derived cell lines: implications for epidemiology of mousepox. Lab Anim Sci 1987;37:28–32.
[109] Buller RM, Wallace GD. Reexamination of the efficacy of vaccination against mousepox. Lab Anim Sci 1985;35:473–6.
[110] Bhatt PN, Jacoby RO. Effect of vaccination on the clinical response, pathogenesis and transmission of mousepox. Lab Anim Sci 1987;37:610–4.
[111] Jacoby RO, Bhatt PN, Johnson EA, Paturzo FX. Pathogenesis of vaccinia (IHD-T) virus infection in BALB/cAnN mice. Lab Anim Sci 1983;33:435–41.
[112] Schriewer J, Buller RM, Owens G. Mouse models for studying orthopoxvirus respiratory infections. Methods Mol Biol 2004;269:289–308.
[113] Chapman JL, Nichols DK, Martinez MJ, Raymond JW. Animal models of orthopoxvirus infection. Vet Pathol 2010;47: 852–70.
[114] Esteban DJ, Buller RM. Ectromelia virus: the causative agent of mousepox. J Gen Virol 2005;86:2645–59.
[115] Xiao Y, Aldaz-Carroll L, Ortiz AM, Whitbeck JC, Alexander E, Lou H, et al. A protein-based smallpox vaccine protects mice from vaccinia and ectromelia virus challenges when given as a prime and single boost. Vaccine 2007;25:1214–24.
[116] Paran N, Suezer Y, Lustig S, Israely T, Schwantes A, Melamed S, et al. Postexposure immunization with modified vaccinia virus Ankara or conventional Lister vaccine provides solid protection in a murine model of human smallpox. J Infect Dis 2009;199:39–48.
[117] Parker S, Siddiqui AM, Oberle C, Hembrador E, Lanier R, Painter G, et al. Mousepox in the C57BL/6 strain provides an improved model for evaluating antipoxvirus therapies. Virology 2009;385:11–21.
[118] Hartley JW, Rowe WP. A new mouse virus apparently related to the adenovirus group. Virology 1960;11:645–7.

[119] Hashimoto K, Sugiyama T, Sasaki S. An adenovirus isolated from the feces of mice I. Isolation and identification. Jpn J Microbiol 1966;10:115-25.
[120] Hamelin C, Lussier G. Genotypic differences between the mouse adenovirus strains FL and K87. Experientia 1988;44:65-6.
[121] Jacques C, Cousineau L, D'Amours B, Lussier G, Hamelin C. Molecular cloning, physical mapping and cross-hybridization of the murine adenovirus type 1 and type 2 genomes. J Gen Virol 1994;75:1311-6.
[122] Jacques C, D'Amours B, Hamelin C. Genetic relationship between mouse adenovirus-2 (strain K87) and human adenovirus-2. FEMS Microbiol Lett 1994;115:7-11.
[123] Spindler KR, Moore ML, Cauthen AN. Mouse adenoviruses. In: Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, Smith AL, editors. The Mouse in Biomedical Research. Diseases 2nd ed., vol. 2. New York: Elsevier Academic Press; 2007. pp. 49-65.
[124] Won YS, Jeong ES, Park HJ, Lee CH, Nam KH, Kim HC, et al. Microbiological contamination of laboratory mice and rats in Korea from 1999 to 2003. Exp Anim 2006;55:11-6.
[125] Smith AL, Singleton GR, Hansen GM, Shellam G. A serologic survey for viruses and *Mycoplasma pulmonis* among wild house mice (*Mus domesticus*) in southeastern Australia. J Wildl Dis 1993;29:219-29.
[126] Smith AL, Winograd DF, Burrage TG. Comparative biological characterization of mouse adenovirus strains FL and K 87 and seroprevalence in laboratory rodents. Arch Virol 1986;91:233-46.
[127] Heck FC, Sheldon WG, Gleiser CA. Pathogenesis of experimentally produced mouse adenovirus infection in mice. Am J Vet Res 1972;33:841-6.
[128] Wigand R. Age and susceptibility of Swiss mice for mouse adenovirus, strain FL. Arch Virol 1980;64:349-57.
[129] Guida JD, Fejer G, Pirofski LA, Brosnan CF, Horwitz MS. Mouse adenovirus type 1 causes a fatal hemorrhagic encephalomyelitis in adult C57BL/6 but not BALB/c mice. J Virol 1995;69:7674-81.
[130] Winters AL, Brown HK. Duodenal lesions associated with adenovirus infection in athymic 'nude' mice. Proc Soc Exp Biol Med 1980;164:280-6.
[131] Pirofski L, Horwitz MS, Scharff MD, Factor SM. Murine adenovirus infection of SCID mice induces hepatic lesions that resemble human Reye syndrome. Proc Natl Acad Sci U S A 1991;88:4358-62.
[132] Margolis G, Kilham L, Hoenig EM. Experimental adenovirus infection of the mouse adrenal gland. I. Light microscopic observations. Am J Pathol 1974;75:363-74.
[133] Takeuchi A, Hashimoto K. Electron microscope study of experimental enteric adenovirus infection in mice. Infect Immun 1976;13:569-80.
[134] van der Veen J, Mes A. Experimental infection with mouse adenovirus in adult mice. Arch Gesamte Virusforsch 1973;42:235-41.
[135] Hashimoto K, Sugiyama T, Yoshikawa M, Sasaki S. Intestinal resistance in the experimental enteric infection of mice with a mouse adenovirus. I. Growth of the virus and appearance of a neutralizing substance in the intestinal tract. Jpn J Microbiol 1970;14:381-95.
[136] Umehara K, Hirakawa M, Hashimoto K. Fluctuation of antiviral resistance in the intestinal tracts of nude mice infected with a mouse adenovirus. Microbiol Immunol 1984;28:679-90.
[137] Wigand R, Gelderblom H, Ozel M. Biological and biophysical characteristics of mouse adenovirus, strain FL. Arch Virol 1977;54:131-42.
[138] Lussier G, Smith AL, Guenette D, Descoteaux JP. Serological relationship between mouse adenovirus strains FL and K87. Lab Anim Sci 1987;37:55-7.
[139] Luethans TN, Wagner JE. A naturally occurring intestinal mouse adenovirus infection associated with negative serologic findings. Lab Anim Sci 1983;33:270-2.
[140] Ehresmann DW, Hogan RN. Acceleration of scrapie disease in mice by an adenovirus. Intervirology 1986;25:103-10.
[141] Klempa B, Kruger DH, Auste B, Stanko M, Krawczyk A, Nickel KF, et al. A novel cardiotropic murine adenovirus representing a distinct species of mastadenoviruses. J Virol 2009;83:5749-59.
[142] Compton SR. Serological diagnosis of murine adenovirus 3. J Am Assoc Lab Anim Sci 2010;49:674 (Abstract).
[143] Bond SB, Howley PM, Takemoto KK. Characterization of K virus and its comparison with polyoma virus. J Virol 1978;28:337-43.
[144] Tegerstedt K, Andreasson K, Vlastos A, Hedlund KO, Dalianis T, Ramqvist T.

