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Invited Review

Pathologic characteristics of infectious diseases in macaque monkeys used in biomedical and toxicologic studies

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Abstract: Nonhuman primates (NHPs), which have many advantages in scientific research and are often the only relevant animals to use in assessing the safety profiles and biological or pharmacological effects of drug candidates, including biologics. In scientific or developmental experiments, the immune systems of animals can be spontaneously compromised possibly due to background infection, experimental procedure-associated stress, poor physical condition, or intended or unintended mechanisms of action of test articles. Under these circumstances, background, incidental, or opportunistic infections can seriously can significantly complicate the interpretation of research results and findings and consequently affect experimental conclusions. Pathologists and toxicologists must understand the clinical manifestations and pathologic features of infectious diseases and the effects of these diseases on animal physiology and experimental results in addition to the spectrum of infectious diseases in healthy NHP colonies. This review provides an overview of the clinical and pathologic characteristics of common viral, bacterial, fungal, and parasitic infectious diseases in NHPs, especially macaque monkeys, as well as methods for definitive diagnosis of these diseases. Opportunistic infections that can occur in the laboratory setting have also been addressed in this review with examples of cases of infection disease manifestation that was observed or influenced during safety assessment studies or under experimental conditions. (DOI: 10.1293/tox.2022-0089; J Toxicol Pathol 2023; 36: 95–122)

Key words: primary infections, opportunistic infections, primate, Macaca, toxicologic pathology

Introduction

Nonhuman primates (NHPs) have important applications in scientific research owing to their genetic similarities to humans[1]. The safety profiles and biological or pharmacological effects of drug candidates, including biologics, can be often evaluated only in NHPs. Hence, the demand for NHPs in drug development research[1, 2] and scientific research in the fields of biology, medicine, and pharmaceutics is increasing in the United States of America (USA), the European Union, and Asia[3–5].

Cynomolgus (*Macaca fascicularis*) and rhesus (*Macaca mulatta*) macaques are the most commonly used NHPs in scientific research[5]. Macaques must be transferred and maintained in the laboratory setting for applications in biomedical and toxicological research. In Japan, monkeys are allowed to be imported only from the USA, the People's Republic of China, the Republic of Indonesia, the Republic of the Philippines, the Socialist Republic of Viet Nam, the Republic of Suriname, the Co-operative Republic of Guyana, and the Kingdom of Cambodia according to the Regulations Concerning the Importation of Animals That May Convey Pathogens of Infectious Diseases under Regulations Concerning the Importation of Animals That May Convey Pathogens of Infectious Diseases (Order of MHLW and MAFF No. 2 of 1999). Monkeys are subject to import and export regulations under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, commonly known as the Washington Convention, Ministry of Economy, Trade, and Industry), while macaque monkeys are subject to import regulations under the Law Concerning the Prevention of Damage to Ecosystems from Specified Invasive Alien Species (commonly known as the Invasive Alien Species Act, Ministry of the Environment). Therefore, additional prior procedures/permissions are required for importing monkeys into Japan.

The spread of Ebola hemorrhagic fever-causing and Marburg fever-causing viruses must be strictly prevented by quarantining monkeys based on Article 55 of the Act on Prevention of Infectious Diseases and Medical Care for Infectious Patients (Act No. 114 of 1998, as amended). Additionally, each facility strives to prevent unexpected outbreaks of infections by performing various tests during the quarantine and acclimatization periods.

Although animal care and handling procedures have

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markedly progressed, the immune system of animals can be spontaneously compromised due to background infection and experimental condition-induced stress, poor physical condition, or the intended or unintended mechanism of the test articles[6, 7]. For example, simian retrovirus (SRV) and measles affect both humoral and cellular immune systems[8, 9], while simian immunodeficiency virus (SIV) compromises cellular immunity[10–12] in infected macaques. Background, incidental, or opportunistic infections can seriously affect the interpretation of the experimental results. To prevent the effects of infectious diseases on experimental results, specific pathogen-free colonies of NHPs can be used. However, eliminating infectious diseases in NHP colonies is challenging when compared with that in several other laboratory animal species. This is because macaques need parental and social interactions for physiological development and additionally considerable numbers of NHP colonies are raised outdoors[13, 14]. Furthermore, the elimination of all infectious agents, including those that are not pathogenic in healthy monkeys, is not ideal because the recurrence of infectious diseases may provide good evidence for assessing or revealing the safety profiles of therapeutic drugs, biomedical agents, or experimental procedures. Therefore, it is important for pathologists to understand the spectrum of infectious diseases in normal NHP colonies, as well as the clinical, gross pathologic, and histopathologic characteristics of these diseases and their effects on physiology and experimental outcomes[14, 15].

This review provides an overview of the clinical and pathologic characteristics of known or common viral, bacterial, parasitic, and fungal infectious diseases in NHPs (Table 1) (represented by macaque monkeys in this study), as well as diagnostic approaches to identify these diseases. Additionally, potential opportunistic infections in laboratory settings have been discussed (Table 1). Furthermore, some examples of cases of infectious disease manifestation that were observed or influenced during safety assessment studies or under experimental conditions are included.

Viral Infection

Simian retrovirus (SRV): synonymous names: simian betaretrovirus, Type D (SRV/D) retrovirus (Serotype: SRV1–5, 7 and SRV-T are reported in Asian macaques)[16]

Overview. The prevalence of seropositivity and/or virus positivity in macaques is known to be relatively high but varies depending on the efforts of the facility. In a facility in Tsukuba, Japan, the prevalence of SRV infection in cynomolgus macaques was 95% in 2010[17]. SRV can be transmitted via contact with saliva, urine, and/or feces of infected individuals as determined during the spread of SRV/D-Tsukuba infection within the facility[18]. SRV is one of the causes of simian acquired immune deficiency (SAIDS), which affects both humoral and cellular immune functions[8, 19]. The hematocrit, white blood cell, lymphocyte, and neutrophil values in seropositive or viremic animals are lower than those in normal animals, However, changes that fit the definition of SAIDS are not commonly found in those seropositive and viremic animals[20]. In one study, only one of the 24 experimentally infected cynomolgus macaques exhibited clinical signs consistent with SAIDS[20]. A $CD8^+$ T-cell-depleted rhesus monkey transfused with whole blood from a monkey positive for SRV1 antibody and polymerase chain reaction (PCR)-positive for viremia did not exhibit clinical or marked hematological or pathologic changes indicative of SRV-related SAIDS within a few months[21]. SRV-4 and SRV-5 are reported to infect Japanese macaques with different pathogenicity[231, 232, 237, 238].

Clinical and anatomical pathologic features. Clinical signs in cynomolgus monkeys include nonspecific anemia, weight loss, and diarrhea. The hallmark of SRV-induced SAIDS is the reactivation of opportunistic infections. Various bacterial infections have been reported in SRV-infected macaques[22] in addition to cytomegalovirus (CMV) infection, oral and esophageal candidiasis, and intestinal cryptosporidiosis, which are relatively common in macaques experimentally infected with the SIV[16, 22, 24]. Histological changes are characterized by increased nonspecific infection and increased incidence or severity of focal lymphoid infiltration (predominantly in the kidney, pancreas, salivary glands, bone marrow, and brain) with occasional germinal center formation, abscess, arthritis, or myositis along with lymphoid hyperplasia in the spleen and splenomegaly due to reactions in the germinal centers and paracortex of the lymph nodes[8, 18, 20]. Lymphoid depletion due to poor conditions associated with the infection may be observed. Some macaques infected with SRV exhibit proliferative lesions of retroperitoneal fibromatosis, which is characterized by the formation of masses with infiltrative growth of fibroblastic cells with collagen fibers[8, 23]. When infected animals exhibit reactivation of opportunistic infections due to SAIDS, the pathologic features discussed in the sections on individual opportunistic infections, including CMV, *Candida*, and *Cryptosporidium* infections, should be referred. In Japanese macaques, SRV-4 or SRV-5 infection is reported to induce related severe thrombocytopenia[231, 232, 237].

Diagnosis. In addition to serological examinations (including commercially available enzyme-linked immunosorbent assay (ELISA) kits), PCR for proviruses, reverse transcription (RT)-PCR, immunohistochemistry (IHC), and *in situ* hybridization (ISH) methods can be used to identify SRV infection[18, 231]. Some detection methods may not exhibit effective performance due to the stage of infection[231]. Hence, a combination of these procedures may be useful. IHC and ISH analyses reveal the viral antigen and genome in the ductal epithelium located between the acinar epithelium of the salivary gland[18].

Simian immunodeficiency virus (SIV) [No natural infection reported in Asian macaques]

Overview. Large proportions of African primates, which are natural hosts of SIV, are serologically positive for SIV. However, clinical disease, which is characterized

Table 1. Viral, Bacterial, Fungal, and Parasitic Infectious Agents That Can Affect Studies in Macaques

Infectious agent	*Prevalence* (reference)
Viral	
Simian retrovirus	*High** [17, 18]
Simian immunodeficiency virus	*Low*** [16, 24]
Measles	*Low*** [27, 28]
Herpes B virus	*High* [16]
Simian T-cell leukemia viruses	*Low* [16, 35]
Cytomegalovirus	*Common opportunistic* [28, 35, 47, 48]
Lymphocryptovirus	*High* [54–56]
Simian adenovirus	*Medium high* [66]
Simian virus 40	*High* [16, 74]
Simian parvovirus	*Low* [79]
Papillomavirus	*High* [82]
Rhesus rhadinovirus	*High* [88, 90]
Simian varicella virus	*Low* [100]
Hepatitis A virus	*Low** [102, 105]
Canine distemper virus	*Low**[226]
Bacterial	
Campylobacter, Shigella, Yersinia	*High* [108, 109]
Mycobacterium tuberculosis complex	*Medium high* [119]
Helicobacter pylori, Helicobacter heilmannii	*High* [117]
Moraxella catarrhalis	*Common opportunistic* [117]
Rhodococcus equi	*Common opportunistic* [136–138]
Enteropathogenic *Escherichia coli*	*High* [146, 147]
Parasitic	
Pulmonary acariasis	*High* [153, 154]
Helminths (*Strongyloides fuelleborni, Trichuris trichiura*, *Oesophagostomum* spp.)	*High**** [157–161]
Gastrointestinal protozoa	
Amoebae (*Entamoeba* spp.)	*High* [166–168]
Coccidia (*Cryptosporidium* spp.)	*Low* [155, 160, 169]
Ciliates (*Balantidium* spp.)	*Low–High* [174]
Flagellates (*Giardia* spp.)	*Low* [155, 160, 169]
Other protozoa	
Flagellates (*Trypanosoma* spp.)	*Low***** [181]
Coccidia (*Toxoplasma gondii*)	*Low* [190, 191]
Coccidia (*Sarcocystis* spp.)	*Low* [196]
Coccidia (*Babesia* spp.)	*Low* [199, 200]
Coccidia (*Plasmodium* spp.)	*High* [203, 204]
Fungal	
Candida albicans	*High* [208–211]
Pneumocystis spp.	*High* [214, 215]

*depends on facility; **not natural host; ***depends on species and facility; ****limited to South America.

by the loss of $CD4^+$ T cells and immunosuppression, is not observed in these monkeys. In cases of cross-species transmission to Asian macaques, SIV infection can decrease the $CD4^+$ T-cell count and compromise the immune system, which are similar to the effects of human immunodeficiency virus (HIV) and to associated acquired immunodeficiency syndrome (AIDS). SIV is not endemic to Asian macaques, and natural cross-species transmission of SIV from African NHPs to Asian macaques is rare[16, 24]. Hence, the probability of SIV infection in Asian laboratory macaques is low. Rhesus monkeys experimentally infected with SIV are frequently used as a model of AIDS. SIV infection in cynomolgus monkeys is reported to be less pathogenic when compared with that in Indian rhesus monkeys[233].

Clinical and anatomical pathologic features. The SIV-specific pathologic features include selective down-regulation of $CD4^+$ lymphocyte counts, lymphoma[10, 16], and the occurrence of opportunistic infections, including manifestations of CMV infection, oral and esophageal candidiasis, and intestinal cryptosporidiosis[16, 24]. For the pathologic features of opportunistic infections, refer to the sections below for each infectious agent. Nonspecific findings that are potentially associated with the manifestations of immune deficiency include decreased body weight, diarrhea, gener-

alized lymphadenopathy, and splenomegaly.

Diagnosis. Antibody responses are induced within weeks of SIV infection. SIV genomes can be detected in peripheral blood $CD4^+$ cells, sometimes within days of infection[25, 26]. A combination of serological and molecular assays can be used to eliminate the risk of SIV-related undesirable immune compromise from colonies used in scientific studies.

Measles virus

Overview. Humans are the only natural hosts that sustain measles virus transmission[27]. Most NHP species are susceptible to measles infections from their infected human handlers[28]. Measles belongs to the family Paramyxoviridae.

Clinical and anatomical pathologic features. The clinical, gross, and histopathologic hallmark of measles in macaques is maculopapular skin rash, which is histologically characterized by multinucleated giant cells in the epidermis and hair follicles with mild inflammation. Although measles is associated with lymphocytopenia, the depletion of peripheral lymphocytes, which as a cause of immune suppression, is often undetectable because lymphocyte counts rapidly return to control levels after virus clearance. Therefore, measles-induced lymphocytopenia may be missed. However, the suppression of humoral and cellular immune functions can last several weeks to months, resulting in mortality predominantly due to secondary infections in the respiratory and digestive tracts[9]. In the lung, the lesions range from mild bronchiolar pneumonia to severe interstitial pneumonia; These changes are associated with the presence of multinucleated giant cells and minimal alveolar edema. Giant cells and intranuclear and cytoplasmic inclusion bodies can also be observed in reticular or phagocytic cells of the lymphoid system and in epithelial cells of the respiratory system, gastrointestinal tract, salivary glands, thyroid gland, liver, pancreas, and urinary system[29, 239]. Representative eosinophilic intranuclear and cytoplasmic inclusion bodies of measles in the infected bronchial epithelium are shown in Fig. 1a and 1b.

Diagnosis. In addition to commercially available detection kits for measles-specific IgM and IgG[30], RT-PCR can also be used for measles virus detection[31, 32].

Herpes B virus (HBV, Cercopithecine herpesvirus 1)

Overview. Most naturally captured Asian macaques are seropositive for HBV. HBV infection is latent and is not associated with clinical signs[16]. In humans, HBV infection is rare but has been sporadically reported[33]. HBV infection can be fatal in humans[34]. To avoid fatal infection from handlers and potential effects on the experiments, HBV seronegative animals are usually used[35]. However, to ensure handler protection, we should be aware that serological testing for antibodies or PCR testing for the virus usually shows negative results in latently infected animals without viremia. HBV infection in macaques is persistent and remains latent in the trigeminal and spinal ganglia. Immunosuppressive conditions, such as under stress can reactivate the infection, leading to the shedding or transmission of virus with increased antibody titers[33, 36, 37].

Clinical and anatomical pathologic features. In macaques, HBV infection is clinically and pathologically characterized by small to large (sometimes erosive) vesicles on the oral and genital mucosa together with a multifocal necrotizing hepatitis with multinucleated syncytial cells and intranuclear inclusion bodies in the epithelial cells of affected organs and tissues[16, 37, 38].

Diagnosis. Serological examination to diagnose HBV infection is challenging owing to the close genetic relationship between herpes simplex virus and HBV and antibodies in the serum can cross react[39, 40]. PCR detection of HBV-specific DNA or viral isolation is used for diagnosis[39–42].

Simian T-cell leukemia viruses (STLVs)

Overview. STLVs can infect Asian macaques[16, 43] although the seroprevalence of STLVs is rare in macaques maintained under laboratory settings[35]. Clinical signs are usually not apparent even in cases of STLV infection is confirmed[16].

Clinical and anatomical pathologic features. Characteristic pathologic changes include lymphoproliferative lesions with changes in cytokine profiles, which are most apparent in baboons (*Papio* spp.)[16] and the African green monkey (*Chlorocebus sabaeus*)[44]. The common findings associated with these lymphomas in NHPs are decreased bodyweight, weakness, lethargy, dyspnea with pneumonia, leukemia with or without multilobulated lymphocytes in the peripheral blood, generalized lymphadenopathy, hepatosplenomegaly, and nodular skin lesions with diffuse infiltration of neoplastic lymphocytes[44–46] and multilobulated lymphocytes[43].

Diagnosis. Antibodies against STLV-1 antigens can be detected in the peripheral blood of animals with suspected infections. The lymph node section containing proliferative lesions can be subjected to IHC to demonstrate the neoplastic increase in T-cell lineages[25].