Murine pneumotropic virus VP1 virus-like particles (VLPs) bind to several cell types independent of sialic acid residues and do not serologically cross react with murine polyomavirus VP1 VLPs. J Gen Virol 2003;84:3443–52.
[145] Nicklas W, Baneux P, Boot R, Decelle T, Deeny AA, Fumanelli M, et al. Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units. Lab Anim 2002; 36:20–42.
[146] Benjamin TL. Polyoma viruses. In: Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, Smith AL, editors. The Mouse in Biomedical Research. Diseases 2nd ed., vol. 2. New York: Elsevier Academic Press; 2007. pp. 105–39.
[147] Greenlee JE, Phelps RC, Stroop WG. The major site of murine K papovavirus persistence and reactivation is the renal tubular epithelium. Microb Pathog 1991; 11:237–47.
[148] Greenlee JE, Clawson SH, Phelps RC, Stroop WG. Distribution of K-papovavirus in infected newborn mice. J Comp Pathol 1994;111:259–68.
[149] Ikeda K, Dorries K, ter Meulen V. Morphological and immunohistochemical studies of the central nervous system involvement in papovavirus K infection in mice. Acta Neuropathol 1988;77:175–81.
[150] Greenlee JE. Chronic infection of nude mice by murine K papovavirus. J Gen Virol 1986;67:1109–14.
[151] Greenlee JE, Dodd WK. Serial passage of murine K-papovavirus in primary cultures of mouse embryo cells. Brief report. Arch Virol 1987;94:169–73.
[152] Groen J, Broeders H, Spijkers I, Osterhaus A. Comparison of an enzyme-linked immunosorbent assay, an immunofluorescence assay and a hemagglutination inhibition assay for detection of antibodies to K-papovavirus in mice. Lab Anim Sci 1989;39:21–4.
[153] Carty AJ, Franklin CL, Riley LK, Besch-Williford C. Diagnostic polymerase chain reaction assays for identification of murine polyomaviruses in biological samples. Comp Med 2001;51:145–9.
[154] Atencio IA, Belli B, Hobbs M, Cheng SF, Villarreal LP, Fan H. A model for mixed virus disease: co-infection with Moloney murine leukemia virus potentiates runting induced by polyomavirus (A2 strain) in Balb/c and NIH Swiss mice. Virology 1995;212:356–66.
[155] Dubensky TW, Murphy FA, Villarreal LP. Detection of DNA and RNA virus genomes in organ systems of whole mice: patterns of mouse organ infection by polyomavirus. J Virol 1984;50:779–83.
[156] McCance DJ, Mims CA. Transplacental transmission of polyoma virus in mice. Infect Immun 1977;18:196–202.
[157] Berke Z, Dalianis T, Feinstein R, Sandstedt K, Evengard B. Persistence of polyomavirus in adult SCID C.B-17 mice. *In Vivo* 1994;8: 339–42.
[158] Rubino MJ, Walker D. Immunosuppression and murine polyomavirus infection. Virus Res 1988;9:1–10.
[159] McCance DJ, Mims CA. Reactivation of polyoma virus in kidneys of persistently infected mice during pregnancy. Infect Immun 1979;25:998–1002.
[160] Wilson JJ, Lin E, Pack CD, Frost EL, Hadley A, Swimm AI, et al. IFN-γ controls mouse polyomavirus infection *in vivo*. J Virol 2011;85:10126–34.
[161] Lin E, Kemball CC, Hadley A, Wilson JJ, Hofstetter AR, Pack CD, et al. Heterogeneity among viral antigen-specific CD4+ T cells and their *de novo* recruitment during persistent polyomavirus infection. J Immunol 2010;185:1692–700.
[162] Nakamichi K, Takayama-Ito M, Nukuzuma S, Kurane I, Saijo M. Long-term infection of adult mice with murine polyomavirus following stereotaxic inoculation into the brain. Microbiol Immunol 2010;54: 475–82.
[163] Tegerstedt K, Franzen AV, Andreasson K, Joneberg J, Heidari S, Ramqvist T, et al. Murine polyomavirus virus-like particles (VLPs) as vectors for gene and immune therapy and vaccines against viral infections and cancer. Anticancer Res 2005;25:2601–8.
[164] Andreasson K, Eriksson M, Tegerstedt K, Ramqvist T, Dalianis T. CD4+, and CD8+ T cells can act separately in tumour rejection after immunization with murine pneumotropic virus chimeric Her2/neu virus-like particles. PLoS ONE 2010;5:e11580.
[165] Ball-Goodrich LJ, Johnson E. Molecular characterization of a newly recognized mouse parvovirus. J Virol 1994;68:6476–86.
[166] Tattersall P, Cotmore SF. The rodent parvoviruses. In: Bhatt PN, Jacoby RO,

Morse III HC, New AE, editors. Viral and Mycoplasmal Infections of Laboratory Rodents: Effects on Biomedical Research. New York: Elsevier Academic Press; 1986. pp. 305–48.

[167] Jacoby RO, Ball-Goodrich LJ, Besselsen DG, McKisic MD, Riley LK, Smith AL. Rodent parvovirus infections. Lab Anim Sci 1996;46:370–80.

[168] Jacoby RO, Ball-Goodrich L. Parvoviruses. In: Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, Smith AL, editors. The Mouse in Biomedical Research. Diseases 2nd ed., vol. 2. New York: Elsevier Academic Press; 2007. pp. 93–104.

[169] Besselsen DG, Franklin CL, Livingston RS, Riley LK. Lurking in the shadows: emerging rodent infectious diseases. ILAR J 2008;49:277–90.

[170] Janus LM, Bleich A. Coping with parvovirus infections in mice: health surveillance and control. Lab Anim 2011;46:14–23.

[171] McKisic MD, Lancki DW, Otto G, Padrid P, Snook S, Cronin DC, et al. Identification and propagation of a putative immunosuppressive orphan parvovirus in cloned T cells. J Immunol 1993;150:419–28.

[172] Besselsen DG, Pintel DJ, Purdy GA, Besch-Williford CL, Franklin CL, Hook RR, et al. Molecular characterization of newly recognized rodent parvoviruses. J Gen Virol 1996;77:899–911.

[173] Besselsen DG, Romero MJ, Wagner AM, Henderson KS, Livingston RS. Identification of novel murine parvovirus strains by epidemiological analysis of naturally infected mice. J Gen Virol 2006;87: 1543–56.

[174] Besselsen DG, Becker MD, Henderson KS, Wagner AM, Banu LA, Shek WR. Temporal transmission studies of mouse parvovirus 1 in BALB/c and C.B-17/Icr-*Prkdc*^{scid} mice. Comp Med 2007;57:66–73.

[175] Filipovska-Naumovska E, Abubakar SM, Thompson MJ, Hopwood D, Pass DA, Wilcox GE. Serologic prevalence of MPV1 in mouse strains in a commercial laboratory mouse colony determined by using VP1 antigen. J Am Assoc Lab Anim Sci 2010;49:437–42.

[176] Livingston RS, Besselsen DG, Steffen EK, Besch-Williford CL, Franklin CL, Riley LK. Serodiagnosis of mice minute virus and mouse parvovirus infections in mice by enzyme-linked immunosorbent assay with baculovirus-expressed recombinant VP2 proteins. Clin Diagn Lab Immunol 2002;9: 1025–31.

[177] Smith AL, Jacoby RO, Johnson EA, Paturzo F, Bhatt PN. *In vivo* studies with an 'orphan' parvovirus of mice. Lab Anim Sci 1993;43:175–82.

[178] Jacoby RO, Johnson EA, Ball-Goodrich L, Smith AL, McKisic MD. Characterization of mouse parvovirus infection by *in situ* hybridization. J Virol 1995;69:3915–9.

[179] Hansen GM, Paturzo FX, Smith AL. Humoral immunity and protection of mice challenged with homotypic or heterotypic parvovirus. Lab Anim Sci 1999;49:380–4.

[180] Christie RD, Marcus EC, Wagner AM, Besselsen DG. Experimental infection of mice with hamster parvovirus: evidence for interspecies transmission of mouse parvovirus 3. Comp Med 2010;60:123–9.

[181] Besselsen DG, Wagner AM, Loganbill JK. Effect of mouse strain and age on detection of mouse parvovirus 1 by use of serologic testing and polymerase chain reaction analysis. Comp Med 2000;50:498–502.

[182] Filipovska-Naumovska E, Thompson MJ, Hopwood D, Pass DA, Wilcox GE. Strain- and age-associated variation in viral persistence and antibody response to mouse parvovirus 1 in experimentally infected mice. J Am Assoc Lab Anim Sci 2010;49:443–7.

[183] Shek WR, Paturzo FX, Johnson EA, Hansen GM, Smith AL. Characterization of mouse parvovirus infection among BALB/c mice from an enzootically infected colony. Lab Anim Sci 1998;48:294–7.

[184] Riley LK, Knowles R, Purdy G, Salome N, Pintel D, Hook RR, et al. Expression of recombinant parvovirus NS1 protein by a baculovirus and application to serologic testing of rodents. J Clin Microbiol 1996;34:440–4.

[185] Ball-Goodrich LJ, Hansen G, Dhawan R, Paturzo FX, Vivas-Gonzalez BE. Validation of an enzyme-linked immunosorbent assay for detection of mouse parvovirus infection in laboratory mice. Comp Med 2002;52:160–6.

[186] Besselsen DG, Besch-Williford CL, Pintel DJ, Franklin CL, Hook RR, Riley LK. Detection of newly recognized rodent parvoviruses by PCR. J Clin Microbiol 1995;33:2859–63.

[187] Redig AJ, Besselsen DG. Detection of rodent parvoviruses by use of fluorogenic nuclease polymerase chain reaction assays. Comp Med 2001;51:326–31. Erratum in Comp Med (2003) 52(2), following table of contents.
[188] Bauer BA, Riley LK. Antemortem detection of mouse parvovirus and mice minute virus by polymerase chain reaction (PCR) of faecal samples. Lab Anim 2006;40: 144–52.
[189] Yagami K, Goto Y, Ishida J, Ueno Y, Kajiwara N, Sugiyama F. Polymerase chain reaction for detection of rodent parvoviral contamination in cell lines and transplantable tumors. Lab Anim Sci 1995;45:326–8.
[190] Besselsen DG, Romero-Aleshire MJ, Munger SJ, Marcus EC, Henderson KS, Wagner AM. Embryo transfer rederivation of C.B-17/Icr-*Prkdc*^{scid} mice experimentally infected with mouse parvovirus 1. Comp Med 2008;58:353–9.
[191] Agca Y, Bauer BA, Johnson DK, Critser JK, Riley LK. Detection of mouse parvovirus in *Mus musculus* gametes, embryos, and ovarian tissues by polymerase chain reaction assay. Comp Med 2007;57:51–6.
[192] McKisic MD, Macy JD, Delano ML, Jacoby RO, Paturzo FX, Smith AL. Mouse parvovirus infection potentiates allogeneic skin graft rejection and induces syngeneic graft rejection. Transplantation 1998;65: 1436–46.
[193] Crawford LV. A minute virus of mice. Virology 1966;29:605–12.
[194] Bonnard GD, Manders EK, Campbell DA, Herberman RB, Collins MJ. Immunosuppressive activity of a subline of the mouse EL-4 lymphoma. Evidence for minute virus of mice causing the inhibition. J Exp Med 1976;143:187–205.
[195] Kimsey PB, Engers HD, Hirt B, Jongeneel CV. Pathogenicity of fibroblast- and lymphocyte-specific variants of minute virus of mice. J Virol 1986;59:8–13.
[196] Brownstein DG, Smith AL, Jacoby RO, Johnson EA, Hansen G, Tattersall P. Pathogenesis of infection with a virulent allotropic variant of minute virus of mice and regulation by host genotype. Lab Invest 1991;65:357–64.
[197] Parker JC, Collins MJ, Cross SS, Rowe WP. Minute virus of mice. II. Prevalence, epidemiology, and occurrence as a contaminant of transplanted tumors. J Natl Cancer Inst 1970;45:305–10.
[198] Garant PR, Baer PN, Kilham L. Electron microscopic localization of virions in developing teeth of young hamsters infected with minute virus of mice. J Dent Res 1980;59:80–6.
[199] Haag A, Wayss K, Rommelaere J, Cornelis JJ. Experimentally induced infection with autonomous parvoviruses, minute virus of mice and H-1, in the African multimammate mouse (*Mastomys coucha*). Comp Med 2000;50:613–21.
[200] Kilham L, Margolis G. Pathogenicity of minute virus of mice (MVM) for rats, mice, and hamsters. Proc Soc Exp Biol Med 1970;133:1447–52.
[201] Kilham L, Margolis G. Fetal infections of hamsters, rats, and mice induced with the minute virus of mice (MVM). Teratology 1971;4:43–61.
[202] Segovia JC, Real A, Bueren JA, Almendral JM. *In vitro* myelosuppressive effects of the parvovirus minute virus of mice (MVMi) on hematopoietic stem and committed progenitor cells. Blood 1991;77: 980–8.
[203] Segovia JC, Bueren JA, Almendral JM. Myeloid depression follows infection of susceptible newborn mice with the parvovirus minute virus of mice (strain i). J Virol 1995;69:3229–32.
[204] Segovia JC, Gallego JM, Bueren JA, Almendral JM. Severe leukopenia and dysregulated erythropoiesis in SCID mice persistently infected with the parvovirus minute virus of mice. J Virol 1999;73: 1774–84.
[205] Naugler SL, Myles MH, Bauer BA, Kennett MJ, Besch-Williford C. Reduced fecundity and death associated with parvovirus infection in B-lymphocyte deficient mice. Contemp. Top Lab Anim Sci 2001;40:66 (Abstract).
[206] Janus LM, Mähler M, Köhl W, Smoczek A, Hedrich HJ, Bleich A. Minute virus of mice: antibody response, viral shedding, and persistence of viral DNA in multiple strains of mice. Comp Med 2008;58:360–8.
[207] Janus LM, Smoczek A, Jörns A, Hedrich HJ, Bleich A. Presence of minute virus of mice in immunocompetent mice despite the onset of host immunity. Vet Microbiol 2010;146:51–8.
[208] Thomas 3rd ML, Morse BC, O'Malley J, Davis JA, St Claire MB, et al. Gender influences infectivity in C57BL/6 mice

exposed to mouse minute virus. Comp Med 2007;57:74–81.
[209] Chang A, Havas S, Borellini F, Ostrove JM, Bird RE. A rapid and simple procedure to detect the presence of MVM in conditioned cell fluids or culture media. Biologicals 1997;25:415–9.
[210] Janus LM, Smoczek A, Hedrich HJ, Bleich A. Risk assessment of minute virus of mice transmission during rederivation: detection in reproductive organs, gametes, and embryos of mice after *in vivo* infection. Biol Reprod 2009;81:1010–5.
[211] Mahabir E, Bulian D, Needham J, Mayer A, Mateusen B, Soom AV, et al. Transmission of mouse minute virus (MMV) but not mouse hepatitis virus (MHV) following embryo transfer with experimentally exposed *in vivo*-derived embryos. Biol Reprod 2007;76:189–97.
[212] Rommelaere J, Cornelis JJ. Antineoplastic activity of parvoviruses. J Virol Methods 1991;33:233–51.
[213] Garnick RL. Experience with viral contamination in cell culture. Dev Biol Stand 1996;88:49–56.
[214] Snijder EJ, Meulenberg JJ. The molecular biology of arteriviruses. J Gen Virol 1998; 79:961–79.
[215] Rowson KE, Mahy BW. Lactic dehydrogenase virus. Virol Monogr 1975:1–121.
[216] Li K, Schuler T, Chen Z, Glass GE, Childs JE, Plagemann PG. Isolation of lactate dehydrogenase-elevating viruses from wild house mice and their biological and molecular characterization. Virus Res 2000;67:153–62.
[217] van den Broek MF, Sporri R, Even C, Plagemann PG, Hanseler E, Hengartner H, et al. Lactate dehydrogenase-elevating virus (LDV): lifelong coexistence of virus and LDV-specific immunity. J Immunol 1997;159:1585–8.
[218] Ammann CG, Messer RJ, Peterson KE, Hasenkrug KJ. Lactate dehydrogenase-elevating virus induces systemic lymphocyte activation via TLR7-dependent IFNalpha responses by plasmacytoid dendritic cells. PLoS ONE 2009;4:e6105.
[219] Le-Thi-Phuong T, Thirion G, Coutelier JP. Distinct gamma interferon-production pathways in mice infected with lactate dehydrogenase-elevating virus. J Gen Virol 2007;88:3063–6.
[220] Anderson GW, Rowland RR, Palmer GA, Even C, Plagemann PG. Lactate dehydrogenase-elevating virus replication persists in liver, spleen, lymph node, and testis tissues and results in accumulation of viral RNA in germinal centers, concomitant with polyclonal activation of B cells. J Virol 1995;69:5177–85.
[221] Riley V, Lilly F, Huerto E, Bardell D. Transmissible agent associated with 26 types of experimental mouse neoplasms. Science 1960;132:545–7.
[222] Peterson NC. From bench to cageside: Risk assessment for rodent pathogen contamination of cells and biologics. ILAR J 2008;49:310–5.
[223] Ohnishi Y, Yoshimura M, Ueyama Y. Lactic dehydrogenase virus (LDHV) contamination in human tumor xenografts and its elimination. J Natl Cancer Inst 1995;87:538–9.
[224] Nicklas W, Giese M, Zawatzky R, Kirchner H, Eaton P. Contamination of a monoclonal antibody with LDH-virus causes interferon induction. Lab Anim Sci 1988;38:152–4.
[225] Anderson GW, Palmer GA, Rowland RR, Even C, Plagemann PG. Infection of central nervous system cells by ecotropic murine leukemia virus in C58 and AKR mice and in *in utero*-infected CE/J mice predisposes mice to paralytic infection by lactate dehydrogenase-elevating virus. J Virol 1995;69:308–19.
[226] Coutelier J-P, Brinton MA. Lactate dehydrogenase-elevating virus. In: Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, Smith AL, editors. The Mouse in Biomedical Research. Diseases 2nd ed., vol. 2. New York: Elsevier Academic Press; 2007. pp. 215–34.
[227] Haven TR, Rowland RR, Plagemann PG, Wong GH, Bradley SE, Cafruny WA. Regulation of transplacental virus infection by developmental and immunological factors: studies with lactate dehydrogenase-elevating virus. Virus Res 1996;41:153–61.
[228] Zitterkopf NL, Haven TR, Huela M, Bradley DS, Cafruny WA. Transplacental lactate dehydrogenase-elevating virus (LDV) transmission: immune inhibition of umbilical cord infection, and correlation of fetal virus susceptibility with development of F4/80 antigen expression. Placenta 2002;23:438–46.
[229] Broen JB, Bradley DS, Powell KM, Cafruny WA. Regulation of maternal-fetal