Cytomegalovirus (CMV): Betaherpesvirinae Macacine herpesviruses 3 (rhesus CMV) and 8 (cynomolgus CMV)

Overview. CMV infects captive rhesus and cynomolgus macaques without clinical signs and seroprevalence of CMV is high (more than 90% in all populations tested and almost 100% in adults[16, 28, 35, 47, 48]). Latent CMV activation is one of the most commonly observed opportunistic infections in immunocompromised macaques, which can be attributed to the high prevalence of CMV (similar to that in humans)[49]. CMV is a highly species-specific virus owing to its long evolutionary history with its host. Therefore, cross-species infection is restricted. Additionally, cross-species infection is rare in primates even under experimental conditions[50, 51]. In cases of CMV activation in monkeys immunosuppressed for tissue transplantation, CMV DNA has been detected in animals with the white blood cell (WBC) counts decreasing to less than 4,500/μL and the lymphocyte counts

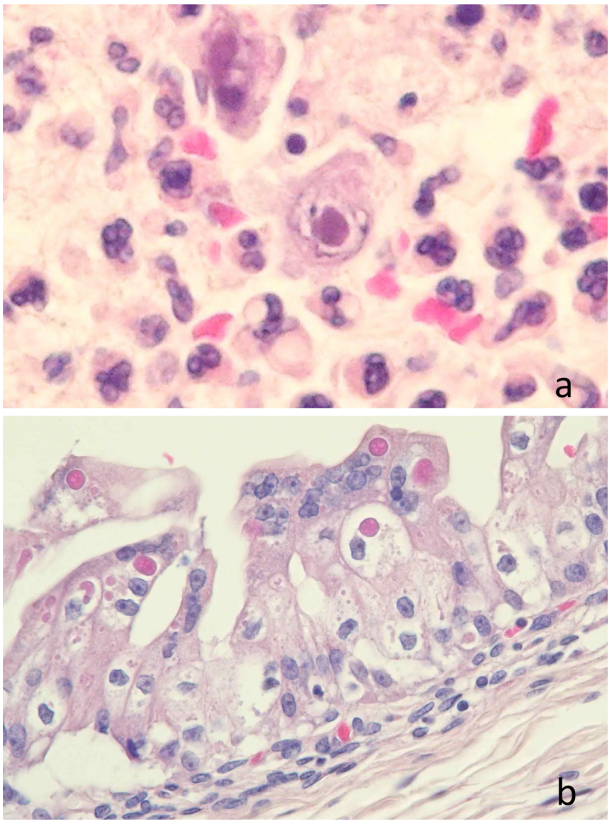

Fig. 1. *Measles virus.* (a) Eosinophilic intranuclear inclusion bodies in the measles-infected cells surrounded by inflammatory cells, including neutrophils. (b) Eosinophilic cytoplasmic inclusion bodies in the infected bronchiolar epithelium. Photographs are kindly provided by Dr. Shinichiro Nakamura, Azabu University.

decreasing to less than 1,800/μL[52]. However, in our experience, a clear decrease in WBC parameters has not been detected before the manifestation of CMV-related clinical signs. Thus, functional alterations in the immune system without marked changes in peripheral WBC counts can also cause CMV reactivation.

Clinical and anatomical pathologic features. In immunosuppressed animals, CMV infection most commonly affects the lungs. Inflammation of the brain, gastrointestinal tract, reproductive organs, and lymphoid system can also be observed[16, 28]. In our experience with opportunistic systemic CMV infection accompanied by clinical manifestations, a severely affected animal exhibits decreased food consumption and decreased activity. At necropsy there was pulmonary effusion with diffuse dark red discoloration of all lung lobes. In the lung, diffuse infiltration of inflammatory cells (predominantly foamy macrophages), serous or fibrinous exudate, and hemorrhage (Fig. 2a) were histologically observed. Enlargement of alveolar epithelial cells and vascular endothelial cells were also observed. These cells frequently contained intracytoplasmic and intranuclear inclusion bodies with or without clear hollows (owl's eyes) (Fig. 2b and 2c). Perivascular edema and inflammatory cell infiltration with hemorrhagic necrosis of blood vessels along with hypertrophy of alveolar epithelial cells, and syncytial cells were also observed. Lymphoid depletion in the bronchiole-associated lymphoid tissue, various lymph nodes with foamy macrophages with intranuclear inclusions, gastric ulceration and inflammation, and inclusion bodies in the systemic vascular endothelium are also frequently observed.

Diagnosis. Electron microscopy revealed the presence of intracytoplasmic virus particles with envelope and core

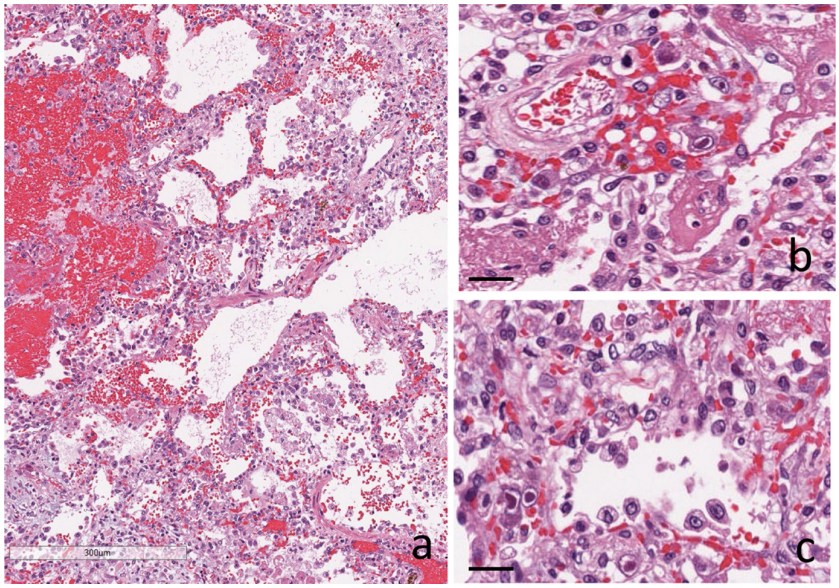

Fig. 2. *Cytomegalovirus* (CMV). (a) Diffuse interstitial pneumonia with hemorrhage and fibrinous exudate in the alveoli were observed in the lung of CMV-infected cynomolgus macaque. (b), (c) Intranuclear inclusion bodies with characteristics of CMV (so-called owl's eyes, with a distinct halo around the inclusion bodies) were found mainly in the enlarged endothelial cells of small vessels and macrophages in the alveoli (bars=50 μm).

characteristics of herpes virus. Immunohistochemical staining with commercially available antibodies against human CMV did not work well in our experience. CMV DNA can be quantified in peripheral blood using real-time PCR with the primers and probes described by Han *et al.*[52] and Kaur *et al*[53].

Lymphocryptovirus (LCV): Gammaherpesvirinae, Macacine herpesvirus 4 (rhesus LCV), Epstein-Barr virus (EBV)

Overview. LCV is a genus of viruses in the order Herpesvirales, in the family Herpesviridae, in the subfamily Gammaherpesvirinae which includes Human gammaherpesvirus 4 (EBV). EBV infection, which is usually asymptomatic but may be symptomatic in humans infected after puberty, is associated with lymphoproliferative lesions in immunocompromised patients. The seroprevalence of LCV in cynomolgus and rhesus macaques is high: almost 100% of adult monkeys in the laboratory are positive for LCV (or EBV)[54–56]. Similar to EBV infection in humans, primary LCV infection in macaque monkeys is not associated with clinical signs. The clinical and histopathologic changes are reportedly limited to macaques immunocompromised by immunosuppressive viral infection, immunosuppression for tissue transplantation, or treatment with immune-modulating drugs[57–60]. LCV has been detected in most malignant lymphomas (non-Hodgkin lymphomas) in macaques infected with SIV[61]. One case report on cynomolgus monkeys demonstrated LCM-related rapidly progressive B-cell lymphoma without marked evidence of immunosuppression in a 3-month toxicity study[242]. In this case, close evaluation using immunophenotyping revealed a low natural killer (NK) cell count before the initiation of the study.

Clinical and anatomical pathologic features. Enlarged lymph nodes were detected as masses in rhesus or cynomolgus monkeys experimentally inoculated with SIV and LCV. The histologic features of these monkeys involve the infiltration of immunoblastic cells, predominantly comprising CD20-positive B cells, and decreased numbers of $CD8^+$ T cells[58]. In Japanese monkeys, lymphomas derived from other lymphocytes, including CD4+ T cells and NK cells, have also been reported[240, 241]. Infected animals with immunosuppression for tissue transplantation can exhibit lymphadenopathy along with lymphoid infiltration of non-lymphoid organs, including the liver, lungs, heart, and kidney[62]. In a monkey treated with immunomodulatory biologics, enlargement of lymph nodes was clinically noted at week 28 of treatment. In a case of B-cell lymphoma observed in a 3-month toxicity study, which was characterized by large unclassified cells with lymphocytosis, monocytosis, and mild neutrophilia in hematology examination and a high cellularity was noted in a bone marrow smear evaluation that mostly consisted of a polymorphic lymphoid cell population[242]. Enlargement of the spleen and various lymph nodes observed at necropsy were histologically associated with loss of the normal lymphoid architecture, which was replaced by neoplastic lymphocytes; the diagnosis was lymphoma[59]. EBV-associated (LCV-associated) focal proliferative lesions have been found on the squamous epithelium of the tongue, esophagus, or penis, or on the haired skin of the lip, hand, or thorax of experimentally SIV-infected and immunocompromised monkeys. The skin lesions consisted of hyperkeratosis, parakeratosis, and acanthosis. Ballooning degeneration of keratinocytes was noted in the tongue, esophagus, and penis, with intranuclear inclusion bodies in cells in the middle and superficial layers of the stratified epithelium[58]. The surface of epithelial lesions is frequently accompanied by colonies of *Candida* species or gram-positive cocci[61].

Diagnosis. By transmission electron microscopy, herpesvirus particles with envelope and core can be found in inclusion-bearing cells are. The virus also can be observed by IHC for LMP-1 (latent membrane protein 1) or EBNA-2 (Epstein-Barr nuclear antigen 2) and ISH for EBV-encoded RNA[60, 61]. In lymphoproliferative lesions, increased numbers of LCV-infected lymphocytes can be observed using ISH[59].

Simian adenovirus

Overview. More than 50 adenovirus serotypes have been identified in NHPs, including macaques. and adenovirus is highly species specific[16, 28]. Generally, simian adenovirus infection is not clinically apparent and can be detected in healthy animals. Infection may target the respiratory and gastrointestinal tracts and conjunctiva (conjunctivitis). The liver, pancreas, and kidney were less frequently affected[63–65]. Adenoviral infection in the intestine is relatively common in macaque monkeys[66]. Infected macaques can shed adenoviruses in the stool[67]. Aerosols from sneezes or coughs and feces from animals exhibiting clinical manifestations or latent infections can be sources of viral transmission.

Clinical and anatomical pathologic features. Gastrointestinal tract infections may be associated with diarrhea with no visible gross lesions but with microscopic enteritis, erosion or ulceration, or villus atrophy of the small intestine with intranuclear inclusion bodies[24, 68–70]. In the infected respiratory tract, necrosis of epithelial cells of the trachea, bronchi, bronchioles, and alveoli with basophilic intranuclear inclusion bodies, is characteristic and accompanied by neutrophil and macrophage infiltration[16]. The intranuclear inclusion bodies of adenovirus are generally large and smudged as reported in cases of pancreatic and renal infections[64, 65].

Diagnosis. Antigens of serotypes 40 and 41 in feces can be detected using commercially available Adenoclone 40/41 kits (Meridian Diagnostics, Cincinnati, OH, USA)[66]. PCR can be performed with fecal or tissue samples using generic primate adenovirus[71, 72]. Meanwhile, the presence of viral structures in tissues with intranuclear inclusion bodies can be confirmed using electron microscopy[63, 73].

Simian virus 40 (SV40): Closely related to cynomolgus polyomavirus

Overview. SV40 and cynomolgus polyomaviruses are a family of small, non-enveloped DNA viruses that commonly cause latent infections in Asian monkeys, including rhesus and cynomolgus macaques. They can cause severe disease in situations of immunocompromised conditions[16, 74].

Clinical and anatomical pathologic features. Polyomaviruses-related lesions are observed in the central nervous system and kidney, and less frequently in the lung[16, 28]. The following two types of SV40-related central nervous system lesions have been reported: lesions similar to those in human progressive multifocal leukoencephalopathy (PML) and meningoencephalitis with renal and pulmonary lesions[74, 75]. Both the PML type and the meningoencephalitis type have the common etiology of latent infection in oligodendrocytes and astrocytes, as detected by ISH[76]. The difference of manifestation can be attributed to the age or duration of infection in animals infected with SV40 before immunosuppression induced by SIV infection. PML type noted in rhesus macaques is characterized by multifocal demyelination, predominantly in the white matter (most commonly near the boundary with the gray matter), with microgliosis, large astrocytes, and oligodendrocytes with swollen, atypical nuclei containing marginated chromatin and intranuclear inclusions[76]. In meningoencephalitis, lesions are observed in the meninges and superficial gray matter and are characterized by inflammation with infiltration of lymphocytes, eosinophils, and macrophages that spread from the perivascular area to the parenchyma. Enlargement of nuclei with smudgy amphophilic intranuclear inclusions in glial cells and/or multinucleation of large, bizarre, gemistocytic astrocytes have also been observed as well as PML-type, but without substantial demyelination[75].

The histological characteristics of nephritis associated with polyomavirus in experimentally immunosuppressed cynomolgus macaques include nuclear enlargement (sometimes with nuclear inclusion bodies), cellular apoptosis and detachment, and destruction of the basement membrane, primarily in the collecting ducts, associated with patchy interstitial infiltration of lymphoplasmacytic inflammatory cells[74, 77]. Involvement of the vascular endothelium or glomeruli is not observed. Mild renal dysfunction with elevated serum creatinine level is sporadically noted.

Diagnosis. The virus can be identified in the affected brain and kidney and sometimes in the ureter using IHC and DNA amplification specific for the large T antigen of the monkey polyomavirus family. Polyomaviruses can also be detected using electron microscopy in the nuclei of infected tubular epithelial cells or astrocytes as a characteristic sheet-like arrangement of non-enveloped virions[16, 75, 77].

Simian parvovirus (SPV)

Overview. SPV is a small, non-enveloped DNA virus with 65% sequence similarity to human parvovirus B19[16, 78]. In humans, the serological prevalence of parvovirus B19 is high, especially in elderly people. However, viremia is rare, and the disease is unapparent or has mild, nonspecific symptoms in immunocompetent hosts[78]. In one facility, a serologic screening test was conducted to prevent SPV outbreaks and all cynomolgus monkeys tested were SPV seronegative without any case of unexpected anemia from 2001 to 2006[79]. Therefore, the prevalence of SPV may not be high. SPV outbreaks are thought to be preventable by examining seronegativity during the initial quarantine period[79]. SPV identified in cynomolgus, rhesus, and pigtailed macaques is usually latent and is activated under immunosuppressive conditions caused by viral infection (e.g. SRV infection) or under experimental conditions, including treatment in preparation for transplantation[78, 80].

Clinical and anatomical pathologic features. Clinically apparent SPV infection is characterized by severe normocytic, normochromic, and non-regenerative anemia (decrease in red blood cells by −80%[83]), whereas the WBC count remains within the normal range. The histologic characteristics involve decreased counts of erythroid and myeloid lineages and intranuclear parvoviral inclusions in erythroid precursors (predominantly in normoblasts and sometimes in pronormoblasts) in the bone marrow[78, 79].

Diagnosis. SPV can be confirmed using electron microscopy based on the presence of a cluster of parvovirus-like particles in erythroid precursor cells in the bone marrow of infected animals. Additionally, SPV can be diagnosed using PCR analysis of DNA isolated from the sera of affected monkeys[80].

Papillomavirus (PV)

Overview. PVs are a diverse family of small, non-enveloped DNA viruses. The prevalence of PV in cynomolgus monkeys is approximately 30% in adults aged >10 years[81]. Recently, 75.2% of wild captive clinically healthy rhesus macaques tested positive for PV DNA next-generation sequencing amplicon assays, most commonly from genital swabs, then less commonly from oral and perianal swabs[82]. In humans it is well known that the incidence and severity of PV-related proliferative lesions are increased in immunocompromised patients[83].

Clinical and anatomical pathologic features. Opportunistic activation of PV can result in proliferative or neoplastic lesions. PV can cause various proliferative lesions in the squamous epithelium, including cutaneous papilloma or cancers of the uterine cervix, oral mucosa, or other epithelia[84–86]. However, PV infection may not manifest with overt clinical symptoms. Cervical and vaginal neoplasms have been observed in only 5.2% (20 out of 385) of cynomolgus monkeys in a facility. The neoplasms were intraepithelial and were detected in routine histological specimens with no or less than obvious gross finding that needs additional sampling[87]. Cancer may require considerable time to develop after PV infection and thus mortality resulting from PV infection in macaques is rare in toxicity studies of pharmaceutical drugs. Cervical and vaginal neoplastic lesions can take the form of intraepithelial dysplasia, benign papilloma,

or invasive cervical carcinoma, with common morphological features, including epithelial dysplasia, epithelial pearls, koilocytosis (cells with vacuoles around nuclei), nuclear atypia, and expansion of the basal epithelium[87].