virus transmission in immunologically reconstituted SCID mice infected with lactate dehydrogenase-elevating virus. Viral Immunol 1992;5:133–40.
[230] Hayashi T, Mori I, Noguchi Y, Itoh T, Saitoh M. Immunofluorescent antibody response to lactic dehydrogenase virus in different strains of mice. J Comp Pathol 1992;107:179–83.
[231] Takahashi-Omoe H, Omoe K, Matsushita S, Inada T. Characterization of lactate dehydrogenase-elevating virus ORF6 protein expressed by recombinant baculoviruses. Comp Immunol Microbiol Infect Dis 2004;27:423–31.
[232] van der Logt JT, Kissing J, Melchers WJ. Enzymatic amplification of lactate dehydrogenase-elevating virus. J Clin Microbiol 1994;32:2003–6.
[233] Chen Z, Plagemann PG. Detection of lactate dehydrogenase-elevating virus in transplantable mouse tumors by biological assay and RT-PCR assays and its removal from the tumor cell. J Virol Methods 1997;65:227–36.
[234] Lipman NS, Henderson K, Shek W. False negative results using RT-PCR for detection of lactate dehydrogenase-elevating virus in a tumor cell line. Comp Med 2000;50:255–6.
[235] Bootz F, Sieber I, Popovic D, Tischhauser M, Homberger FR. Comparison of the sensitivity of *in vivo* antibody production tests with *in vitro* PCR-based methods to detect infectious contamination of biological materials. Lab Anim 2003;37:341–51.
[236] Goto K, Takakura A, Yoshimura M, Ohnishi Y, Itoh T. Detection and typing of lactate dehydrogenase-elevating virus RNA from transplantable tumors, mouse liver tissues, and cell lines, using polymerase chain reaction. Lab Anim Sci 1998;48:99–102.
[237] Bauer BA, Besch-Williford CL, Riley LK. Comparison of the mouse antibody production (MAP) assay and polymerase chain reaction (PCR) assays for the detection of viral contaminants. Biologicals 2004;32:177–82.
[238] Plagemann PG, Swim HE. Relationship between the lactic dehydrogenase-elevating virus and transplantable murine tumors. Proc Soc Exp Biol Med 1966;121:1142–6.
[239] Liu H, Bockhorn J, Dalton R, Chang YF, Qian D, Zitzow LA, et al. Removal of lactate dehydrogenase-elevating virus from human-in-mouse breast tumor xenografts by cell-sorting. J Virol Methods 2011;173:266–70.
[240] Cafruny WA, Hovinen DE. Infection of mice with lactate dehydrogenase-elevating virus leads to stimulation of autoantibodies. J Gen Virol 1988;69:723–9.
[241] Takei I, Asaba Y, Kasatani T, Maruyama T, Watanabe K, Yanagawa T, et al. Suppression of development of diabetes in NOD mice by lactate dehydrogenase virus infection. J Autoimmun 1992;5:665–73.
[242] Markine-Goriaynoff D, Hulhoven X, Cambiaso CL, Monteyne P, Briet T, Gonzalez MD, et al. Natural killer cell activation after infection with lactate dehydrogenase-elevating virus. J Gen Virol 2002;83:2709–16.
[243] Gomez KA, Longhi SA, Marino VJ, Mathieu PA, Loureiro ME, Coutelier JP, et al. Effects of various adjuvants and a viral infection on the antibody specificity toward native or cryptic epitopes of a protein antigen. Scand J Immunol 2003;57:144–50.
[244] Sasaki Y, Hayashi T, Hasegawa K. Lactate dehydrogenase-elevating virus infection at the sensitization and challenge phases reduces the development of delayed eosinophilic allergic rhinitis in BALB/c mice. Scand J Immunol 2007;66:628–35.
[245] Robertson SJ, Ammann CG, Messer RJ, Carmody AB, Myers L, Dittmer U, et al. Suppression of acute anti-friend virus CD8+ T-cell responses by coinfection with lactate dehydrogenase-elevating virus. J Virol 2008;82:408–18.
[246] Salazar-Bravo J, Ruedas LA, Yates TL. Mammalian reservoirs of arenaviruses. Curr Top Microbiol Immunol 2002;262:25–63.
[247] Ackermann R. Risk to humans through contact with golden hamsters carrying lymphocytic choriomeningitis virus (author's transl). Dtsch Med Wochenschr. 1977;102:1367–70.
[248] Barthold SW, Smith AL. Lymphocytic choriomeningitis virus. In: Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, Smith AL, editors. The Mouse in Biomedical Research. Diseases 2nd ed., vol. 2. New York: Elsevier Academic Press; 2007. pp. 179–213.
[249] Asper M, Hofmann P, Osmann C, Funk J, Metzger C, Bruns M, et al. First outbreak of

callitrichid hepatitis in Germany: genetic characterization of the causative lymphocytic choriomeningitis virus strains. Virology 2001;284:203-13.
[250] Lukashevich IS, Tikhonov I, Rodas JD, Zapata JC, Yang Y, Djavani M, et al. Arenavirus-mediated liver pathology: acute lymphocytic choriomeningitis virus infection of rhesus macaques is characterized by high-level interleukin-6 expression and hepatocyte proliferation. J Virol 2003;77:1727-37.
[251] Lehmann-Grube F. Lymphocytic choriomeningitis virus. In: Foster HL, Small DD, Fox JG, editors. The Mouse in Biomedical Research. Diseases, vol. 2. New York: Elsevier Academic Press; 1982.
[252] Tagliapietra V, Rosa R, Hauffe HC, Laakkonen J, Voutilainen L, Vapalahti O, et al. Spatial and temporal dynamics of lymphocytic choriomeningitis virus in wild rodents, northern Italy. Emerg Infect Dis 2009;15:1019-25.
[253] el Karamany RM, Imam IZ. Antibodies to lymphocytic choriomeningitis virus in wild rodent sera in Egypt. J Hyg Epidemiol Microbiol Immunol 1991;35:97-103.
[254] Morita C, Tsuchiya K, Ueno H, Muramatsu Y, Kojimahara A, Suzuki H, et al. Seroepidemiological survey of lymphocytic choriomeningitis virus in wild house mice in China with particular reference to their subspecies. Microbiol Immunol 1996;40:313-5.
[255] Childs JE, Glass GE, Korch GW, Ksiazek TG, Leduc JW. Lymphocytic choriomeningitis virus infection and house mouse (*Mus musculus*) distribution in urban Baltimore. Am J Trop Med Hyg 1992;47:27-34.
[256] Bhatt PN, Jacoby RO, Barthold SW. Contamination of transplantable murine tumors with lymphocytic choriomeningitis virus. Lab Anim Sci 1986;36:136-9.
[257] Childs JE, Glass GE, Ksiazek TG, Rossi CA, Oro JG, Leduc JW. Human-rodent contact and infection with lymphocytic choriomeningitis and Seoul viruses in an inner-city population. Am J Trop Med Hyg 1991; 44:117-21.
[258] Marrie TJ, Saron MF. Seroprevalence of lymphocytic choriomeningitis virus in Nova Scotia. Am J Trop Med Hyg 1998;58:47-9.
[259] Lledo L, Gegundez MI, Saz JV, Bahamontes N, Beltran M. Lymphocytic choriomeningitis virus infection in a province of Spain: analysis of sera from the general population and wild rodents. J Med Virol 2003;70:273-5.
[260] Emonet S, Retornaz K, Gonzalez JP, de Lamballerie X, Charrel RN. Mouse-to-human transmission of variant lymphocytic choriomeningitis virus. Emerg Infect Dis 2007;13:472-5.
[261] Knust B, Macneil A, Wong SJ, Backenson PB, Gibbons A, Rollin PE, et al. Exposure to lymphocytic choriomeningitis virus, New York, USA. Emerg Infect Dis 2011;17:1324-5.
[262] Dykewicz CA, Dato VM, Fisher-Hoch SP, Howarth MV, Perez-Oronoz GI, Ostroff SM, et al. Lymphocytic choriomeningitis outbreak associated with nude mice in a research institute. JAMA 1992; 267:1349-53.
[263] Bowen GS, Calisher CH, Winkler WG, Kraus AL, Fowler EH, Garman RH, et al. Laboratory studies of a lymphocytic choriomeningitis virus outbreak in man and laboratory animals. Am J Epidemiol 1975; 102:233-40.
[264] Rousseau MC, Saron MF, Brouqui P, Bourgeade A. Lymphocytic choriomeningitis virus in southern France: four case reports and a review of the literature. Eur J Epidemiol 1997;13:817-23.
[265] Biggar RJ, Schmidt TJ, Woodall JP. Lymphocytic choriomeningitis in laboratory personnel exposed to hamsters inadvertently infected with LCM virus. J Am Vet Med Assoc 1977;171:829-32.
[266] Amman BR, Pavlin BI, Albarino CG, Comer JA, Erickson BR, Oliver JB, et al. Pet rodents and fatal lymphocytic choriomeningitis in transplant patients. Emerg Infect Dis 2007;13:719-25.
[267] Hinman AR, Fraser DW, Douglas RG, Bowen GS, Kraus AL, Winkler WG, et al. Outbreak of lymphocytic choriomeningitis virus infections in medical center personnel. Am J Epidemiol 1975;101:103-10.
[268] Mahy BW, Dykewicz C, Fisher-Hoch S, Ostroff S, Tipple M, Sanchez A. Virus zoonoses and their potential for contamination of cell cultures. Dev Biol Stand 1991;75:183-9.
[269] Fischer SA, Graham MB, Kuehnert MJ, Kotton CN, Srinivasan A, Marty FM, et al. Transmission of lymphocytic choriomeningitis virus by organ transplantation. N Engl J Med 2006;354:2235-49.