In cynomolgus monkeys, beta PV-related papilloma has been observed on the skin of the hand and foot[85, 86]. The lesions are histologically characterized by diffuse thickening of the epidermis with foci of large and pale superficial cells and rarely with eosinophilic intranuclear inclusion bodies[86].

Diagnosis. IHC analysis of AU-1, which is an antigen of the genus-specific PV epitope, can be performed to diagnose PV. Additionally, other antibodies that cross-react with PV in monkeys can be used to detect PV capsid proteins expressed in the nuclei of surface epithelial cells of lesions. PCR[85, 87] and whole-genome sequencing can also be used[86].

Rhesus rhadinovirus (RRV): Gammaherpesvirinae, Macacine herpesvirus 5

Overview. RRV is highly endemic in socially housed and captive rhesus macaques, and the seroprevalence of RRV is almost 100% in adults[88, 89]. Viral DNA is detected in the blood or saliva, or both, in clinically healthy rhesus monkeys, and the detection rates of viral DNA are high in young (aged <2 years) monkeys[88, 90]. Infection is not usually associated with clinical signs but is associated with lymphoproliferative changes, resembling those of non-Hodgkin's lymphoma or multicentric Castleman's disease[91, 92]. Kaposi's sarcoma-associated herpesvirus (KSHV) and primate retroperitoneal fibromatosis-associated herpesvirus (RFHV), which can cause mesenchymal proliferative lesions, are closely associated with RRV[16]. RRV has been detected in areas of retroperitoneal fibromatosis in SIV-infected rhesus macaques and is considered one of the causative agents of mesenchymal proliferative lesions in macaques[91].

Clinical and anatomical pathologic features. In macaques experimentally infected with SIV and RRV, lymphoproliferative lesions resembling those of multicentric Castleman disease have been reported. In these animals, marked peripheral lymphadenopathy was observed with pronounced splenomegaly 10 weeks after RRV infection; and in the animals with severe hemolytic anemia was observed 30 weeks after RRV infection[92]. The affected lymph nodes and spleen there are many lymph follicles with large reactive germinal centers, which are often irregular in shape and have indistinct mantle zones. In enlarged follicles of the spleen, hyalinized cell-poor areas with increased numbers of blood vessels were observed[92]. The presence of plasma cell clusters in the spleen and lymph nodes may also be a characteristic feature. Enlargement of the liver and spleen along with lymphadenopathy and increased erythropoiesis in the bone marrow has been observed[91, 92].

Diagnosis. In addition to the characteristic histopathologic features, detection of RRV can be done using PCR for definitive diagnosis[91, 92].

Simian varicella virus (SVV): Cercopithecine herpesvirus 9

Overview. Simian varicella epizootics occur sporadically in NHP facilities, including Japanese facilities[93, 94]. After natural infection in primates, SVV becomes latent and latent viral DNA can be detected in the ganglia[95]. SVV becomes latent in ganglionic neurons and can be reactivated spontaneously or opportunistically in monkeys immunosuppressed by social or environmental stress[96, 97] or by experimental treatment with irradiation or immunosuppressive agents[98–101]. The seroprevalence of SVV may not be high. The reported incidence of SVV antibodies in newly captured wild pigtailed and cynomolgus macaque monkeys in Malaysia is only 0.8%[93]. However, SVV can spread in the colonies at research facilities. The seroprevalences at facilities where clinical manifestations of SVV were observed were 35% and 20% in rhesus macaques[101] and pigtailed macaques[100], respectively. In one rhesus macaque facility in which 57 animals received total body irradiation, SVV activation was observed in 2 animals[101].

Clinical and anatomical pathologic features. SVV causes skin erythematous lesions in macaques and other monkeys. The outbreaks of SVV infections can result in high morbidity and mortality[93]. The most characteristic cutaneous changes are diffuse multifocal papules/vesicles often associated with hemorrhage (maculopapulovesicular rashes). Many vesicles can appear successively and form crusts[16, 100]. Macroscopically, hemorrhage or ulcerative lesions may also be observed in other squamous epithelium including the oral cavity and mucosal and serosal surfaces of thoracic and abdominal organs. Skin vesicles are histologically characterized as intra-epidermal vesicles containing cellular debris and/or erythrocytes. In the epithelial cells, eosinophilic intranuclear inclusion bodies consistent with herpesvirus infection (Cowdry type A) can be observed. Visceral organs, including the lung and liver, and gastric mucosa, can be infected and manifest necrosis with hemorrhage and intranuclear inclusion bodies in infected epithelial cells[93, 100]. In a case of cynomolgus monkey in which anti-SVV antibody was detected by serological evaluation, eruption was observed whole body surface especially on the neck, chest and inner thighs and were histologically vesicles in the skin with intranuclear inclusion bodies in the basal cells of epidermis (Fig. 3a and 3b). Necrotizing inflammation with hemorrhage was observed in the lungs of this monkey (Fig. 3c). The presence of a bacterial colony was indicative of immunosuppression in this animal.

Diagnosis. Serological detection of anti-SVV antibodies is one of the most reliable diagnostic methods[93, 98]. IHC can also be used to detect herpesviruses in vesicles[100, 101].

Hepatitis A virus (HAV)

Overview. HAV, an RNA virus, infects humans and NHPs naturally, and there are species-specific variations in its sequences[102–105]. Rhesus and cynomolgus macaques are the natural hosts of HAV[102]. Infection is usually self-limiting in immunologically normal animals, and baseline preva-

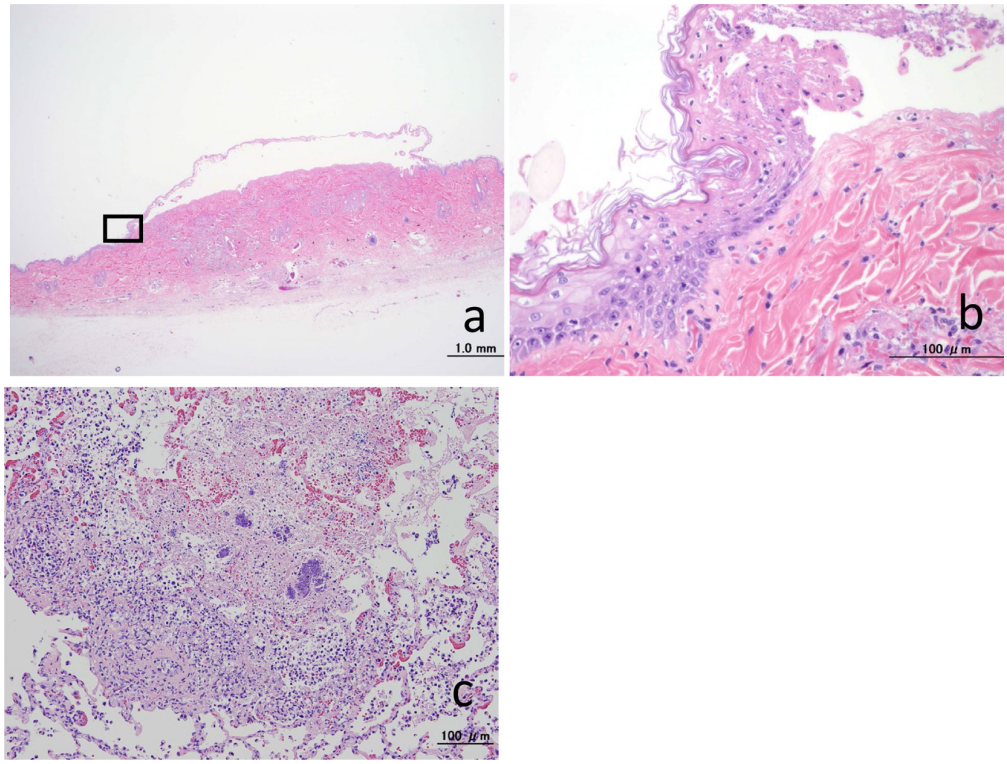

Fig. 3. *Simian varicella virus* (SVV). (a) A vesicle in the skin with cell debris. (b) An image of higher magnification of the area denoted using a square in Fig. 3a. Intercellular edema and intranuclear inclusion bodies are identified based on hematoxylin and eosin staining. Photographs were kindly provided by Dr. Junko Sato and Dr. Takuya Doi, LSIM Safety Institute Corporation.

lence may be low. As HAV infection can be transmitted via the fecal-oral route[105], the infection may spread, especially under group-housing conditions.

Clinical and anatomical pathologic features. In cynomolgus macaques experimentally infected with human HAV, no clinical manifestations of the disease are observed. Histopathologic changes in the liver may be characterized by inflammatory cell infiltration in the portal area and parenchyma with microvesicular vacuolation of the hepatocytes. Inflammation associated with infection is characterized by the presence of diffuse and scattered inflammatory cells (predominantly macrophages, lymphocytes, and a few neutrophils)[106, 107]. In immunocompromised animals, the infection may be serious. The livers of severely affected humans and chimpanzees are characterized by hepatocellular swelling, ballooning, and necrosis. Additionally, associated elevation of serum liver enzymes and the manifestation of jaundice have been reported[107].

Diagnosis. As the histopathologic characteristics and clinical manifestations are usually nonspecific, a definitive diagnosis can be performed using a combination of determining the presence of hepatitis, serological examination, and RT-PCR analysis of the serum, feces, saliva, or tissues[106].

Canine distemper virus (CDV)

Overview. CDV belongs to the genus Morbillivirus and the family Paramyxoviridae. Paramyxoviridae includes various highly pathogenic viruses, such as measles virus, rinderpest, and peste-des-petits-ruminants virus. In addition to canines, CDV is known to infect several carnivorous and non-carnivorous species, including Japanese rhesus and cynomolgus monkeys, but does not infect humans[223–227]. In macaque monkeys, CDV infection outbreaks have been reported in China and Japan[224–227]. CDV infection can be lethal with a mortality rate of approximately 10%[224], which is less than that in canines (approximately 50%).

Clinical and anatomical pathologic features. Clinical signs of infected macaque monkeys include fever, conjunctivitis and rhinitis with mucous discharge, cough, anorexia, diarrhea, generalized red rash, and periocular or plantar swelling[224, 226, 228]. Necropsy of severely affected monkeys revealed focal red or dark-red discolored areas on the lung or focal or diffuse hemorrhage on the brain surface[225, 227]. Histopathologic changes in cynomolgus monkeys sacrificed moribund were predominantly observed in the lungs, brain, and lymphoid organs[224]. Lung pneumonia with syncytial giant cell formation in the alveoli was observed (Fig. 4a and 4c). Gliosis (Fig. 4g) and/or demyelination were focally observed in the cerebrum and/or cerebellum[224]. Severe lymphoid depletion in the lymphoid organs suggesting immune suppression, has also been reported[224].

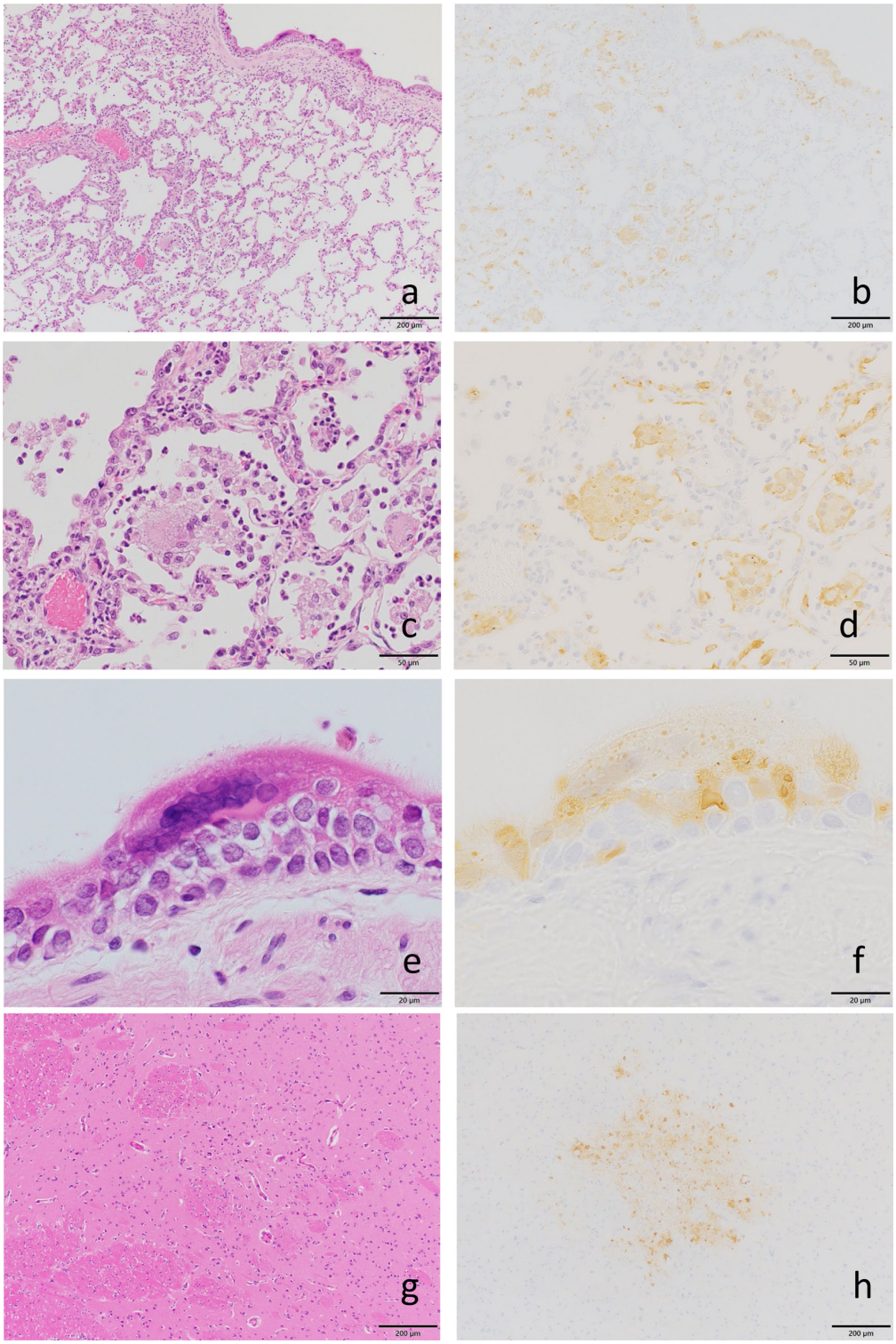

Fig. 4. *Canine distemper virus* (CDV). (a) The lung of a cynomolgus macaque with CDV infection sacrificed moribund. Cellular infiltration in the alveoli and thickening of the alveolar wall are noted. (b) Same area of "a" immunohistochemically stained for CDV. Cells in the alveoli and bronchial epithelium are positive for CDV. (c) Higher magnification of "a." Inflammatory cell infiltration and syncytial giant cell formation in the alveoli. (d) Syncytial giant cells and some cells in the alveolar wall are immunohistochemically positive for CDV. (e) Syncytial cells in the bronchiolar epithelium. (f) Syncytial cells in the bronchiolar epithelium are also immunohistochemically positive for CDV. (g) The brain of the same animal. Slight focal gliosis is noted. (h) The focus of gliosis is immunohistochemically positive for CDV. (a, c, e, and g) Hematoxylin and eosin staining. (b, d, f, and h) Immunohistochemical staining of CDV antigen. Photographs of sections obtained from animals sacrificed moribund are kindly provided by Dr. Noriyo Nagata, National Institute of Infectious Diseases and Dr. Shigeru Morikawa, Okayama University of Science[224].

In such cases, characteristic perivascular cuffing by lymphocytes may not be observed. Giant cells may be observed in other organs, including the skin, intestines, kidneys, salivary glands, and testes[224, 229]. Eosinophilic inclusion bodies are observed in epithelial cells of these affected organs in other animal species[229, 230]. The causes of death could be severe pneumonia or neurological clinical signs[224, 227].

Diagnosis. CDV can be diagnosed using IHC analysis of viral antigens in mononuclear cells in lymphoid organs, glial cells in the brain, and giant cells in the lung. Additionally, CDV can be diagnosed based on characteristic histopathologic changes (Fig. 4a–4h). Furthermore, CDV antigen detection kits can also be used to diagnose animals with clinical signs.

Bacterial Infection

Campylobacter, Shigella, and Yersinia (causes of bacterial diarrhea): Campylobacter spp., S. flexneri, Y. enterocolitica, or Y. pseudotuberculosis

Overview. *Campylobacter* spp. (*Campylobacter coli* and *Campylobacter jejuni*), *Shigella flexneri*, and *Yersinia enterocolitica* have been identified as causative infectious agents as well as adenovirus and the parasite *Strongyloides fuelleborni* in samples collected from macaque monkeys with chronic diarrhea[108, 109]. Among the bacteria that can cause diarrhea in macaque monkeys, *Campylobacter* spp. (especially *C. jejuni* and *C. coli*) and *S. flexneri* are the most commonly isolated bacteria, while *Y. enterocolitica*, *Y pseudotuberculosis*, and *Salmonella* spp. are less commonly isolated[24, 110]. Routine quarantine procedures in which only clinical signs are evaluated do not guarantee freedom *from either shigella* or *salmonella*[110]. *Campylobacter jejuni* and *C. coli*, which are the main organisms responsible for infectious enteritis, are difficult to eradicate because simple isolation or treatment with antibiotics does not prevent recurrent infections with diarrheal bacteria in a colony[110]. Diligent serial testing and treatment are needed to control infection with these bacteria.

Clinical and anatomical pathologic features. Diarrhea caused by *Shigella flexneri* can be observed during quarantine within the first month after import from the country of origin. *Shigella flexneri*-induced diarrhea often exhibits antibiotic resistance and causes lethality[110]. The clinical signs of shigellosis include mucus and bloody diarrhea, abdominal pain, vomiting, and fever[111]. Shigellosis-related lesions, which are primarily observed in the cecum and colon, may be focal or diffuse and are characterized by edema, hemorrhage, erosions, and ulceration often accompanied by crypt abscesses but less commonly accompanied by pseudomembrane formation. Shigella occasionally causes periodontitis in monkeys[111, 112].

Campylobacter spp.-related diarrhea can be observed later in the quarantine period. In contrast to *S. flexneri*-related diarrhea, *Campylobacter* spp.-related diarrhea is chronic. Clinical signs associated with experimental *C. jejuni* infection include fever and stool changes (soft feces or diarrhea). *Campylobacter* spp.-related lesions and evidence of direct damage in the intestine are not evident although lymphoreticular cells can infiltrate the lamina propria of the ileum, cecum, and colon[113]. The clinical syndrome of bacterial diarrhea can be complicated at all stages by the presence of helminths or viral infections[110]. Histologically, gram-negative S-shaped or spiral shaped bacteria may be observed but identification of Campylobacter spp. is difficult because of the presence of many other organisms in the intestinal flora.

Limited number of studies have reported *Y. enterocolitica* infections in macaque monkeys. In one case of yersiniosis in cynomolgus monkeys, both *Y. pseudotuberculosis* and *Y. enterocolitica* were detected. At necropsy, that enterocolitis was occasionally accompanied by enlargement of the mesenteric lymph nodes and presence of necrotic foci in the liver and spleen[114]. Gastrointestinal lesions comprise superficial erosions or ulcerations with masses of gram-negative coccobacilli and acute inflammatory exudate[115].

Diagnosis. Diagnosis of shigellosis is based on clinical signs and isolation of the organism from deep rectal swabs and fresh stool specimens. Bacterial strains can be identified by isolation culture on selective media or based on biochemical characterization and PCR analysis[234, 235].

Campylobacter spp.-related diarrhea can be definitively diagnosed based on the recovery of the organism in samples obtained from animals with clinical signs[113, 116].

Yersinia spp. infection can be definitively identified by recovering organisms from samples collected from animals with clinical signs and analyzing the histopathologic characteristics, including necrotic foci in the liver and spleen.

Mycobacterium tuberculosis: aerobic gram-positive rods, Mycobacterium avium complex

Overview. Tuberculosis (TB) caused by *M. tuberculosis* and its complex (*Mycobacterium tuberculosis* complex: *M. tuberculosis, M. africanum*, *M. bovis*, *M. canettii*, *M. microti*, and others, together known as MTBC) is an insidious disease[117] that can be latent in some animals and exacerbated by immunosuppressive condition[118]. In macaque monkeys from Asian countries and Gibraltar, PCR testing revealed a 32% positivity rate for MTBC. Generally, the prevalence of MTBC is high among NHPs in countries where the World Health Organization has reported an increased prevalence of human MTBC infection[119]. Facilities conducting transplant experiments perform repeated tests to detect MTBC owing to its high prevalence[118].

Clinical and anatomical pathologic features. In cynomolgus monkeys experimentally infected with *M. tuberculosis*, clinical signs include anorexia, weight loss, cachexia, tachypnea, and dyspnea. X-ray examination can reveal the signs of bronchopneumonia. At necropsy, disseminated miliary nodules (0.5–3 mm in diameter) with a caseous cut surface are observed throughout the lung lobes. Lymphadenopathy with caseation and necrosis along with the involvement of the liver, spleen, and mesenteric tissues have been observed[120]. Animals with the active progression of disease exhibit macroscopic pulmonary changes comprising nod-

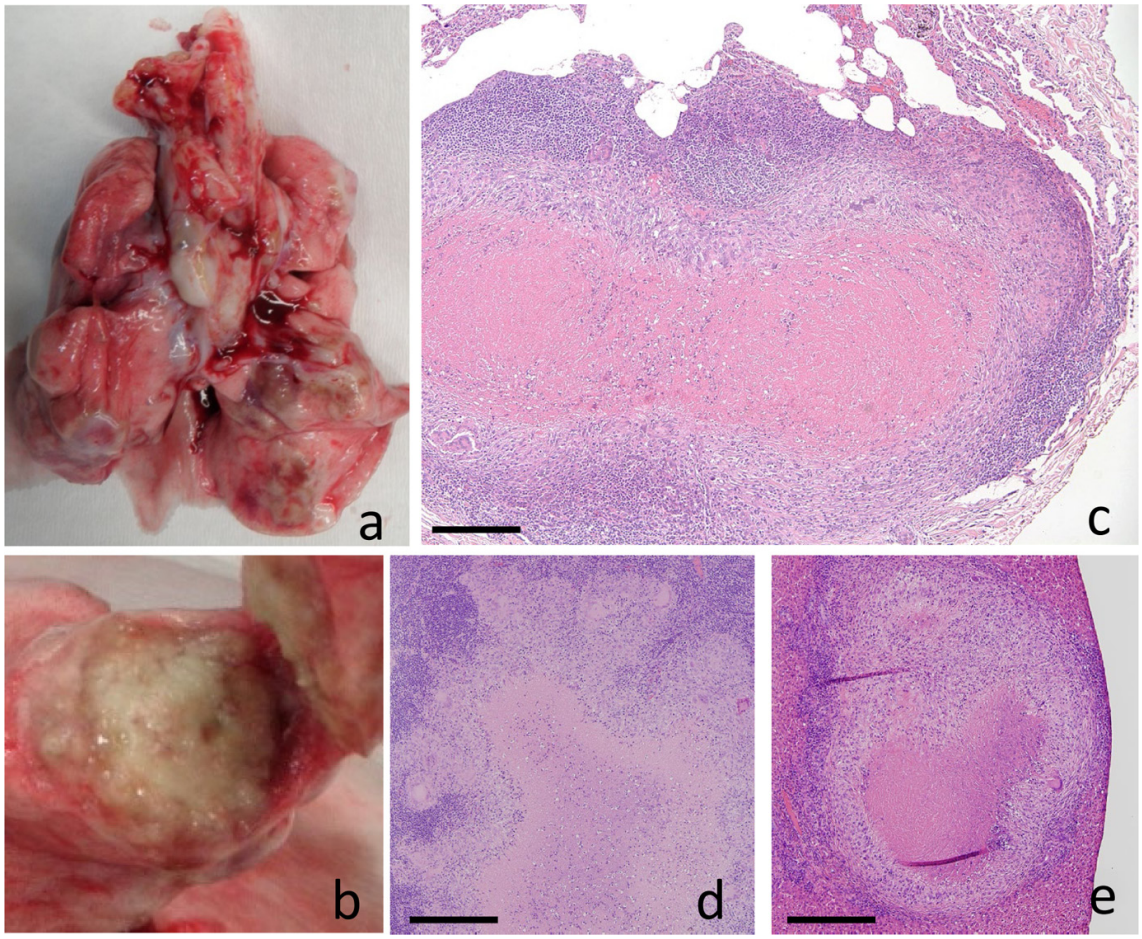

Fig. 5. *Mycobacterium tuberculosis.* (a) Lung of a cynomolgus macaque exhibiting active tuberculosis. Multifocal yellowish-white nodules are apparent. (b) Caseous cut surface of the nodules. Granulomas in the (c) lung, (d) lymph node, and (e) liver. (bars=200 μm). (c, d, e) Hematoxylin and eosin staining. Photographs are kindly provided by Dr. Takuya Doi, LSIM Safety Institute Corporation.

ules of various sizes with caseous cut surfaces (Fig. 5a–5b). The histopathologic characteristics include multifocal caseous and non-necrotizing granulomas consisting of epithelioid macrophages with a surrounding peripheral infiltration of lymphocytes and neutrophils (Fig. 5c). Granulomas were observed in histologic sections of various organs, including the liver and lymph nodes (Fig. 5d and 5e). In granulomas with active necrosis or inflammation, acid-fast bacilli can be observed, which can be confirmed using Ziehl-Neelsen staining[118, 121]. In latently infected monkeys, TB-related lesions may be limited to mineralization of the hilar lymph nodes or small sclerotic granulomas in the lung.

Diagnosis. Macaques with TB are diagnosed based on the characteristic macroscopic and microscopic features, including caseous nodules indicative of granulomatous inflammation with the presence of acid-fast positive bacilli, which can be confirmed using Ziehl-Neelsen staining[118, 121] A positive tuberculin skin test (TST or Mantoux test) can be evidence of a history of TB infection[118]. The detection of TB based on clinical signs is challenging, especially during the early phase of infection or in the case of latent infection. TST yields positive results in NHPs from week 4 to week 8 post-infection[120, 122]. Serological assays based on the detection of MTBC-specific antibodies and the measurement of cytokine (TB-specific IFNγ) levels using ELISA are available for diagnosis. However, these methods are not effective owing to false-positive and false-negative results[236]. PCR methods have been suggested to detect the presence of MTBC DNA in bronchoalveolar lavage, gastric aspirate, and, for humans, sputum samples[119].

Helicobacter pylori and Helicobacter heilmannii

Overview. *Helicobacter pylori* is a curved, spiral-shaped, flagellated gram-negative bacterium commonly detected in rhesus and cynomolgus monkeys[117]. Natural *H. pylori* infections do not cause clinical disease, but microscopic gastric lesions are sometimes obvious and may obscure, or cover, the mild effects of test articles on the stomach of monkeys in preclinical safety or pharmacological studies. Rhesus monkeys are used as a model for human *H. pylori* infection owing to the similarity in pathologic changes[123]. In rhesus monkeys, *H. pylori* infection occurs in about 40% of socially housed NHPs by 12 weeks of age and increased up to 90% by 1 year of age[124]. A high prevalence has also been reported in cynomolgus monkeys; the prevalence likely depends on the source country of origin (up to 93% in

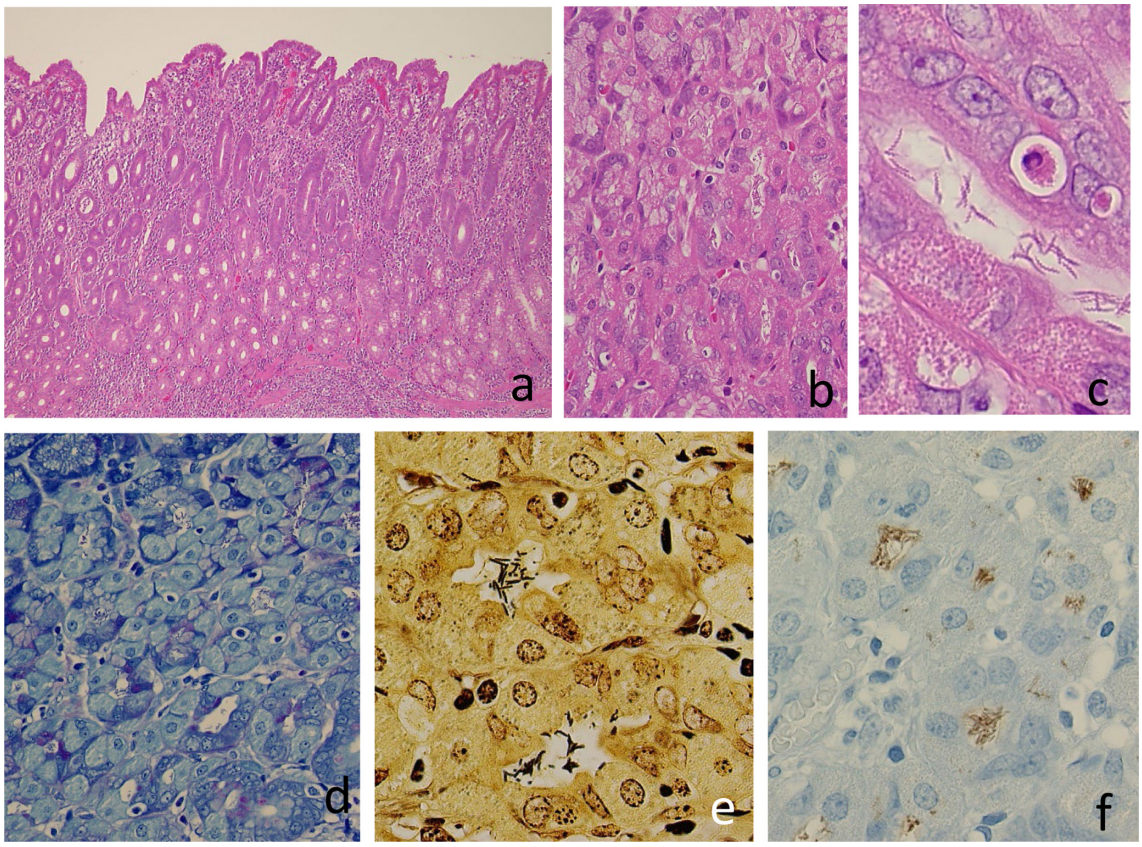

Fig. 6. *Helicobacter pylori.* (a) Chronic inflammation of the pyloric mucosa of a cynomolgus macaque infected with *H. pylori* (hematoxylin and eosin (H&E) staining). (b) Bacilli in the glandular lumen (H&E and Giemsa staining). (c) Higher magnification of bacilli in the lumen. Spiral shapes can be seen. Bacilli in the glandular lumen stained with (d) Giemsa and (e) Warthin-Starry stains, and (f) subjected to immunohistochemistry for *H. pylori*.

adolescent cynomolgus monkeys from the Philippines, 20% in monkeys bred in Japan, and 0% in those from Indonesia[125–127]).

Clinical and anatomical pathologic features. In the stomachs of cynomolgus monkeys infected with *H. pylori*, inflammatory cell infiltration of the lamina propria of the fundus or pylorus[128] was the most characteristic finding. This is consistent with chronic inflammation characterized by lymphoplasmacytic infiltration with frequent loss of parietal cells and reactive proliferation of the mucosal epithelium, resulting in mucosal thickening in severe cases[125, 127] (Fig. 6a). Clusters of bacteria may be observed in the mucosal pits and upper glands with hematoxylin and eosin (H&E) staining alone although they are difficult to detect in regenerative proliferative lesions. Warthin-Starry staining can reveal the presence of curved or spiral-shaped bacteria, i.e. *H. pylori* and *H. heilmannii*-like organisms, on the surfaces and in the lumens of the gastric glands, as well as in the parietal cells[126, 129, 130] (Fig. 6b–6e).

Diagnosis. The presence of curved or spiral-shaped bacteria detected by Warthin-Starry staining and electron microscopy can be evidence. Small comma-shaped bacteria are considered *H. pylori*, while large bacteria with 6 to 8 spirals were considered *H. heilmannii*-like organisms[127]. *H. pylori* can also be diagnosed using IHC (Fig. 6f). Coinfection with several species of *Helicobacter* bacteria (the so-called *H. heilmannii*-like organisms) may yield positive results for *H. pylori* in IHC analysis[129–132]. For the precise identification of *Helicobacter* species, PCR and other molecular techniques can be used.

Moraxella catarrhalis (M. macacae in macaques)

Overview. *Moraxella catarrhalis* is a gram-negative aerobic diplococcus formerly known as *Branhamella catarrhalis*[28, 117]. *M. catarrhalis* is a common inhabitant of the nasopharynx in humans and NHPs, and is an opportunistic pathogen associated with otitis media in infants and children. In macaques, a similar *Moraxella* sp., possibly *M. macacae*, causes epistaxis (nasal hemorrhage, known as bloody nose syndrome)[133–135]. Moraxella macacae can be transmitted in closed facilities. Epistaxis caused by M. macacae can be treated with antibiotics[134].

Clinical and anatomical pathologic features. M. macacae infection in monkeys is associated with clinical signs including sneezing, epistaxis (clear to serosanguineous, mucohemorrhagic, or bloody nasal discharge), and periocular swelling[133, 135]. Examination of nasal swab smears revealed

WBCs and red blood cells and large diplococcal organisms[135].

Diagnosis. In addition to nasal swab cultures, real-time PCR can be used to identify the infecting species[134].

Rhodococcus equi

Overview. *Rhodococcus equi* is a gram-positive coccoid or bacillary facultative anaerobe that is found in large numbers on dry soil surfaces. *R. equi* is an important pathogen in foals and is also pathogenic in immunocompromised animals and humans[136–138].