[270] Barton LL, Peters CJ, Ksiazek TG. Lymphocytic choriomeningitis virus: an unrecognized teratogenic pathogen. Emerg Infect Dis 1995;1:152–3.
[271] Barton LL, Hyndman NJ. Lymphocytic choriomeningitis virus: reemerging central nervous system pathogen. Pediatrics. 2000; 105:E35.
[272] Barton LL, Mets MB, Beauchamp CL. Lymphocytic choriomeningitis virus: emerging fetal teratogen. Am J Obstet Gynecol 2002;187:1715–6.
[273] Ciurea A, Klenerman P, Hunziker L, Horvath E, Odermatt B, Ochsenbein AF, et al. Persistence of lymphocytic choriomeningitis virus at very low levels in immune mice. Proc Natl Acad Sci U S A 1999;96:11964–9.
[274] Baldridge JR, Buchmeier MJ. Mechanisms of antibody-mediated protection against lymphocytic choriomeningitis virus infection: mother-to-baby transfer of humoral protection. J Virol 1992;66:4252–7.
[275] Gossmann J, Lohler J, Utermohlen O, Lehmann-Grube F. Murine hepatitis caused by lymphocytic choriomeningitis virus. II. Cells involved in pathogenesis. Lab Invest 1995;72:559–70.
[276] Oldstone MB. Biology and pathogenesis of lymphocytic choriomeningitis virus infection. Curr Top Microbiol Immunol 2002; 263:83–117.
[277] Kang SS, McGavern DB. Lymphocytic choriomeningitis infection of the central nervous system. Front Biosci 2008;13: 4529–43.
[278] Rai SK, Cheung DS, Wu MS, Warner TF, Salvato MS. Murine infection with lymphocytic choriomeningitis virus following gastric inoculation. J Virol 1996; 70:7213–8.
[279] Rai SK, Micales BK, Wu MS, Cheung DS, Pugh TD, Lyons GE, et al. Timed appearance of lymphocytic choriomeningitis virus after gastric inoculation of mice. Am J Pathol 1997;151:633–9.
[280] Ike F, Bourgade F, Ohsawa K, Sato H, Morikawa S, Saijo M, et al. Lymphocytic choriomeningitis infection undetected by dirty-bedding sentinel monitoring and revealed after embryo transfer of an inbred strain derived from wild mice. Comp Med 2007;57:272–81.
[281] Takimoto K, Taharaguchi M, Morikawa S, Ike F, Yamada YK. Detection of the antibody to lymphocytic choriomeningitis virus in sera of laboratory rodents infected with viruses of laboratory and newly isolated strains by ELISA using purified recombinant nucleoprotein. Exp Anim 2008;57:357–65.
[282] Park JY, Peters CJ, Rollin PE, Ksiazek TG, Gray B, Waites KB, et al. Development of a reverse transcription-polymerase chain reaction assay for diagnosis of lymphocytic choriomeningitis virus infection and its use in a prospective surveillance study. J Med Virol 1997;51:107–14.
[283] Besselsen DG, Wagner AM, Loganbill JK. Detection of lymphocytic choriomeningitis virus by use of fluorogenic nuclease reverse transcriptase-polymerase chain reaction analysis. Comp Med 2003;53:65–9.
[284] McCausland MM, Crotty S. Quantitative PCR technique for detecting lymphocytic choriomeningitis virus *in vivo*. J Virol Methods 2008;147:167–76.
[285] U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories (BMBL). 5th ed. Washington, DC: U.S. Government Printing Office, http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf; 2009.
[286] Slifka MK. Mechanisms of humoral immunity explored through studies of LCMV infection. Curr Top Microbiol Immunol 2002;263:67–81.
[287] Zinkernagel RM. Lymphocytic choriomeningitis virus and immunology. Curr Top Microbiol Immunol 2002;263:1–5.
[288] Oldstone MB. Viral persistence: parameters, mechanisms and future predictions. Virology 2006;344:111–8.
[289] Gumenscheimer M, Balkow S, Simon MM, Jirillo E, Galanos C, Freudenberg MA. Stage of primary infection with lymphocytic choriomeningitis virus determines predisposition or resistance of mice to secondary bacterial infections. Med Microbiol Immunol 2007;196:79–88.
[290] Barthold SW. Reovirus type 3 infection, liver, mouse. In: Jones TC, Popp JA, Mohr U, editors. Monographs on Pathology of Laboratory Animals: Digestive System. 2nd ed. Berlin: Springer Verlag; 1997. pp. 196–200.
[291] Ward RL, McNeal MM, Faron MB, Faron AL. Reoviridae. In: Fox JG,

Barthold SW, Davisson MT, Newcomer CE, Quimby FW, Smith AL, editors. The Mouse in Biomedical Research. Diseases 2nd ed., vol. 2. New York: Elsevier Academic Press; 2007. pp. 235-68.
[292] Tyler KL, Fields BN. Reovirus infection in laboratory rodents. In: Bhatt PN, Jacoby RO, Morse III HC, New AE, editors. Viral and Mycoplasmal Infections of Laboratory Rodents: Effects on Biomedical Research. New York: Elsevier Academic Press; 1986. pp. 277-303.
[293] Morrison LA, Sidman RL, Fields BN. Direct spread of reovirus from the intestinal lumen to the central nervous system through vagal autonomic nerve fibers. Proc Natl Acad Sci U S A 1991;88:3852-6.
[294] Mann MA, Knipe DM, Fischbach GD, Fields BN. Type 3 reovirus neuro-invasion after intramuscular inoculation: direct invasion of nerve terminals and age-dependent pathogenesis. Virology 2002;303:222-31.
[295] Tyler KL, Clarke P, DeBiasi RL, Kominsky D, Poggioli GJ. Reoviruses and the host cell. Trends Microbiol 2001;9: 560-4.
[296] Schiff LA, Nibert ML, Tyler KL. Orthoreoviruses and their replication. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE, editors. Field's Virology. 5th ed., vol. 2. Philadelphia, PA: Lippincott Williams & Wilkins; 2007. pp. 1853-915.
[297] Cuff CF, Lavi E, Cebra CK, Cebra JJ, Rubin DH. Passive immunity to fatal reovirus serotype 3-induced meningoencephalitis mediated by both secretory and transplacental factors in neonatal mice. J Virol 1990;64:1256-63.
[298] Barthold SW, Smith AL, Bhatt PN. Infectivity, disease patterns, and serologic profiles of reovirus serotypes 1, 2, and 3 in infant and weanling mice. Lab Anim Sci 1993;43:425-30.
[299] George A, Kost SI, Witzleben CL, Cebra JJ, Rubin DH. Reovirus-induced liver disease in severe combined immunodeficient (SCID) mice. A model for the study of viral infection, pathogenesis, and clearance. J Exp Med 1990;171:929-34.
[300] Carthew P. Histopathological characterization of the naturally occurring hepatotropic virus infections of nude mice. J Pathol 1984;142:79-85.
[301] American Committee on Laboratory Animal Disease. Detection methods for the identification of rodent viral and mycoplasmal infections. Lab Anim Sci 1991;41: 199-225.
[302] Wright MH, Cera LM, Sarich NA, Lednicky JA. Reverse transcription-polymerase chain reaction detection and nucleic acid sequence confirmation of reovirus infection in laboratory mice with discordant serologic indirect immunofluorescence assay and enzyme-linked immunosorbent assay results. Comp Med 2004; 54:410-7.
[303] Kraft V, Meyer B. Diagnosis of murine infections in relation to test methods employed. Lab Anim Sci 1986;36:271-6.
[304] Steele MI, Marshall CM, Lloyd RE, Randolph VE. Reovirus 3 not detected by reverse transcriptase-mediated polymerase chain reaction analysis of preserved tissue from infants with cholestatic liver disease. Hepatology 1995;21:697-702.
[305] Uchiyama A, Besselsen DG. Detection of reovirus type 3 by use of fluorogenic nuclease reverse transcriptase polymerase chain reaction. Lab Anim 2003;37:352-9.
[306] Leary TP, Erker JC, Chalmers ML, Wetzel JD, Desai SM, Mushahwar IK, et al. Detection of reovirus by reverse transcription-polymerase chain reaction using primers corresponding to conserved regions of the viral L1 genome segment. J Virol Methods 2002;104:161-5.
[307] Bennette JG. Isolation of a non-pathogenic tumour-destroying virus from mouse ascites. Nature 1960;187:72-3.
[308] Nelson JB, Tarnowski GS. An oncolytic virus recovered from Swiss mice during passage of an ascites tumour. Nature 1960;188:866-7.
[309] Barthold SW, Smith AL. Mouse hepatitis virus. In: Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, Smith AL, editors. The Mouse in Biomedical Research. Diseases. 2nd ed. vol. 2. New York: Elsevier Academic Press; 2007. pp. 141-78.
[310] Homberger FR. Enterotropic mouse hepatitis virus. Lab Anim 1997;31:97-115.
[311] Casanova LM, Jeon S, Rutala WA, Weber DJ, Sobsey MD. Effects of air temperature and relative humidity on coronavirus survival on surfaces. Appl Environ Microbiol 2010;76:2712-7.