Clinical and anatomical pathologic features. Infection usually manifests as pneumonia but can also as wound infection[136, 137, 139]. In the lungs of infected foals, gross lesions are characterized by multifocal to coalescing nodules ranging from a few millimeters to 10 cm in diameter. The Histopathologic features include multifocal pyogranulomatous inflammation characterized by the infiltration of neutrophils and macrophages, occupying the alveolar spaces and bronchioles. Macrophages occasionally contain myriad coccobacillary basophilic gram-positive bacteria. Lung lesions may also include multinucleated giant cells, multifocal thrombosis, interstitial and alveolar edema, and tissue necrosis[140]. In humans treated with immunosuppressants for organ transplantation, *R. equi*-related lung lesions include inflammation of the lung parenchyma, with foamy histiocytic infiltration. In these foamy macrophages, intracellular bacteria that are positive for periodic acid Schiff or Gram staining can be observed[141]. Pyogranulomatous inflammation can also be observed in the liver, kidney, spleen, and nervous tissue[139]. Previously, one case of *R. equi* isolation from monkeys has been reported[142].

Diagnosis. In addition to the characteristic histopathologic findings of pyogranulomatous pneumonia with intracytoplasmic bacteria in the macrophages infiltrating the lesions, positive staining for *R. equi* in intralesional macrophages is a potential tool for definitive diagnosis[140]. However, obtaining an appropriate antibody for IHC analysis is sometimes difficult. Various PCR assays have been developed[143–145] and can be used to detect *R. equi* in lesions.

Enteropathogenic Escherichia coli (EPEC)

Overview. Diarrheagenic *E. coli* strains are the most common etiological agents for diarrhea in mammals. *E. coli* strains are categorized as follows based on their specific virulence factors and phenotypic traits: enteropathogenic *E. coli* (EPEC), causes diarrhea in children and animals; enterotoxigenic *E. coli* (ETEC), causes traveler's diarrhea and porcine and bovine diarrhea; enterohemorrhagic *E. coli* (EHEC), causes hemorrhagic colitis and hemolytic uremic syndrome and includes verotoxin-producing/Shiga toxin-producing *E. coli* (VTEC/STEC); enteroinvasive *E. coli* (EIEC), causes watery diarrhea and dysentery; enteroaggregative *E. coli* (EAEC), causes persistent diarrhea in humans; diffusely adherent E. coli (DAEC), a subclass of EAEC that causes diarrhea in children[146, 147]. EPEC strains are important agents that can cause chronic diarrhea and are closely related to morbidity in infants and children less than 2 years in the developing world[147, 148]. *E. coli* is also one of the most common bacterial pathogens causing diarrhea in HIV-infected immunocompromised humans in whom both ETEC and EPEC are important opportunistic diarrheagenic pathogens[149]. EPEC strains have also been identified in SIV-infected rhesus[150] and cynomolgus monkeys used in preclinical safety studies[151].

Clinical and anatomical pathologic features. EPEC-induced diarrhea associated histologic changes are observed in the large or small intestine or both. To identify the characteristic histologic lesions, several sections must be evaluated[148, 150, 151]. In the small intestine of infected cynomolgus monkeys, the villi exhibit clubbing and shortening with vacuolation in the subepithelial lamina propria[150, 151]. In the colons of infected marmosets and rhesus monkeys, bacteria can be detected on the surface of the affected epithelium along with decreased crypt size or onset of reactive crypt hyperplasia, epithelial vacuolation, and neutrophilic inflammatory infiltration[148, 150]. EPEC may be observed in lesions on the surfaces of villi in toluidine blue-stained semithin sections subjected to electron microscopy examination. The tight attachment of bacteria to the cell surface causes epithelial cell injury, which is characterized by degeneration and loss of cilia in the brush border[151].

Diagnosis. As several *E. coli* can cause diarrhea, fecal culture and serotype identification are the traditional methods to obtain a definitive diagnosis. Commercial PCR diagnostic kits are also available.

Parasitic Infections

Pulmonary acariasis (Pneumonyssus simicola)

Overview. Pulmonary acariasis (*Pneumonyssus simicola*) is parasitic infections observed in NHPs, including cynomolgus monkeys[152]. The prevalence of pulmonary acariasis in a colony increases with the age of the monkeys[153, 154]. Recently, mite bodies were not so commonly observed in the lung sections in toxicological studies. In combination with other evidence of acariasis, including granulomatous inflammation with or without bronchiolar dilatation, dark pigments phagocytosed by macrophages around the lesions are indicators of acariasis even if the mite is not observed in the lung section[128, 152].

Clinical and anatomical pathologic features. When infested with a small number of pulmonary achalasia, animals do not show clinical signs. In heavily infested monkeys, scattered small (1–5 mm) yellow or pale green nodules can be observed grossly in the lung[155, 156]. The definitive histopathologic finding is the presence of mite bodies in a section. Mites are surrounded by a thin wall of connective tissue, forming cysts or bullae. Around the cysts, granulomatous tissue with inflammatory cells, including pigmented or unpigmented macrophages, lymphocytes, neutrophils, and eosinophils, is observed. Bronchitis or peribronchiolitis, which is sometimes accompanied by bronchiolar dilatation, is evident[128, 154–156]. Ivermectin treatment effectively

eliminates mites but cyst-like structures with granulomatous inflammation may persist[153, 154].

Diagnosis: Infested monkeys usually do not exhibit clinical signs[154, 155]. Pneumothorax due to cyst or bulla rupture can occur in severely infested macaques. Clinical diagnosis of acariasis using X-ray is difficult in monkeys[153, 155]. Tracheobronchial lavage examination and computed tomography are potential tools for detecting the presence of *Pneumonyssus* spp. or multiple bullae in the lung[153].

Helminths, including nematodes, cestodes, and trematodes

Overview. Helminths include nematodes and platyhelminths (cestodes and trematodes). Wild-caught macaque monkeys can be infested with a large number of a wide variety of helminths[155]. Of these, *Strongyloides fuelleborni*, *Trichuris trichiura*, and *Oesophagostomum* spp. are intestinal nematodes that have been detected with high incidence in macaque monkeys[36, 152, 157–161] and are recommended to be monitored[36, 160]. Although cestodes and trematodes have also been reported to infest macaque monkeys[155], nematodes are frequently reported and are the focus of this section. In NHPs maintained in internal housing, the incidence of nematode infestations is low. Anthelmintics, including ivermectin and moxidectin, effectively decrease the egg counts per gram of feces in the laboratory[160–162]. However, the complete elimination of nematodes from the intestine is difficult, requiring sequential or combined anthelmintics[138, 160, 161].

Clinical and anatomical pathologic features. Severe infestations of nematodes that cause clinical signs in macaque monkeys are rare[36]. Pathologists may encounter helminths in specimens during histopathologic examination in studies and investigations (Fig. 7a and 7b).

Oesophagostomum spp. form small (approximately 8 mm in diameter) dark nodules in the large intestine that are can be observed via gross examination[152]. Lesions can be distributed from the submucosa to the serosal surface and are sometimes detected in the mesentery or intestinal wall[152, 155]. The histopathologic characteristics of the nodules included multiple cross-sections of the nematode. The inflammatory cells are predominantly neutrophils and macrophages with occasional sometimes foreign-body giant cells. The nodules may be encapsulated by fibrous tissue, and forming granulomatous lesions[152, 155]. Granulomatous lesions with mineralization in the center can be observed. These are old lesions that suggest evidence of previous infestation of helminths[155].

Strongyloides spp. are usually observed in the mucosa of the small intestine and occasionally in the large intestine with inflammation in the lamina propria[152, 155]. In the case of severe autoinfection, the lymphatics of the intestine and lung may be obstructed by the larvae[155].

Trichuris trichiura on the mucosal surface of the large intestine does not cause clinical signs. Histologically, cross- (or longitudinal) sections of *T. trichiura* can be observed on the surface of the mucosa or embedded in the mucosal folds without any reaction or inflammation.

Diagnosis. To determine whether animals were infested with intestinal helminths, microscopic examination of fecal samples is performed to detect parasite eggs[36, 157, 160, 162]. Pooled fecal samples can also be used for the diagnosis[36]. The number of eggs per gram (EPG) of feces indicates the severity of infestation[160, 162, 163]. Molecular techniques have also been used to identify infectious species[164, 165].

Protozoa: general overview

In macaque monkeys, protozoa are more common than nematodes. Infections with some species can be opportunistically activated in immunosuppressed macaques[138, 160]. Protozoal parasites that may be encountered during the histopathologic examination of laboratory macaque monkeys include flagellates (*Giardia* spp., *Trypanosoma* spp., and *Trichomonas* spp.), amoebae (*Entamoeba* spp.), coccidia (*Cryptosporidium* spp., *Plasmodium* spp., *Hepatocystis* spp., *Toxoplasma gondii*, *Sarcocystis* spp., and *Babesia* spp.), and ciliates (*Balantidium* spp.)[155]. In macaques housed in laboratory settings, infections may have occurred and carried in from outdoor breeding colonies.

Gastrointestinal protozoa

Overview. Protozoa infecting the gastrointestinal tract of macaque monkeys include *Entamoeba* spp., *Giardia* spp., *Balantidium* spp., and *Cryptosporidium* spp. Of these, *Balantidium* spp. are frequently observed in the lumen and on the surface of mucosa of the large intestine during routine histopathologic examinations. The prevalence of *Entamoeba* spp. in macaques in East Asia is high although their virulence is low. Meanwhile, the prevalence of the virulent *Entamoeba histolytica* is low[166–168]. *Cryptosporidium* and *Giardia* spp. are uncommon in normal healthy macaques[155, 160, 169]. However, gastrointestinal infection with *Cryptosporidium* sp. has been reported in juvenile and immunocompromised macaques[169, 170].

Clinical and anatomical pathologic features.

Entamoeba: The pathogenicity of *Entamoeba* depends on the strain and the host species, nutritional status, environmental factors, and bacterial flora[171]. *Entamoeba* spp. infection becomes pathogenic when protozoa invade the mucosa, leading to amebic dysentery[172, 173]. In clinically apparent cases, anorexia, vomiting, severe diarrhea, and ulcerative hemorrhagic colitis with trophozoites in ulcerated lesions, as well as liver abscesses, are reported under immunosuppressive conditions[155, 166, 171]. Trophozoites may not be visible in H&E-stained sections but are stained bright red in periodic acid Schiff (PAS)-stained sections[155].

Cryptosporidium: Cryptosporidium spp. are detected on the epithelial surfaces of gastric pits or intestinal crypts[169, 170] and can be activated under immunosuppressive conditions. In the stomachs of monkeys with the proliferation of *Cryptosporidium* spp., small (1–4 μm) and round protozoa (stained blue with Giemsa staining[170]) have been detected on the surface of the affected mucosa. Additionally, associated reactive mucosal hyperplasia with increased mu-

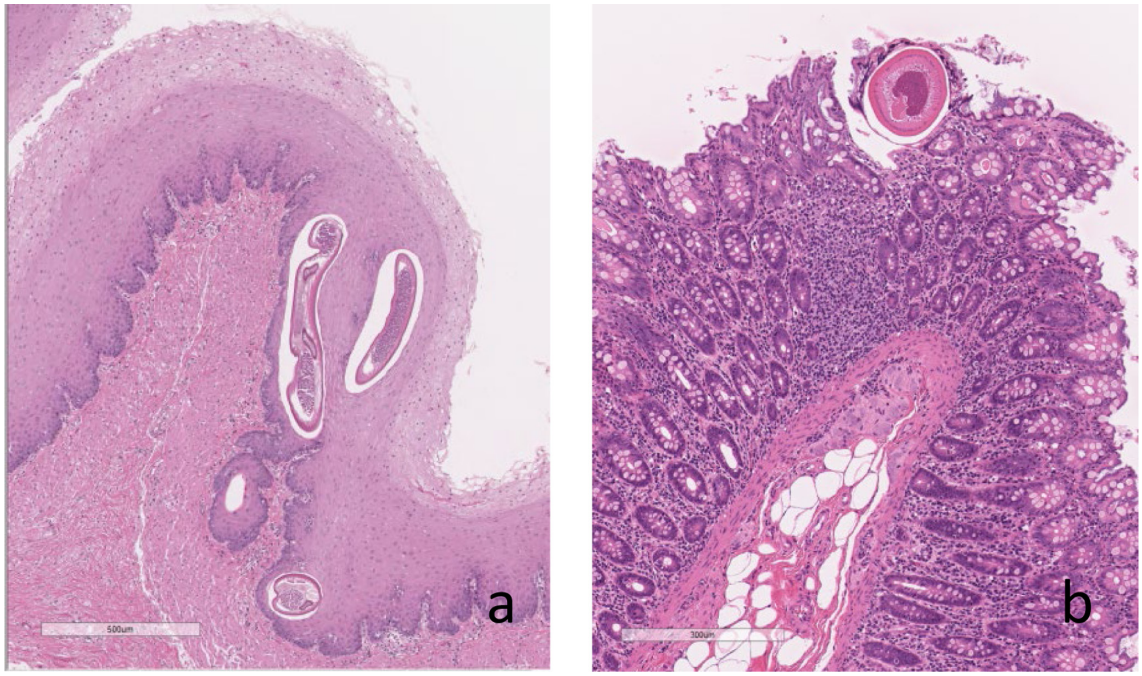

Fig. 7. *Helminths (Nematode).* (a) A cross and longitudinal section of nematode in the esophageal mucosa. (b) A cross-section of nematode in the cecal mucosa. Hematoxylin and eosin (H&E) staining.

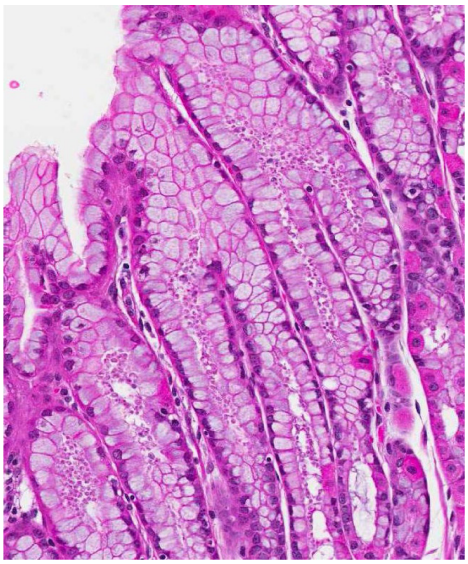

Fig. 8. *Cryptosporidium* sp. Numerous cocci were found in the fundic pits of the stomach of cynomolgus monkeys with hypertrophy/hyperplasia of superficial mucus cells. Hematoxylin and eosin (H&E) staining.

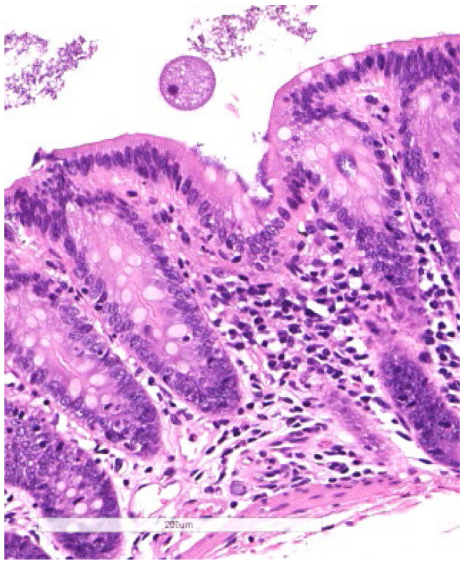

Fig. 9. *Balantidium coli*. *Balantidium coli* on the mucosa of the cecum. Hematoxylin and eosin (H&E) staining.

cus secretion has also been reported (Fig. 8). *Cryptosporidium* spp. infection in the small intestine of juvenile macaques (rhesus and cynomolgus) can result in life-threatening diarrhea with histological lesions, including epithelial vacuolation, necrosis, and villus atrophy.

Balantidium coli: In most cases, *B. coli* infection in macaques is asymptomatic[174]. *B. coli*, which can be identified in the intestinal lumen and mucosal folds (Fig. 9), can become pathogenic in severely immunocompromised animals, causing severe diarrhea with the multiplication of protozoa and their invasion into the mucosa, and mucosal erosions or ulcerations[175].

Giardia spp.: Although the prevalence of intestinal

Giardia spp. in rhesus macaques is high[176], limited information is available on its pathologic features. This may be because similar to humans, infection by a small number of parasites does not induce clinical signs in monkeys. However, in humans, symptomatic diarrhea with gas formation has been reported[177].

Diagnosis. In addition to the identification of characteristic protozoa in intestinal sections and fecal examination, which is one of the most common tools to screen for intestinal protozoal infestation[166], other laboratory methods, including biochemical analysis of isoenzymes of *Entamoeba* spp., ELISA, or PCR evaluation should be employed to identify the species and virulence of infectious protozoa to detect virulent *E. histolytica*[168, 178–180].

Other protozoa: Trypanosoma spp. (flagellates)

Overview. The prevalence of *Trypanosoma cruzi* in Central and South America is higher than that in other parts of the world because of the dissemination of the insect vector kissing-bug[181, 182]. The prevalence of *T. cruzi* in NHPs ranges from 2% to 10% in facilities in the southern USA[181].