[312] Taguchi F, Yamada A, Fujiwara K. Asymptomatic infection of mouse hepatitis virus in the rat. Brief report. Arch Virol 1979;59:275–9.
[313] Silverman J, Paturzo F, Smith AL. Effects of experimental infection of the deer mouse (*Peromyscus maniculatus*) with mouse hepatitis virus. Lab Anim Sci 1982;32:273–4.
[314] Sabesin SM. Isolation of a latent murine hepatitis virus from cultured mouse liver cells. Am J Gastroenterol 1972;58:259–74.
[315] Yoshikura H, Taguchi F. Induction of lytic plaques by murine leukemia virus in murine sarcoma virus-transformed non-producer mouse cells persistently infected with mouse hepatitis virus MHV-S. Intervirology 1979;11:69–73.
[316] Barthold SW. Mouse hepatitis virus biology and epizootiology. In: Bhatt PN, Jacoby RO, Morse III HC, New AE, editors. Viral and Mycoplasmal Infections of Laboratory Rodents: Effects on Biomedical Research. Orlando, FL: Academic Press; 1986. pp. 571–601.
[317] Compton SR, Barthold SW, Smith AL. The cellular and molecular pathogenesis of coronaviruses. Lab Anim Sci 1993;43:15–28.
[318] Barthold SW, Beck DS, Smith AL. Enterotropic coronavirus (mouse hepatitis virus) in mice: influence of host age and strain on infection and disease. Lab Anim Sci 1993;43:276–84.
[319] Barthold SW, Smith AL. Response of genetically susceptible and resistant mice to intranasal inoculation with mouse hepatitis virus JHM. Virus Res 1987;7:225–39.
[320] Barthold SW, Smith AL. Duration of mouse hepatitis virus infection: studies in immunocompetent and chemically immunosuppressed mice. Lab Anim Sci 1990;40:133–7.
[321] Williamson JS, Stohlman SA. Effective clearance of mouse hepatitis virus from the central nervous system requires both CD4+ and CD8+ T cells. J Virol 1990;64:4589–92.
[322] Kyuwa S, Machii K, Shibata S. Role of CD4+ and CD8+ T cells in mouse hepatitis virus infection in mice. Exp Anim 1996;45:81–3.
[323] Lin MT, Hinton DR, Marten NW, Bergmann CC, Stohlman SA. Antibody prevents virus reactivation within the central nervous system. J Immunol 1999;162:7358–68.
[324] Haring J, Perlman S. Mouse hepatitis virus. Curr Opin Microbiol 2001;4:462–6.
[325] Barthold SW, Smith AL, Povar ML. Enterotropic mouse hepatitis virus infection in nude mice. Lab Anim Sci 1985;35:613–8.
[326] Rehg JE, Blackman MA, Toth LA. Persistent transmission of mouse hepatitis virus by transgenic mice. Comp Med 2001;51:369–74.
[327] Barthold SW, Smith AL. Duration of challenge immunity to coronavirus JHM in mice. Arch Virol 1989;107:171–7.
[328] Barthold SW, Smith AL. Virus strain specificity of challenge immunity to coronavirus. Arch Virol 1989;104:187–96.
[329] Homberger FR, Barthold SW, Smith AL. Duration and strain-specificity of immunity to enterotropic mouse hepatitis virus. Lab Anim Sci 1992;42:347–51.
[330] Homberger FR, Barthold SW. Passively acquired challenge immunity to enterotropic coronavirus in mice. Arch Virol 1992;126:35–43.
[331] Barthold SW, Smith AL, Lord PF, Bhatt PN, Jacoby RO, Main AJ. Epizootic coronaviral typhlocolitis in suckling mice. Lab Anim Sci 1982;32:376–83.
[332] Ishida T, Taguchi F, Lee YS, Yamada A, Tamura T, Fujiwara K. Isolation of mouse hepatitis virus from infant mice with fatal diarrhea. Lab Anim Sci 1978;28:269–76.
[333] Godfraind C, Holmes KV, Coutelier JP. Thymus involution induced by mouse hepatitis virus A59 in BALB/c mice. J Virol 1995;69:6541–7.
[334] Fox JG, Murphy JC, Igras VE. Adverse effects of mouse hepatitis virus on ascites myeloma passage in the BALB/eJ mouse. Lab Anim Sci 1977;27:173–9.
[335] De Albuquerque N, Baig E, Ma X, Zhang J, He W, Rowe A, et al. Murine hepatitis virus strain 1 produces a clinically relevant model of severe acute respiratory syndrome in A/J mice. J Virol 2006;80:10382–94.
[336] Khanolkar A, Hartwig SM, Haag BA, Meyerholz DK, Harty JT, Varga SM. Toll-like receptor 4 deficiency increases disease and mortality after mouse hepatitis virus type 1 infection of susceptible C3H mice. J Virol 2009;83:8946–56.
[337] France MP, Smith AL, Stevenson R, Barthold SW. Granulomatous peritonitis and pleuritis in interferon-gamma gene knockout mice naturally infected with mouse hepatitis virus. Aust Vet J 1999;77:600–4.

[338] Compton SR, Ball-Goodrich LJ, Johnson LK, Johnson EA, Paturzo FX, Macy JD. Pathogenesis of enterotropic mouse hepatitis virus in immunocompetent and immunodeficient mice. Comp Med 2004;54:681–9.
[339] Katami K, Taguchi F, Nakayama M, Goto N, Fujiwara K. Vertical transmission of mouse hepatitis virus infection in mice. Jpn J Exp Med 1978;48:481–90.
[340] Scavizzi F, Raspa M. Tissue distribution and duration of mouse hepatitis virus in naturally infected immunocompetent ICR (CD-1) and immunodeficient athymic nude-nu mouse strains used for ovarian transplantation and *in vitro* fertilization. Lab Anim 2004;38:189–99.
[341] Reetz IC, Wullenweber-Schmidt M, Kraft V, Hedrich HJ. Rederivation of inbred strains of mice by means of embryo transfer. Lab Anim Sci 1988;38:696–701.
[342] Peters DD, Marschall S, Mahabir E, Boersma A, Heinzmann U, Schmidt J, et al. Risk assessment of mouse hepatitis virus infection via *in vitro* fertilization and embryo transfer by the use of zona-intact and laser-microdissected oocytes. Biol Reprod 2006;74:246–52.
[343] Brownstein DG, Barthold SW. Mouse hepatitis virus immunofluorescence in formalin- or Bouin's-fixed tissues using trypsin digestion. Lab Anim Sci 1982;32:37–9.
[344] de Souza M, Smith AL. Comparison of isolation in cell culture with conventional and modified mouse antibody production tests for detection of murine viruses. J Clin Microbiol 1989;27:185–7.
[345] Casebolt DB, Stephensen CB. Monoclonal antibody solution hybridization assay for detection of mouse hepatitis virus infection. J Clin Microbiol 1992;30:608–12.
[346] Homberger FR, Smith AL, Barthold SW. Detection of rodent coronaviruses in tissues and cell cultures by using polymerase chain reaction. J Clin Microbiol 1991;29:2789–93.
[347] Kunita S, Terada E, Goto K, Kagiyama N. Sequence analysis and molecular detection of mouse hepatitis virus using the polymerase chain reaction. Lab Anim Sci 1992;42:593–8.
[348] Yamada YK, Yabe M, Yamada A, Taguchi F. Detection of mouse hepatitis virus by the polymerase chain reaction and its application to the rapid diagnosis of infection. Lab Anim Sci 1993;43:285–90.
[349] Besselsen DG, Wagner AM, Loganbill JK. Detection of rodent coronaviruses by use of fluorogenic reverse transcriptase-polymerase chain reaction analysis. Comp Med 2002;52:111–6.
[350] Smith AL. An immunofluorescence test for detection of serum antibody to rodent coronaviruses. Lab Anim Sci 1983;33:157–60.
[351] Kunita S, Kato K, Ishida M, Hagiwara K, Kameda S, Ishida T, et al. Simultaneous detection of antibodies to mouse hepatitis virus recombinant structural proteins by a microsphere-based multiplex fluorescence immunoassay. Clin Vaccine Immunol 2011;18:758–66.
[352] Nakanaga K, Ishida T, Fujiwara K. Differences in antibody production against mouse hepatitis virus (MHV) among mouse strains. Lab Anim 1983;17:90–4.
[353] Homberger FR. Maternally-derived passive immunity to enterotropic mouse hepatitis virus. Arch Virol 1992;122:133–41.
[354] Holmes KV, Boyle JF, Frana MF. Mouse hepatitis virus: molecular biology and implications for pathogenesis. In: Bhatt PN, Jacoby RO, Morse III HC, New AE, editors. Viral and Mycoplasmal Infections of Laboratory Rodents: Effects on Biomedical Research. Orlando, FL: Academic Press; 1986. pp. 603–24.
[355] Okumura A, Machii K, Azuma S, Toyoda Y, Kyuwa S. Maintenance of pluripotency in mouse embryonic stem cells persistently infected with murine coronavirus. J Virol 1996;70:4146–9.
[356] Kyuwa S. Replication of murine coronaviruses in mouse embryonic stem cell lines. *in vitro*. Exp Anim 1997;46:311–3.
[357] Smith PC, Nucifora M, Reuter JD, Compton SR. Reliability of soiled bedding transfer for detection of mouse parvovirus and mouse hepatitis virus. Comp Med 2007;57:90–6.
[358] Compton SR, Homberger FR, Paturzo FX, MacArthur Clark J. Efficacy of three microbiological monitoring methods in a ventilated cage rack. Comp Med 2004;54:382–92.
[359] Lipman NS, Newcomer CE, Fox JG. Rederivation of MHV and MEV antibody positive mice by cross-fostering and use of the microisolator caging system. Lab Anim Sci 1987;37:195–9.

[360] Weir EC, Bhatt PN, Barthold SW, Cameron GA, Simack PA. Elimination of mouse hepatitis virus from a breeding colony by temporary cessation of breeding. Lab Anim Sci 1987;37:455–8.
[361] Adami C, Pooley J, Glomb J, Stecker E, Fazal F, Fleming JO, et al. Evolution of mouse hepatitis virus (MHV) during chronic infection: quasispecies nature of the persisting MHV RNA. Virology 1995;209:337–46.
[362] Rülicke T, Hassam S, Autenried P, Briner J. The elimination of mouse hepatitis virus by temporary transplantation of human tumors from infected athymic nude mice into athymic nude rats (rnuN/rnuN). J Exp Anim Sci 1991;34:127–31.
[363] Hsu CC, Wobus CE, Steffen EK, Riley LK, Livingston RS. Development of a microsphere-based serologic multiplexed fluorescent immunoassay and a reverse transcriptase PCR assay to detect murine norovirus 1 infection in mice. Clin Diagn Lab Immunol 2005;12:1145–51.
[364] Karst SM, Wobus CE, Lay M, Davidson J, Virgin HW. STAT1-dependent innate immunity to a Norwalk-like virus. Science. 2003;299:1575–8.
[365] Mumphrey SM, Changotra H, Moore TN, Heimann-Nichols ER, Wobus CE, Reilly MJ, et al. Murine norovirus 1 infection is associated with histopathological changes in immunocompetent hosts, but clinical disease is prevented by STAT1-dependent interferon responses. J Virol 2007;81:3251–63.
[366] Wobus CE, Karst SM, Thackray LB, Chang KO, Sosnovtsev SV, Belliot G, et al. Replication of norovirus in cell culture reveals a tropism for dendritic cells and macrophages. PLoS Biol 2004;2:e432.
[367] Hsu CC, Riley LK, Wills HM, Livingston RS. Persistent infection with and serologic cross-reactivity of three novel murine noroviruses. Comp Med 2006;56:247–51.
[368] Thackray LB, Wobus CE, Chachu KA, Liu B, Alegre ER, Henderson KS, et al. Murine noroviruses comprising a single genogroup exhibit biological diversity despite limited sequence divergence. J Virol 2007;81:10460–73.
[369] Goto K, Hayashimoto N, Yasuda M, Ishida T, Kameda S, Takakura A, et al. Molecular detection of murine norovirus from experimentally and spontaneously infected mice. Exp Anim 2009;58:135–40.
[370] Perdue KA, Green KY, Copeland M, Barron E, Mandel M, Faucette LJ, et al. Naturally occurring murine norovirus infection in a large research institution. J Am Assoc Lab Anim Sci 2007;46:39–45.
[371] Manuel CA, Hsu CC, Riley LK, Livingston RS. Soiled-bedding sentinel detection of murine norovirus 4. J Am Assoc Lab Anim Sci 2008;47:31–6.
[372] Artwohl JE, Purcell JE, Fortman JD. The use of cross-foster rederivation to eliminate murine norovirus, *Helicobacter* spp., and murine hepatitis virus from a mouse colony. J Am Assoc Lab Anim Sci 2008;47: 19–24.
[373] Achard M, Mähler M, Neumann D, Zschemisch NH, Janus LM, Köhl W, et al. Impact of murine norovirus on a mouse model of IBD. Gastroenterology 2009;136: A706 (Abstract).
[374] Cadwell K, Patel KK, Maloney NS, Liu TC, Ng AC, Storer CE, et al. Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine Cell 2011;141:1135–45.
[375] Lencioni KC, Seamons A, Treuting PM, Maggio-Price L, Brabb T. Murine norovirus: an intercurrent variable in a mouse model of bacteria-induced inflammatory bowel disease. Comp Med 2008;58: 522–33.
[376] Doom CM, Turula HM, Hill AB. Investigation of the impact of the common animal facility contaminant murine norovirus on experimental murine cytomegalovirus infection. Virology 2009;392:153–61.
[377] Paik J, Fierce Y, Drivdahl R, Treuting PM, Seamons A, Brabb T, et al. Effects of murine norovirus infection on a mouse model of diet-induced obesity and insulin resistance. Comp Med 2010;60:189–95.
[378] Richter CB. Mouse adenovirus, K virus, and pneumonia virus of mice. In: Bhatt PN, Jacoby RO, Morse III HC, New AE, editors. Viral and Mycoplasmal Infections of Laboratory Rodents: Effects on Biomedical Research. Orlando, FL: Academic Press; 1986. pp. 137–92.
[379] Brownstein DG. Sendai virus and pneumonia virus of mice (PVM). In: Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, Smith AL, editors. The Mouse in Biomedical Research. Diseases 2nd ed., vol. 2. New York: Elsevier Academic Press; 2007. pp. 281–309.

[380] Rosenberg HF, Domachowske JB. Pneumonia virus of mice: severe respiratory infection in a natural host. Immunol Lett 2008;118:6–12.
[381] Brownstein DG. Pneumonia virus of mice infection, lung, mouse, and rat. In: Jones TC, Dungworth DL, Mohr U, editors. Monographs on Pathology of Laboratory Animals: Respiratory System. 2nd ed. Berlin: Springer Verlag; 1996. pp. 317–21.
[382] Carthew P, Sparrow S. Persistence of pneumonia virus of mice and Sendai virus in germ-free (nu/nu) mice. Br J Exp Pathol 1980;61:172–5.
[383] Richter CB, Thigpen JE, Richter CS, Mackenzie JM. Fatal pneumonia with terminal emaciation in nude mice caused by pneumonia virus of mice. Lab Anim Sci 1988;38:255–61.
[384] Weir EC, Brownstein DG, Smith AL, Johnson EA. Respiratory disease and wasting in athymic mice infected with pneumonia virus of mice. Lab Anim Sci 1988;38:133–7.
[385] Cook PM, Eglin RP, Easton AJ. Pathogenesis of pneumovirus infections in mice: detection of pneumonia virus of mice and human respiratory syncytial virus mRNA in lungs of infected mice by *in situ* hybridization. J Gen Virol 1998;79:2411–7.
[386] Anh DB, Faisca P, Desmecht DJ. Differential resistance/susceptibility patterns to pneumovirus infection among inbred mouse strains. Am J Physiol Lung Cell Mol Physiol. 2006;291:L426–435.
[387] Lagace-Simard J, Descoteaux JP, Lussier G. Experimental pneumovirus infections: 1. Hydrocephalus of mice due to infection with pneumonia virus of mice (PVM). Am J Pathol 1980;101:31–40.
[388] Wagner AM, Loganbill JK, Besselsen DG. Detection of sendai virus and pneumonia virus of mice by use of fluorogenic nuclease reverse transcriptase polymerase chain reaction analysis. Comp Med 2003;53:173–7.
[389] Roths JB, Smith AL, Sidman CL. Lethal exacerbation of *Pneumocystis murina*. pneumonia in severe combined immunodeficiency mice after infection by pneumonia virus of mice. J Exp Med 1993;177:1193–8.
[390] Ward RL, McNeal MM. Adult mouse model for rotavirus. In: Zak O, Sande MA, editors. Handbook of Animal Models of Infection. New York: Elsevier Academic Press; 1999. pp. 1049–60.
[391] Sheridan JF, Vonderfecht S. Mouse rotavirus. In: Bhatt PN, Jacoby RO, Morse III HC, New AE, editors. Viral and Mycoplasmal Infections of Laboratory Rodents: Effects on Biomedical Research. New York: Elsevier Academic Press; 1986. pp. 217–43.
[392] Barthold SW. Murine rotavirus infection, mouse. In: Jones TC, Popp JA, Mohr U, editors. Monographs on Pathology of Laboratory Animals: Digestive System. 2nd ed. Berlin: Springer Verlag; 1997. pp. 384–8.
[393] Held N, Hedrich HJ, Bleich A. Successful sanitation of an EDIM-infected mouse colony by breeding cessation. Lab Anim 2011;45:276–9.
[394] Lundgren O, Peregrin AT, Persson K, Kordasti S, Uhnoo I, Svensson L. Role of the enteric nervous system in the fluid and electrolyte secretion of rotavirus diarrhoea Science 2000;287:491–5.
[395] Ball JM, Tian P, Zeng CQ, Morris AP, Estes MK. Age-dependent diarrhoea induced by a rotaviral nonstructural glycoprotein. Science 1996;272:101–4.
[396] Rosé J, Franco M, Greenberg H. The immunology of rotavirus infection in the mouse. Adv Virus Res 1998;51:203–35.
[397] Feng N, Franco MA, Greenberg HB. Murine model of rotavirus infection. Adv Exp Med Biol 1997;412:233–40.
[398] McNeal MM, Rae MN, Ward RL. Evidence that resolution of rotavirus infection in mice is due to both CD4 and CD8 cell-dependent activities. J Virol 1997;71:8735–42.
[399] Pott J, Mahlakoiv T, Mordstein M, Duerr CU, Michiels T, Stockinger S, et al. IFN-λ determines the intestinal epithelial antiviral host defense. Proc Natl Acad Sci U S A 2011;108:7944–9.
[400] Riepenhoff-Talty M, Dharakul T, Kowalski E, Michalak S, Ogra PL. Persistent rotavirus infection in mice with severe combined immunodeficiency. J Virol 1987; 61:3345–8.
[401] Franco MA, Greenberg HB. Role of B cells and cytotoxic T lymphocytes in clearance of and immunity to rotavirus infection in mice. J Virol 1995;69:7800–6.
[402] Ferner WT, Miskuff RL, Yolken RH, Vonderfecht SL. Comparison of methods for detection of serum antibody to murine rotavirus. J Clin Microbiol 1987;25: 1364–9.