Clinical and anatomical pathologic features. Infected animals may be asymptomatic. *Trypanosoma* spp. induce histopathologic lesions, including lymphocytic myocarditis and gastritis, with increased levels of proinflammatory cytokines in the blood. These characteristics are similar to those of Chagas disease in humans. The myocardium, muscle fibers, or esophagus may also be affected and may accompanied by arrythmia or esophageal dilatation[181–186].

Diagnosis. In addition to clinical and histopathologic characteristics, examination of Giemsa-stained thin or thick blood smears can be used to detect the active phase of *Trypanosoma* spp. PCR can also be used to detect parasite-specific DNA in the blood or tissues[187].

Other protozoa: Toxoplasma gondii (coccidia)

Overview. Although *Toxoplasma gondii* is widely distributed in wild and domestic animals[188, 189], its seroprevalence in breeding colonies of rhesus and cynomolgus monkeys is low[190, 191].

Clinical and anatomical pathologic features. Toxoplasmosis in macaques is less prevalent and there is little information on its clinical manifestations, perhaps because it is asymptomatic as in humans. Some studies have reported pathologic lesions associated with natural *T. gondii* infection in macaques. In humans and other animals, *Toxoplasma*-induced lesions include cellular necrosis and inflammation associated with invasion by tachyzoites, as well as the formation of bradyzoite cysts in various organs and tissues, including the blood vessels, lung, liver, and brain[188, 189].

Diagnosis. Serological examination can be used to identify characteristic cysts and tachyzoites in the affected tissues[191, 192]. PCR can be performed to detect *Toxoplasma* DNA in the blood, tissues, and cerebrospinal fluid[193–195].

Other protozoa: Sarcocystis spp. (coccidia)

Overview. The prevalence of *Sarcocystis* infection is high in monkeys caught from the wild and low in captive-born monkeys[196].

Clinical and anatomical pathologic features. In one reported case of a rhesus monkey with clinically apparent sarcocystosis, the animal became moribund with anorexia and systemic edema. In this animal, coalescing myocardial edema and necrosis of the myocardium with infiltration of macrophages and lymphocytes, as well as *Sarcocystis* spp. infestation in endothelial cells, were observed[197]. Cysts of *Sarcocystis* are incidentally observed in skeletal muscle in the absence of any associated degenerative or inflammatory lesions (Fig. 10).

Diagnosis. In addition to histopathologic analysis, PCR can be used[197].

Other protozoa: Babesia spp., including Entopolypoides macaci (coccidia)

Overview. *Babesia*, which is an intra-erythrocytic parasite transmitted by ticks in rhesus and cynomolgus monkeys, can cause opportunistic infections in immunocompromised monkeys[198]. Although the tick, vector of *Babesia* spp., is not observed in the laboratory setting, latent infection can be caused by exposure to ticks outside breeding colonies. Babesiosis is recognized worldwide as a zoonotic disease and has been sporadically reported in mammals, including humans and NHPs[199, 200].

Clinical and anatomical pathologic features. Babesia infections are generally latent and not clinically apparent. Recurrence can occur when animals are immunocompromised or have undergone splenectomy[198, 201, 202]. Anemia, parasites in red blood cells, and splenomegaly are observed in animals with clinically apparent babesiosis[198, 202]. In contrast to the malaria-causing *Plasmodium*, this organism does not produce pigments (hemozoin) in the affected red blood cells[202].

Diagnosis. The presence of parasites without hemozoin pigments is confirmed in red blood cells in Giemsa-stained thin blood smears. PCR detection of *Babesia*-specific DNA is used to differentiate *Babesia* from *Plasmodium* infection.

Other protozoa: Plasmodium spp. (coccidia)

Overview. *Plasmodium* spp. cause malaria in various animals. Plasmodium cynomolgi, P. semiovale, and P. fieldi have been identified in macaques. Infections are usually latent and take the form of hepatocellular hypnozoites that can lead to relapse. Although infection rates are high in macaque monkeys[203, 204], clinical manifestations of malaria are rare in healthy macaques. Relapse can be induced in laboratory macaques under immune suppressive conditions due to viral infection, treatment with immunosuppressive chemicals or biologics, splenectomy, or increased turnover of red blood cells[59, 203–205].

Clinical and anatomical pathologic features. In the case of clinically evident malaria that we experienced in a 13-week toxicity study, severe anemia with a marked decrease

in bodyweight (by −15% compared with controls), spontaneous activity, erythrocyte count (by 0.89 × 10^6/μL), and hematocrit values (by 9.0%) was observed. In this animal, cyclic decreases in erythroid parameters were observed. The presence of hemoparasites morphologically consistent with Plasmodium spp. in red blood cells was confirmed by the examination of thin blood smears (Fig. 11a–11c). At necropsy, massive enlargement and dark discoloration of the spleen (Fig. 11d) and liver were observed. Histopathologic evaluation revealed depositions of black-brown hemozoin pigment in macrophages in the spleen and Kupffer cells in the liver (Fig. 11e and 11f) along with Berlin-blue-positive hemosiderin. Hemozoin pigments are birefringent under polarized light and are negative for iron staining (Berlin blue)[207]. These changes are accompanied by lymphoid depletion in the spleen[204].

Diagnosis. To detect low-level parasitemia, Giemsa-stained thick blood smears have been reported to be better than tin blood smear examinations in NHPs. Other diagnostic methods, including PCR, can be used although their efficacy has not been thoroughly established in NHPs[203, 204, 206, 207].

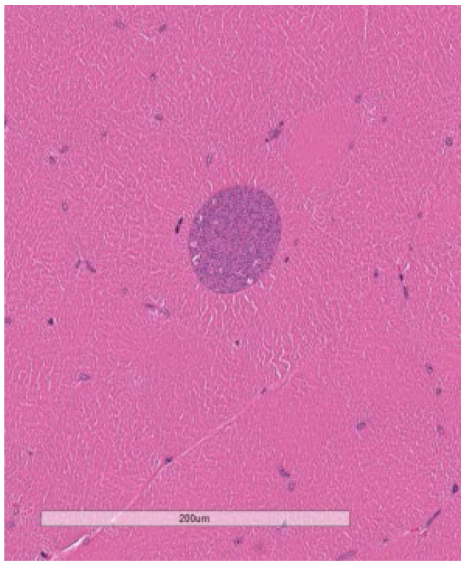

Fig. 10. *Sarcocystis spp.* Sarcocystis in the skeletal muscle without any associated degenerative or inflammatory changes.

Fungi

Many fungal infections are regarded as opportunistic and may not have clinical or histopathologic manifestations unless the immune function of the host is compromised by the disease or experimental treatment. In addition to *Candida albicans* and *Pneumocystis* spp. described in this section, *Histoplasma capsulatum*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* have been identified as rare opportunistic agents in monkeys[22, 208].

Candida albicans

Overview. Candidiasis is the most common opportunistic yeast infection worldwide. Infection can be superficial or deep/systemic and can affect the skin, mucosa of the mouth and alimentary tract, genital or urinary tract, or respiratory system[208–211]. In immunocompromised humans, endocarditis, meningitis, and *Candida* sepsis have been reported as forms of deep systemic candidiasis[211].

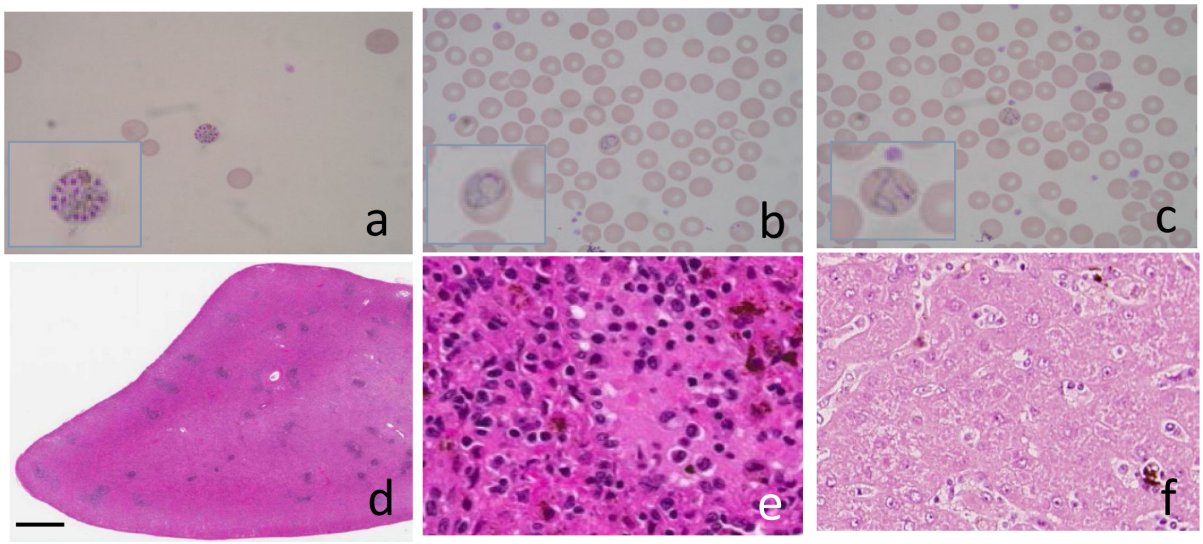

Fig. 11. *Plasmodium* spp. In thin blood smears, various stages of parasites in red blood cells were observed: (a) schizont, (b) trophozoite, and (c) ring form. The spleen was enlarged (d) with the expansion of the red pulp and lymphoid depletion (bar=3 mm) and (e) several brown-black pigments in the macrophages in the red pulp. (f) Pigments were observed in the livers of the Kupffer cells. (a, b, and c) Wright's staining and (d, e, and f) hematoxylin and eosin (H&E) staining. Photographs are from a previous study from the author of this manuscript (E. Ohta).

Clinical and anatomical pathologic features. In the upper alimentary tracts of macaques affected with symptomatic candidiasis, clinical signs related to ulceration of mucosa thought to be observed. The ulcers can be observed as macroscopic whitish streaks or plaques on the mucosa of the tongue, oral cavity, and esophagus. A yellowish pseudomembrane is also observed in the intestine. Histologically, clusters of fungus (3–5-μm pseudohyphae and blastospores that are PAS-positive and Gomori methenamine silver [GMS]-positive) can be observed on the mucosal epithelium. If the fungus invades the tissue, ulceration with inflammatory cell infiltration can occur[208].

Diagnosis. In addition to the characteristic gross and histological findings in the presence of PAS-positive or GMS-positive yeasts in the affected organs, fungal culture, PCR, and serum antibodies against *Candida* or its glycoproteins can be used to diagnose candidiasis[208, 212, 213].

Pneumocystis spp.

Overview. *Pneumocystis* is a yeast-like fungus that is predominantly observed in the lung tissues. Strains of *Pneumocystis* spp. have a high host specificity[214, 215]. Only the genomes of human *P. jirovecii*, rat *P. carinii*, and mouse *P. murina* have been identified[215, 216]. Additionally, the genome of *P. macacae* in macaque monkeys[215, 217] has been proposed. Similar to their pathologic effects in humans, *Pneumocystis* spp. are opportunistic fungal organisms that cause *Pneumocystis* pneumonia in macaque monkeys. Clinical manifestations can be observed in immunologically compromised animals[22, 218].

Clinical and anatomical pathologic features. In lungs with symptomatic pneumonia, the alveolar septa are thickened with infiltration of lymphocytes and neutrophils. In alveolar spaces filled with eosinophilic foamy material, the presence of *Pneumocystis* spp. can be confirmed using GMS staining and IHC for *Pneumocystis* antigen[219–221]. A nodular type of *Pneumocystis* pneumonia has been reported in monkeys with AIDS. This pneumonia is characterized by numerous grossly apparent large nodules. Histological analysis revealed foamy material, central necrosis, and necrotizing vasculitis in the nodules[220].

Diagnosis. Infection can be diagnosed based on the typical histopathologic features, as well as based on IHC detection of *Pneumocystis*-specific antigens and PCR detection of DNA[220, 221].

Discussion and Conclusion

This review presents a summary of the clinical and anatomical manifestations, pathologic features, and diagnosis of representative infectious diseases, including opportunistic infections, in NHPs, especially macaque monkeys. The macaque monkeys that were the focus of this review were predominantly the cynomolgus macaques (*Macaca fascicularis*) and rhesus macaques (*Macaca mulatta*), which are the most common NHP models in biomedical research, including preclinical toxicity studies[222]. Several efforts have been undertaken to screen out representative infectious agents via periodic examination during breeding and quarantine at export or import. However, latent or incidental background infections can be observed. In particular, the potential recurrence of latent infection should be considered under experimental conditions in which the immune system can be compromised. Recurrence of latent infection can be severe in immunocompromised animals with systemic or local manifestations and can mask or complicate the real pathologic features induced by the test agent. In such case the interpretation of the study results is complicated and challenging. For example, changes in erythroid parameters in a toxicity study described in the *Plasmodium* spp. section can be misunderstood as being directly caused by the test article because the article was not an intended immune modulator. In this case, a thin blood smear examination was decided to conduct because careful monitoring of hematological parameters revealed cyclic changes in red blood cells. The smear examination revealed parasites in the red blood cells and thus the changes in the animal were diagnosed to be related to the relapse of latent malaria in the animal and not directly related to the test article. In particular, when the intended pharmacological effects of test articles are not related to the immune system, the changes related to incidental infection or relapse of latent spontaneous infection are misinterpreted as direct effects of test articles.

In addition to the effects of test article on the immune system, background or incidental infection with immunosuppressive viruses (e.g. measles transmitted from humans to monkeys), malnutrition, and stress due to experimental procedures, changes in animal husbandry conditions (including changes in the animal room), or shipping can cause recurrence. To accurately interpret changes associated with the test article or experiment, toxicologic pathologists must understand the prevalence of these infections, their basic pathologic features, and the current diagnostic methods. When unexpected findings are observed and infectious diseases are suspected in studies, pathologists should not only collect information on the origin of the monkeys, the quarantine methods, and the results of routine testing for infectious agents but also on the time-course changes in clinical findings, the health status of the handlers, and any hints of accidents or procedures that could have stressed the monkeys. If an intervention has been performed on an animal diagnosed with an infection, pathologists should also be aware of the type of intervention (e.g. allowing dosing holidays for animals or drugs that were used). This information will help pathologists to distinguish both infectious disease-related and test-item-related lesions and precisely interpret the results of experiments or studies. If the pathogen is identified, the pathologist and onsite veterinarian should check whether the laws of each country require notification of the infectious disease and take appropriate action.