[403] Jure MN, Morse SS, Stark DM. Identification of nonspecific reactions in laboratory rodent specimens tested by Rotazyme rotavirus ELISA. Lab Anim Sci 1988;38: 273–8.
[404] Wilde J, Eiden J, Yolken R. Removal of inhibitory substances from human fecal specimens for detection of group A rotaviruses by reverse transcriptase and polymerase chain reactions. J Clin Microbiol 1990;28:1300–7.
[405] Newsome PM, Coney KA. Synergistic rotavirus and Escherichia coli diarrhoeal infection of mice. Infect Immun 1985;47: 573–4.
[406] Machii K, Otsuka Y, Iwai H, Ueda K. Infection of rabbits with Sendai virus. Lab Anim Sci 1989;39:334–7.
[407] Percy DH, Palmer DJ. Pathogenesis of Sendai virus infection in the Syrian hamster. Lab Anim Sci 1997;47:132–7.
[408] Tashiro M, McQueen NL, Seto JT. Determinants of organ tropism of Sendai virus. Front Biosci 1999;4:D642–645.
[409] Brownstein DG. Sendai virus infection, lung, mouse, and rat. In: Jones TC, Dungworth DL, Mohr U, editors. Monographs on Pathology of Laboratory Animals: Respiratory System. 2nd ed. Berlin: Springer Verlag; 1996. pp. 308–16.
[410] Simon AY, Moritoh K, Torigoe D, Asano A, Sasaki N, Agui T. Multigenic control of resistance to Sendai virus infection in mice. Infect Genet Evol 2009;9:1253–9.
[411] Ward JM, Houchens DP, Collins MJ, Young DM, Reagan RL. Naturally-occurring Sendai virus infection of athymic nude mice. Vet Pathol 1976;13:36–46.
[412] Percy DH, Auger DC, Croy BA. Signs and lesions of experimental Sendai virus infection in two genetically distinct strains of SCID/beige mice. Vet Pathol 1994;31: 67–73.
[413] Kast WM, Bronkhorst AM, de Waal LP, Melief CJ. Cooperation between cytotoxic and helper T lymphocytes in protection against lethal Sendai virus infection. Protection by T cells is MHC-restricted and MHC-regulated; a model for MHC-disease associations. J Exp Med 1986;164: 723–38.
[414] Hou S, Doherty PC, Zijlstra M, Jaenisch R, Katz JM. Delayed clearance of Sendai virus in mice lacking class I MHC-restricted CD8+ T cells. J Immunol 1992;149:1319–25.
[415] Ward JM. Naturally occurring Sendai virus disease of mice. Lab Anim Sci 1974;24: 938–42.
[416] Shimokata K, Nishiyama Y, Ito V, Kimura Y, Nagata I. Affinity of Sendai virus for the inner ear of mice. Infect Immun 1977;16:706–8.
[417] Hayase Y, Tobita K, Kii M, Hakamada Y, Arai T. Detection of nucleoprotein gene of Sendai virus in the lungs of rats by touchdown nested reverse transcription polymerase chain reaction. Exp Anim 1997;46: 307–10.
[418] Dillehay DL, Lehner ND, Huerkamp MJ. The effectiveness of a microisolator cage system and sentinel mice for controlling and detecting MHV and Sendai virus infections. Lab Anim Sci 1990;40:367–70.
[419] Artwohl JE, Cera LM, Wright MF, Medina LV, Kim LJ. The efficacy of a dirty bedding sentinel system for detecting Sendai virus infection in mice: a comparison of clinical signs and seroconversion. Lab Anim Sci 1994;44:73–5.
[420] Lipton HL, Kim BS, Yahikozawa H, Nadler CF. Serological evidence that *Mus musculus* is the natural host of Theiler's murine encephalomyelitis virus. Virus. Res 2001;76:79–86.
[421] Hemelt IE, Huxsoll DL, Warner AR. Comparison of MHG virus with mouse encephalomyelitis viruses. Lab Anim Sci 1974;24:523–9.
[422] Ohsawa K, Watanabe Y, Miyata H, Sato H. Genetic analysis of a Theiler-like virus isolated from rats. Comp Med 2003;53: 191–6.
[423] Hansen AK, Thomsen P, Jensen HJ. A serological indication of the existence of a guineapig poliovirus. Lab Anim 1997;31: 212–8.
[424] Brownstein D, Bhatt P, Ardito R, Paturzo F, Johnson E. Duration and patterns of transmission of Theiler's mouse encephalomyelitis virus infection. Lab Anim Sci 1989;39:299–301.
[425] Rozengurt N, Sanchez S. A spontaneous outbreak of Theiler's encephalomyelitis in a colony of severe combined immunodeficient mice in the UK. Lab Anim 1993;27: 229–34.
[426] McGavern DB, Murray PD, Rivera-Quinones C, Schmelzer JD, Low PA, Rodriguez M. Axonal loss results in spinal cord atrophy, electrophysiological

abnormalities and neurological deficits following demyelination in a chronic inflammatory model of multiple sclerosis. Brain 2000;123:519–31.
[427] Lipton HL, Rozhon EJ. The Theiler's murine encephalomyelitis viruses. In: Bhatt PN, Jacoby RO, Morse III HC, New AE, editors. Viral and Mycoplasmal Infections of Laboratory Rodents: Effects on Biomedical Research. Orlando, FL: Academic Press; 1986. pp. 253–75.
[428] Lipton HL, Dal Canto MC. Susceptibility of inbred mice to chronic central nervous system infection by Theiler's murine encephalomyelitis virus. Infect Immun 1979;26:369–74.
[429] Rossi CP, Cash E, Aubert C, Coutinho A. Role of the humoral immune response in resistance to Theiler's virus infection. J Virol 1991;65:3895–9.
[430] Brahic M, Bureau JF, Michiels T. The genetics of the persistent infection and demyelinating disease caused by Theiler's virus. Annu Rev Microbiol 2005;59:279–98.
[431] Palma JP, Kwon D, Clipstone NA, Kim BS. Infection with Theiler's murine encephalomyelitis virus directly induces proinflammatory cytokines in primary astrocytes via NF-kappaB activation: potential role for the initiation of demyelinating disease. J Virol 2003;77:6322–31.
[432] Kim BS, Palma JP, Kwon D, Fuller AC. Innate immune response induced by Theiler's murine encephalomyelitis virus infection. Immunol Res 2005;31:1–12.
[433] Lipton HL, Kumar ASM, Hertzler S. Cardioviruses: encephalomyocarditis virus and Theiler's murine encephalomyelitis virus. In: Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, Smith AL, editors. The Mouse in Biomedical Research. Diseases 2nd ed., vol. 2. New York: Elsevier Academic Press; 2007. pp. 311–23.
[434] Crane MA, Yauch R, Dal Canto MC, Kim BS. Effect of immunization with Theiler's virus on the course of demyelinating disease. J Neuroimmunol 1993;45:67–73.
[435] Kurtz CI, Sun XM, Fujinami RS. Protection of SJL/J mice from demyelinating disease mediated by Theiler's murine encephalomyelitis virus. Microb Pathog 1995;18:11–27.
[436] Rodriguez M, Lindsley MD. Immunosuppression promotes CNS remyelination in chronic virus-induced demyelinating disease. Neurology 1992;42:348–57.
[437] Yamada M, Zurbriggen A, Fujinami RS. Pathogenesis of Theiler's murine encephalomyelitis virus. Adv Virus Res 1991;39:291–320.
[438] Rosenthal A, Fujinami RS, Lampert PW. Mechanism of Theiler's virus-induced demyelination in nude mice. Lab Invest 1986;54:515–22.
[439] Fujinami RS, Rosenthal A, Lampert PW, Zurbriggen A, Yamada M. Survival of athymic (nu/nu) mice after Theiler's murine encephalomyelitis virus infection by passive administration of neutralizing monoclonal antibody. J Virol 1989;63:2081–7.
[440] Rozengurt N, Sanchez S. Vacuolar neuronal degeneration in the ventral horns of SCID mice in naturally occurring Theiler's encephalomyelitis. J Comp Pathol 1992;107:389–98.
[441] Abzug MJ, Rotbart HA, Magliato SA, Levin MJ. Evolution of the placental barrier to fetal infection by murine enteroviruses. J Infect Dis 1991;163:1336–41.
[442] Trottier M, Schlitt BP, Lipton HL. Enhanced detection of Theiler's virus RNA copy equivalents in the mouse central nervous system by real-time RT-PCR. J Virol Methods 2002;103:89–99.
[443] Krinke GJ, Zurbriggen A. Spontaneous demyelinating myelopathy in aging laboratory mice. Exp Toxicol Pathol 1997;49:501–3.