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References

1. Friedman H, Ator N, Haigwood N, Newsome W, Allan JS, Golos TG, Kordower JH, Shade RE, Goldberg ME, Bailey MR, and Bianchi P. The critical role of nonhuman primates in medical research. Pathog Immun. **2**: 352–365. 2017. [Medline] [CrossRef]
2. Hobson W. Safety assessment studies in nonhuman primates. Int J Toxicol. **19**: 141–147. 2000. [CrossRef]
3. Grimm D. U.S. labs using a record number of monkeys. Science. **362**: 630. 2018. [Medline] [CrossRef]
4. Cyranoski D. Monkey kingdom. Nature. **532**: 300–302. 2016. [Medline] [CrossRef]
5. Zhang XL, Pang W, Hu XT, Li JL, Yao YG, and Zheng YT. Experimental primates and non-human primate (NHP) models of human diseases in China: current status and progress. Zool Res. **35**: 447–464. 2014. [Medline]
6. Bailey J. Does the stress inherent to laboratory life and experimentation on animals adversely affect research data? Altern Lab Anim. **45**: 299–301. 2017. [Medline] [CrossRef]
7. Nehete PN, Shelton KA, Nehete BP, Chitta S, Williams LE, Schapiro SJ, and Abee CR. Effects of transportation, relocation, and acclimation on phenotypes and functional characteristics of peripheral blood lymphocytes in rhesus monkeys (*Macaca mulatta*). PLoS One. **12**: e0188694. 2017. [Medline] [CrossRef]
8. Henrickson RV, Maul DH, Lerche NW, Osborn KG, Lowenstine LJ, Prahalada S, Sever JL, Madden DL, and Gardner MB. Clinical features of simian acquired immunodeficiency syndrome (SAIDS) in rhesus monkeys. Lab Anim Sci. **34**: 140–145. 1984. [Medline]
9. de Vries RD, McQuaid S, van Amerongen G, Yüksel S, Verburgh RJ, Osterhaus AD, Duprex WP, and de Swart RL. Measles immune suppression: lessons from the macaque model. PLoS Pathog. **8**: e1002885. 2012. [Medline] [CrossRef]
10. Kahnt K, Mätz-Rensing K, Hofmann P, Stahl-Hennig C, and Kaup FJ. SIV-associated lymphomas in rhesus monkeys (*Macaca mulatta*) in comparison with HIV-associated lymphomas. Vet Pathol. **39**: 42–55. 2002. [Medline] [CrossRef]
11. Lerche NW. Simian retroviruses: infection and disease—implications for immunotoxicology research in primates. J Immunotoxicol. **7**: 93–101. 2010. [Medline] [CrossRef]
12. Montiel NA. An updated review of simian betaretrovirus (SRV) in macaque hosts. J Med Primatol. **39**: 303–314. 2010. [Medline] [CrossRef]
13. Council NR. The psychological well-being of nonhuman primates. 1998, from National Academy Press: https://nap.nationalacademies.org/catalog/4909/the-psychological-well-being-of-nonhuman-primates
14. Mansfield KG, and Kemnitz JW. Introduction: challenges in microbial quality control for nonhuman primate. ILAR J. **49**: 133–136. 2008. [Medline] [CrossRef]
15. Bailey C, and Mansfield K. Emerging and reemerging infectious diseases of nonhuman primates in the laboratory setting. Vet Pathol. **47**: 462–481. 2010. [Medline] [CrossRef]
16. Wachtman L, and Mansfield K. Viral diseases of nonhuman primates. In: Nonhuman Primates in Biomedical Research. 2nd ed. Volume 2: Disease. CR Abee, K Mansfield, SD Tardif, and T Morris (eds). Academic Press, London.1–104. 2012.
17. Fujiomto K, Takano J, Narita T, Hanari K, Shimozawa N, Sankai T, Yosida T, Terao K, Kurata T, and Yasutomi Y. Simian betaretrovirus infection in a colony of cynomolgus monkeys (*Macaca fascicularis*). Comp Med. **60**: 51–53. 2010. [Medline]
18. Hara M, Kikuchi T, Sata T, Nakajima N, Ami Y, Sato Y, Tanaka K, Narita T, Ono F, Akari H, Terao K, and Mukai R. Detection of SRV/D shedding in body fluids of cynomolgus macaques and comparison of partial gp70 sequences in SRV/D-T isolates. Virus Genes. **35**: 281–288. 2007. [Medline] [CrossRef]
19. Lerche NW, Marx PA, Osborn KG, Maul DH, Lowenstine LJ, Bleviss ML, Moody P, Henrickson RV, and Gardner MB. Natural history of endemic type D retrovirus infection and acquired immune deficiency syndrome in group-housed rhesus monkeys. J Natl Cancer Inst. **79**: 847–854. 1987. [Medline]
20. Guzman RE, Kerlin RL, and Zimmerman TE. Histologic lesions in cynomolgus monkeys (*Macaca fascicularis*) naturally infected with simian retrovirus type D: comparison of seropositive, virus-positive, and uninfected animals. Toxicol Pathol. **27**: 672–677. 1999. [Medline] [CrossRef]
21. Yee JL, Grant R, Van Rompay KK, Kuller L, Carpenter A, Watanabe R, Huebner R, Agricola B, Smedley J, and Roberts JA. Emerging diagnostic challenges and characteristics of simian betaretrovirus infections in captive macaque colonies. J Med Primatol. **46**: 149–153. 2017. [Medline] [CrossRef]
22. Wachtman LM, and Mansfield KG. Opportunistic infections in immunologically compromised nonhuman primates. ILAR J. **49**: 191–208. 2008. [Medline] [CrossRef]
23. Yanai T, Teranishi M, Takaoka M, Manabe S, Sakai H, Masegi T, and Yoshikawa Y. Retroperitoneal fibromatosis in a cynomolgus monkey (*Macaca fascicularis*). J Toxicol Pathol. **16**: 117–121. 2003. [CrossRef]
24. Sasseville VG, and Diters RW. Impact of infections and normal flora in nonhuman primates on drug development. ILAR J. **49**: 179–190. 2008. [Medline] [CrossRef]
25. Lerche NW, Yee JL, and Jennings MB. Establishing specific retrovirus-free breeding colonies of macaques: an approach to primary screening and surveillance. Lab Anim Sci. **44**: 217–221. 1994. [Medline]
26. Yee JL, Vanderford TH, Didier ES, Gray S, Lewis A, Roberts J, Taylor K, and Bohm RP. Specific pathogen free ma-

caque colonies: a review of principles and recent advances for viral testing and colony management. J Med Primatol. **45**: 55–78. 2016. [Medline] [CrossRef]
27. Centers for Disease Control and Prevention (CDC). https://www.cdc.gov/measles/hcp/index.html (Accessed on May 9, 2022)
28. Saravanan C, Sasseville VG, and Mansfield KG. Chapter 10: in nonhuman primate diseases of relevance in drug development and their Impact on the Interpretation of Study Findings. In: J Bluemel, S Korte, E Schenck, and GF Weinbauer (eds). The Nonhuman Primate in Nonclinical Drug Development and Safety Assessment: Academic Press San Diego. 187–213. 2015.
29. Hall WC, Kovatch RM, Herman PH, and Fox JG. Pathology of measles in rhesus monkeys. Vet Pathol. **8**: 307–319. 1971. [Medline] [CrossRef]
30. World Health Organization. Manual for the Laboratory Diagnosis of Measles and Rubella Virus Infection. 2nd ed. WHO/ IVB/07.1. Geneva. 2007.
31. Oliveira SA, Siqueira MM, Camacho LA, Castro-Silva R, Bruno BF, and Cohen BJ. Use of RT-PCR on oral fluid samples to assist the identification of measles cases during an outbreak. Epidemiol Infect. **130**: 101–106. 2003. [Medline] [CrossRef]
32. Willy ME, Woodward RA, Thornton VB, Wolff AV, Flynn BM, Heath JL, Villamarzo YS, Smith S, Bellini WJ, and Rota PA. Management of a measles outbreak among Old World nonhuman primates. Lab Anim Sci. **49**: 42–48. 1999. [Medline]
33. Rohrman M. Macacine herpes virus (B virus). Workplace Health Saf. **64**: 9–12. 2016. [Medline] [CrossRef]
34. Huff JL, and Barry PA. B-virus (Cercopithecine herpesvirus 1) infection in humans and macaques: potential for zoonotic disease. Emerg Infect Dis. **9**: 246–250. 2003. [Medline] [CrossRef]
35. Kaul A, Schönmann U, and Pöhlmann S. Seroprevalence of viral infections in captive rhesus and cynomolgus macaques. Primate Biol. **6**: 1–6. 2019. [Medline] [CrossRef]
36. Balansard I, Cleverley L, and Cutler KL. SpÅngberg MG, Thibault-Duprey K, and Langermans JA. Revised recommendations for health monitoring of non-human primate colonies (2018). FELASA Working Group Report. Lab Anim. **53**: 429–446. 2019.
37. Carlson CS, O'Sullivan MG, Jayo MJ, Anderson DK, Harber ES, Jerome WG, Bullock BC, and Heberling RL. Fatal disseminated cercopithecine herpesvirus 1 (herpes B infection in cynomolgus monkeys (*Macaca fascicularis*). Vet Pathol. **34**: 405–414. 1997. [Medline] [CrossRef]
38. Pöhlmann S, Suntz M, Akimkin V, Bleyer M, and Kaul A. Herpes B virus replication and viral lesions in the liver of a cynomolgus macaque which died from severe disease with rapid onset. J Med Primatol. **46**: 256–259. 2017. [Medline] [CrossRef]
39. Centers for Disease Control and Prevention (CDC). Laboratory testing and diagnosis: https://www.cdc.gov/herpesbvirus/laboratory.html (Accessed on May 9, 2022)
40. Fukushi S, Yamada S, Saijo M, and Suzuki T. Gentai manual B virus Kokuritsukansenshokenkyujo kanja chiryo oyobi kanja kentia ni kanshite. NIID, National Institute of Infectious Diseases; 2020.
41. Scinicariello F, Eberle R, and Hilliard JK. Rapid detection of B virus (herpesvirus simiae) DNA by polymerase chain reaction. J Infect Dis. **168**: 747–750. 1993. [Medline] [CrossRef]
42. Oya C, Ochiai Y, Taniuchi Y, Takano T, Ueda F, Yoshikawa Y, and Hondo R. Specific detection and identification of herpes B virus by a PCR-microplate hybridization assay. J Clin Microbiol. **42**: 1869–1874. 2004. [Medline] [CrossRef]
43. Ishikawa K, Fukasawa M, Tsujimoto H, Else JG, Isahakia M, Ubhi NK, Ishida T, Takenaka O, Kawamoto Y, Shotake T, Ohsawa H, Ivanoff B, Cooper RW, Frost E, Grant FC, Spriatna Y, Sutarman, Abe K, Yamamoto K, Hayami M. Serological survey and virus isolation of simian T-cell leukemia/T-lymphotropic virus type I (STLV-I) in nonhuman primates in their native countries. Int J Cancer. **40**: 233–239. 1987. [Medline] [CrossRef]
44. Tsujimoto H, Noda Y, Ishikawa K, Nakamura H, Fukasawa M, Sakakibara I, Sasagawa A, Honjo S, and Hayami M. Development of adult T-cell leukemia-like disease in African green monkey associated with clonal integration of simian T-cell leukemia virus type I. Cancer Res. **47**: 269–274. 1987. [Medline]
45. Allan JS, Leland M, Broussard S, Mone J, and Hubbard G. Simian T-cell lymphotropic Viruses (STLVs) and lymphomas in African nonhuman primates. Cancer Invest. **19**: 383–395. 2001. [Medline] [CrossRef]
46. Yamaguchi I, Myojo K, Sanada H, Sudo E, Ootsuka S, Okumura H, Takami A, Yoneshige T, Suzuki Y, Imaizumi M, Takada C, Kimoto N, Saeki K, and Takaba K. Spontaneous malignant T cell lymphoma in a young male common marmoset (*Callithrix jacchus*). J Toxicol Pathol. **26**: 301–307. 2013. [Medline] [CrossRef]
47. Andrade MR, Yee J, Barry P, Spinner A, Roberts JA, Cabello PH, Leite JP, and Lerche NW. Prevalence of antibodies to selected viruses in a long-term closed breeding colony of rhesus macaques (*Macaca mulatta*) in Brazil. Am J Primatol. **59**: 123–128. 2003. [Medline] [CrossRef]
48. Ambagala AP, Marsh A, Chan J, Pilon R, Fournier J, Mazzulli T, Sandstrom P, Willer DO, and MacDonald KS. Isolation and characterization of cynomolgus macaque (Macaca fascicularis) cytomegalovirus (CyCMV). Virology. **412**: 125–135. 2011. [Medline] [CrossRef]
49. Griffiths P, and Reeves M. Pathogenesis of human cytomegalovirus in the immunocompromised host. Nat Rev Microbiol. **19**: 759–773. 2021. [Medline] [CrossRef]
50. Marsh AK, Ambagala AP, Perciani CT, Russell JN, Chan JK, Janes M, Antony JM, Pilon R, Sandstrom P, Willer DO, and MacDonald KS. Examining the species-specificity of rhesus macaque cytomegalovirus (RhCMV) in cynomolgus macaques. PLoS One. **10**: e0121339. 2015. [Medline] [CrossRef]
51. Burwitz BJ, Malouli D, Bimber BN, Reed JS, Ventura AB, Hancock MH, Uebelhoer LS, Bhusari A, Hammond KB, Espinosa Trethewy RG, Klug A, Legasse AW, Axthelm MK, Nelson JA, Park BS, Streblow DN, Hansen SG, Picker LJ, Früh K, and Sacha JB. Cross-species rhesus cytomegalovirus infection of cynomolgus macaques. PLoS Pathog. **12**: e1006014. 2016. [Medline] [CrossRef]
52. Han D, Berman DM, Willman M, Buchwald P, Rothen D, Kenyon NM, and Kenyon NS. Choice of immunosuppression influences cytomegalovirus DNAemia in cynomolgus monkey (Macaca fascicularis) islet allograft recipients. Cell Transplant. **19**: 1547–1561. 2010. [Medline] [CrossRef]
53. Kaur A, Hale CL, Noren B, Kassis N, Simon MA, and John-

son RP. Decreased frequency of cytomegalovirus (CMV)-specific CD4+ T lymphocytes in simian immunodeficiency virus-infected rhesus macaques: inverse relationship with CMV viremia. J Virol. **76**: 3646–3658. 2002. [Medline] [CrossRef]
54. Fujimoto K, and Honjo S. Presence of antibody to Cyno-EBV in domestically bred cynomolgus monkeys (*Macaca fascicularis*). J Med Primatol. **20**: 42–45. 1991. [Medline] [CrossRef]
55. Kamperschroer C, Tartaro K, and Kumpf SW. Quantitative PCR assays reveal high prevalence of lymphocryptovirus as well as lytic phase gene expression in peripheral blood cells of cynomolgus macaques. J Virol Methods. **207**: 220–225. 2014. [Medline] [CrossRef]
56. Rao P, Jiang H, and Wang F. Cloning of the rhesus lymphocryptovirus viral capsid antigen and Epstein-Barr virus-encoded small RNA homologues and use in diagnosis of acute and persistent infections. J Clin Microbiol. **38**: 3219–3225. 2000. [Medline] [CrossRef]
57. Kutok JL, Klumpp S, Simon M, MacKey JJ, Nguyen V, Middeldorp JM, Aster JC, and Wang F. Molecular evidence for rhesus lymphocryptovirus infection of epithelial cells in immunosuppressed rhesus macaques. J Virol. **78**: 3455–3461. 2004. [Medline] [CrossRef]
58. Carville A, and Mansfield KG. Comparative pathobiology of macaque lymphocryptoviruses. Comp Med. **58**: 57–67. 2008. [Medline]
59. Hutto DL. Opportunistic infections in non-human primates exposed to immunomodulatory biotherapeutics: considerations and case examples. J Immunotoxicol. **7**: 120–127. 2010. [Medline] [CrossRef]
60. Blaschke S, Hannig H, Buske C, Kaup FJ, Hunsmann G, and Bodemer W. Expression of the simian Epstein-Barr virus-encoded latent membrane protein-1 in malignant lymphomas of SIV-infected rhesus macaques. J Med Virol. **65**: 114–120. 2001. [Medline] [CrossRef]
61. Baskin GB, Roberts ED, Kuebler D, Martin LN, Blauw B, Heeney J, and Zurcher C. Squamous epithelial proliferative lesions associated with rhesus Epstein-Barr virus in simian immunodeficiency virus-infected rhesus monkeys. J Infect Dis. **172**: 535–539. 1995. [Medline] [CrossRef]
62. Schmidtko J, Wang R, Wu CL, Mauiyyedi S, Harris NL, Della Pelle P, Brousaides N, Zagachin L, Ferry JA, Wang F, Kawai T, Sachs DH, Cosimi BA, and Colvin RB. Post-transplant lymphoproliferative disorder associated with an Epstein-Barr-related virus in cynomolgus monkeys. Transplantation. **73**: 1431–1439. 2002. [Medline] [CrossRef]
63. Chandler FW, and McClure HM. Adenoviral pancreatitis in rhesus monkeys: current knowledge. Vet Pathol Suppl. **7**: 171–180. 1982. [Medline] [CrossRef]
64. Waldman M, Marshall V, Whitby D, and Kopp JB. Viruses and kidney disease: beyond HIV. Semin Nephrol. **28**: 595–607. 2008. [Medline] [CrossRef]
65. Baskin GB, Murphey-Corb M, Watson EA, and Martin LN. Necropsy findings in rhesus monkeys experimentally infected with cultured simian immunodeficiency virus (SIV)/delta. Vet Pathol. **25**: 456–467. 1988. [Medline] [CrossRef]
66. Wang Y, Tu X, Humphrey C, McClure H, Jiang X, Qin C, Glass RI, and Jiang B. Detection of viral agents in fecal specimens of monkeys with diarrhea. J Med Primatol. **36**: 101–107. 2007. [Medline] [CrossRef]
67. Roy S, Vandenberghe LH, Kryazhimskiy S, Grant R, Calcedo R, Yuan X, Keough M, Sandhu A, Wang Q, Medina-Jaszek CA, Plotkin JB, and Wilson JM. Isolation and characterization of adenoviruses persistently shed from the gastrointestinal tract of non-human primates. PLoS Pathog. **5**: e1000503. 2009. [Medline] [CrossRef]
68. Stuker G, Oshiro LS, Schmidt NJ, Holmberg CA, Anderson JH, Glaser CA, and Henrickson RV. Virus detection in monkeys with diarrhea: the association of adenoviruses with diarrhea and the possible role of rotaviruses. Lab Anim Sci. **29**: 610–616. 1979. [Medline]
69. Baskin GB, and Soike KF. Adenovirus enteritis in SIV-infected rhesus monkeys. J Infect Dis. **160**: 905–907. 1989. [Medline] [CrossRef]
70. Handley SA, Thackray LB, Zhao G, Presti R, Miller AD, Droit L, Abbink P, Maxfield LF, Kambal A, Duan E, Stanley K, Kramer J, Macri SC, Permar SR, Schmitz JE, Mansfield K, Brenchley JM, Veazey RS, Stappenbeck TS, Wang D, Barouch DH, and Virgin HW. Pathogenic simian immunodeficiency virus infection is associated with expansion of the enteric virome. Cell. **151**: 253–266. 2012. [Medline] [CrossRef]
71. Wevers D, Leendertz FH, Scuda N, Boesch C, Robbins MM, Head J, Ludwig C, Kühn J, and Ehlers B. A novel adenovirus of Western lowland gorillas (*Gorilla gorilla gorilla*). Virol J. **7**: 303. 2010. [Medline] [CrossRef]
72. Wevers D, Metzger S, Babweteera F, Bieberbach M, Boesch C, Cameron K, Couacy-Hymann E, Cranfield M, Gray M, Harris LA, Head J, Jeffery K, Knauf S, Lankester F, Leendertz SA, Lonsdorf E, Mugisha L, Nitsche A, Reed P, Robbins M, Travis DA, Zommers Z, Leendertz FH, and Ehlers B. Novel adenoviruses in wild primates: a high level of genetic diversity and evidence of zoonotic transmissions. J Virol. **85**: 10774–10784. 2011. [Medline] [CrossRef]
73. Doane FW, Anderson N, Zbitnew A, and Rhodes AJ. Application of electron microscopy to the diagnosis of virus infections. Can Med Assoc J. **100**: 1043–1049. 1969. [Medline]
74. Simon MA. Polyomaviruses of nonhuman primates: implications for research. Comp Med. **58**: 51–56. 2008. [Medline]
75. Simon MA, Ilyinskii PO, Baskin GB, Knight HY, Pauley DR, and Lackner AA. Association of simian virus 40 with a central nervous system lesion distinct from progressive multifocal leukoencephalopathy in macaques with AIDS. Am J Pathol. **154**: 437–446. 1999. [Medline] [CrossRef]
76. Horvath CJ, Simon MA, Bergsagel DJ, Pauley DR, King NW, Garcea RL, and Ringler DJ. Simian virus 40-induced disease in rhesus monkeys with simian acquired immunodeficiency syndrome. Am J Pathol. **140**: 1431–1440. 1992. [Medline]
77. van Gorder MA, Della Pelle P, Henson JW, Sachs DH, Cosimi AB, and Colvin RB. Cynomolgus polyoma virus infection: a new member of the polyoma virus family causes interstitial nephritis, ureteritis, and enteritis in immunosuppressed cynomolgus monkeys. Am J Pathol. **154**: 1273–1284. 1999. [Medline] [CrossRef]
78. Simon MA. Simian parvoviruses: biology and implications for research. Comp Med. **58**: 47–50. 2008. [Medline]
79. Schröder C, Pfeiffer S, Wu G, Azimzadeh AM, Aber A, Pierson RN 3rd, and O'Sullivan MG. Simian parvovirus infection in cynomolgus monkey heart transplant recipients causes death related to severe anemia. Transplantation. **81**:

1165–1170. 2006. [Medline] [CrossRef]
80. O'Sullivan MG, Anderson DC, Fikes JD, Bain FT, Carlson CS, Green SW, Young NS, and Brown KE. Identification of a novel simian parvovirus in cynomolgus monkeys with severe anemia. A paradigm of human B19 parvovirus infection. J Clin Invest. **93**: 1571–1576. 1994. [Medline] [CrossRef]
81. Wood CE, Chen Z, Cline JM, Miller BE, and Burk RD. Characterization and experimental transmission of an oncogenic papillomavirus in female macaques. J Virol. **81**: 6339–6345. 2007. [Medline] [CrossRef]
82. Chen Z, Long T, Wong PY, Ho WCS, Burk RD, and Chan PKS. Non-human primate papillomaviruses share similar evolutionary histories and niche adaptation as the human counterparts. Front Microbiol. **10**: 2093. 2019. [Medline] [CrossRef]
83. Leiding JW, and Holland SM. Warts and all: human papillomavirus in primary immunodeficiencies. J Allergy Clin Immunol. **130**: 1030–1048. 2012. [Medline] [CrossRef]
84. Antonsson A, and Hansson BG. Healthy skin of many animal species harbors papillomaviruses which are closely related to their human counterparts. J Virol. **76**: 12537–12542. 2002. [Medline] [CrossRef]
85. Joh J, Hopper K, Van Doorslaer K, Sundberg JP, Jenson AB, and Ghim SJ. Macaca fascicularis papillomavirus type 1: a non-human primate betapapillomavirus causing rapidly progressive hand and foot papillomatosis. J Gen Virol. **90**: 987–994. 2009. [Medline] [CrossRef]
86. Wood CE, Tannehill-Gregg SH, Chen Z, Doorslaer K, Nelson DR, Cline JM, and Burk RD. Novel betapapillomavirus associated with hand and foot papillomas in a cynomolgus macaque. Vet Pathol. **48**: 731–736. 2011. [Medline] [CrossRef]
87. Wood CE, Borgerink H, Register TC, Scott L, and Cline JM. Cervical and vaginal epithelial neoplasms in cynomolgus monkeys. Vet Pathol. **41**: 108–115. 2004. [Medline] [CrossRef]
88. White JA, Todd PA, Yee JL, Kalman-Bowlus A, Rodgers KS, Yang X, Wong SW, Barry P, and Lerche NW. Prevalence of viremia and oral shedding of rhesus rhadinovirus and retroperitoneal fibromatosis herpesvirus in large age-structured breeding groups of rhesus macaques (*Macaca mulatta*). Comp Med. **59**: 383–390. 2009. [Medline]
89. Desrosiers RC, Sasseville VG, Czajak SC, Zhang X, Mansfield KG, Kaur A, Johnson RP, Lackner AA, and Jung JU. A herpesvirus of rhesus monkeys related to the human Kaposi's sarcoma-associated herpesvirus. J Virol. **71**: 9764–9769. 1997. [Medline] [CrossRef]
90. Ruff K, Baskin GB, Simpson L, Murphey-Corb M, and Levy LS. Rhesus rhadinovirus infection in healthy and SIV-infected macaques at Tulane National Primate Research Center. J Med Primatol. **32**: 1–6. 2003. [Medline] [CrossRef]
91. Orzechowska BU, Powers MF, Sprague J, Li H, Yen B, Searles RP, Axthelm MK, and Wong SW. Rhesus macaque rhadinovirus-associated non-Hodgkin lymphoma: animal model for KSHV-associated malignancies. Blood. **112**: 4227–4234. 2008. [Medline] [CrossRef]
92. Wong SW, Bergquam EP, Swanson RM, Lee FW, Shiigi SM, Avery NA, Fanton JW, and Axthelm MK. Induction of B cell hyperplasia in simian immunodeficiency virus-infected rhesus macaques with the simian homologue of Kaposi's sarcoma-associated herpesvirus. J Exp Med. **190**: 827–840. 1999. [Medline] [CrossRef]
93. Gray WL. Simian varicella in old world monkeys. Comp Med. **58**: 22–30. 2008. [Medline]
94. Takasaka M. An outbreak of nonhuman primate varicella-like herpesvirus infection in the established breeding colony of cynomolgus monkeys. Tsukuba Primate Center News. **9**: 5–11. 1990.
95. Mahalingam R, Smith D, Wellish M, Wolf W, Dueland AN, Cohrs R, Soike K, and Gilden D. Simian varicella virus DNA in dorsal root ganglia. Proc Natl Acad Sci USA. **88**: 2750–2752. 1991. [Medline] [CrossRef]
96. Soike KF, Rangan SR, and Gerone PJ. Viral disease models in primates. Adv Vet Sci Comp Med. **28**: 151–199. 1984. [Medline] [CrossRef]
97. Soike KF. Simian varicella virus infection in African and Asian monkeys. The potential for development of antivirals for animal diseases. Ann N Y Acad Sci. **653**: 323–333. 1992. [Medline] [CrossRef]
98. Mahalingam R, Traina-Dorge V, Wellish M, Lorino R, Sanford R, Ribka EP, Alleman SJ, Brazeau E, and Gilden DH. Simian varicella virus reactivation in cynomolgus monkeys. Virology. **368**: 50–59. 2007. [Medline] [CrossRef]
99. Kolappaswamy K, Mahalingam R, Traina-Dorge V, Shipley ST, Gilden DH, Kleinschmidt-Demasters BK, McLeod CG Jr, Hungerford LL, and DeTolla LJ. Disseminated simian varicella virus infection in an irradiated rhesus macaque (*Macaca mulatta*). J Virol. **81**: 411–415. 2007. [Medline] [CrossRef]
100. Hukkanen RR, Gillen M, Grant R, Liggitt HD, Kiem HP, and Kelley ST. Simian varicella virus in pigtailed macaques (*Macaca nemestrina*): clinical, pathologic, and virologic features. Comp Med. **59**: 482–487. 2009. [Medline]
101. Gulani J, Koch A, Chappell MG, Christensen CL, Facemire P, Singh VK, Ossetrova NI, Srinivasan V, and Holt RK. Cercopithecine herpesvirus 9 (simian varicella virus) infection after total-body irradiation in a rhesus macaque (*Macaca mulatta*). Comp Med. **66**: 150–153. 2016. [Medline]
102. Balayan MS. Natural hosts of hepatitis A virus. Vaccine. **10**(Suppl 1): S27–S31. 1992. [Medline] [CrossRef]
103. Brown EA, Jansen RW, and Lemon SM. Characterization of a simian hepatitis A virus (HAV): antigenic and genetic comparison with human HAV. J Virol. **63**: 4932–4937. 1989. [Medline] [CrossRef]
104. Nainan OV, Margolis HS, Robertson BH, Balayan M, and Brinton MA. Sequence analysis of a new hepatitis A virus naturally infecting cynomolgus macaques (*Macaca fascicularis*). J Gen Virol. **72**: 1685–1689. 1991. [Medline] [CrossRef]
105. Lanford RE, Walker CM, and Lemon SM. Nonhuman primate models of hepatitis A virus and hepatitis E virus infections. Cold Spring Harb Perspect Med. **9**: a031815. 2019. [Medline] [CrossRef]
106. Amado LA, Marchevsky RS, de Paula VS, Hooper C, Freire MS, Gaspar AM, and Pinto MA. Experimental hepatitis A virus (HAV) infection in cynomolgus monkeys (Macaca fascicularis): evidence of active extrahepatic site of HAV replication. Int J Exp Pathol. **91**: 87–97. 2010. [Medline] [CrossRef]
107. Cullen JM, and Lemon SM. Comparative pathology of hepatitis A virus and hepatitis E virus infection. Cold Spring

Harb Perspect Med. **9**: a033456. 2019. [Medline] [CrossRef]
108. Sestak K, Merritt CK, Borda J, Saylor E, Schwamberger SR, Cogswell F, Didier ES, Didier PJ, Plauche G, Bohm RP, Aye PP, Alexa P, Ward RL, and Lackner AA. Infectious agent and immune response characteristics of chronic enterocolitis in captive rhesus macaques. Infect Immun. **71**: 4079–4086. 2003. [Medline] [CrossRef]
109. Zhang Q, Han S, Liu K, Luo J, Lu J, and He H. Occurrence of selected zoonotic fecal pathogens and first molecular identification of *Hafnia paralvei* in wild Taihangshan macaques (*Macaca mulatta tcheliensis*) in China. BioMed Res Int. **2019**: 2494913. 2019. [Medline]
110. Tribe GW, and Fleming MP. Biphasic enteritis in imported cynomolgus (*Macaca fascicularis*) monkeys infected with Shigella, Salmonella and Campylobacter species. Lab Anim. **17**: 65–69. 1983. [Medline] [CrossRef]
111. Lee JI, Kim SJ, and Park CG. Shigella flexneri infection in a newly acquired rhesus macaque (*Macaca mulatta*). Lab Anim Res. **27**: 343–346. 2011. [Medline] [CrossRef]
112. Takeuchi A. Early colonic lesions in experimental Shigella infection in rhesus monkeys: revisited. Vet Pathol Suppl. **7**: 1–8. 1982. [Medline] [CrossRef]
113. Fitzgeorge RB, Baskerville A, and Lander KP. Experimental infection of Rhesus monkeys with a human strain of *Campylobacter jejuni*. J Hyg (Lond). **86**: 343–351. 1981. [Medline] [CrossRef]
114. MacArthur JA, and Wood M. Yersiniosis in a breeding unit of *Macaca fascicularis* (cynomolgus monkeys). Lab Anim. **17**: 151–155. 1983. [Medline] [CrossRef]
115. Bronson RT, May BD, and Ruebner BH. An outbreak of infection by *Yersinia pseudotuberculosis* in nonhuman primates. Am J Pathol. **69**: 289–308. 1972. [Medline]
116. Russell RG, Blaser MJ, Sarmiento JI, and Fox J. Experimental *Campylobacter jejuni* infection in Macaca nemestrina. Infect Immun. **57**: 1438–1444. 1989. [Medline] [CrossRef]
117. Simmons J, and Gibson S. Bacterial and mycotic diseases of nonhuman primates. In: Nonhuman Primates in Biomedical Research: 2nd ed. Volume 2: Disease. CR Abee, K Mansfield, SD Tardif, and T Morris (eds). Academic Press, London. 105–172. 2012.
118. Choi EW, Lee KW, Kim TM, Park H, Jeon MR, Cho CW, Park JB, and Kim S. *Mycobacterium tuberculosis* infections in cynomolgus monkey transplant recipients and institution of a screening program for the prevention and control of tuberculosis. BMC Vet Res. **12**: 289. 2016. [Medline] [CrossRef]
119. Wilbur AK, Engel GA, Rompis A, A Putra IG, Lee BP, Aggimarangsee N, Chalise M, Shaw E, Oh G, Schillaci MA, and Jones-Engel L. From the mouths of monkeys: detection of *Mycobacterium tuberculosis* complex DNA from buccal swabs of synanthropic macaques. Am J Primatol. **74**: 676–686. 2012. [Medline] [CrossRef]
120. Capuano SV 3rd, Croix DA, Pawar S, Zinovik A, Myers A, Lin PL, Bissel S, Fuhrman C, Klein E, and Flynn JL. Experimental Mycobacterium tuberculosis infection of cynomolgus macaques closely resembles the various manifestations of human M. tuberculosis infection. Infect Immun. **71**: 5831–5844. 2003. [Medline] [CrossRef]
121. Payne KS, Novak JJ, Jongsakul K, Imerbsin R, Apisitsaowapa Y, Pavlin JA, and Hinds SB. Mycobacterium tuberculosis infection in a closed colony of rhesus macaques (*Macaca mulatta*). J Am Assoc Lab Anim Sci. **50**: 105–108. 2011. [Medline]
122. Cadena AM, Flynn JL, and Fortune SM. The importance of first impressions: early events in *Mycobacterium tuberculosis* infection influence outcome. MBio. **7**: e00342–e16. 2016. [Medline] [CrossRef]
123. Burkitt MD, Duckworth CA, Williams JM, and Pritchard DM. *Helicobacter pylori*-induced gastric pathology: insights from in vivo and ex vivo models. Dis Model Mech. **10**: 89–104. 2017. [Medline] [CrossRef]
124. Solnick JV, Chang K, Canfield DR, and Parsonnet J. Natural acquisition of *Helicobacter pylori* infection in newborn rhesus macaques. J Clin Microbiol. **41**: 5511–5516. 2003. [Medline] [CrossRef]
125. Reindel JF, Fitzgerald AL, Breider MA, Gough AW, Yan C, Mysore JV, and Dubois A. An epizootic of lymphoplasmacytic gastritis attributed to Helicobacter pylori infection in cynomolgus monkeys (Macaca fascicularis). Vet Pathol. **36**: 1–13. 1999. [Medline] [CrossRef]
126. Katsuta O, Tomonari Y, Okazaki Y, Kawamura M, Watanabe K, Matsui H, Sakuma Y, and Tsuchitani M. Naturally occurring *Helicobacter pylori* infection in the cynomolgus monkey (*Macaca fascicularis*) used in subclinical studies. J Toxicol Pathol. **14**: 45–49. 2001. [CrossRef]
127. McKeag S, and McInnes EF. The incidence of lymphoplasmacytic gastritis in the fundus and antrum of cynomolgus monkey (*Macaca fascicularis*) stomachs. J Toxicol Pathol. **25**: 249–256. 2012. [Medline] [CrossRef]
128. Sato J, Doi T, Wako Y, Hamamura M, Kanno T, Tsuchitani M, and Narama I. Histopathology of incidental findings in beagles used in toxicity studies. J Toxicol Pathol. **25**: 103–134. 2012. [Medline] [CrossRef]
129. Dubois A, Fiala N, Heman-Ackah LM, Drazek ES, Tarnawski A, Fishbein WN, Perez-Perez GI, and Blaser MJ. Natural gastric infection with Helicobacter pylori in monkeys: a model for spiral bacteria infection in humans. Gastroenterology. **106**: 1405–1417. 1994. [Medline] [CrossRef]
130. Haesebrouck F, Pasmans F, Flahou B, Chiers K, Baele M, Meyns T, Decostere A, and Ducatelle R. Gastric helicobacters in domestic animals and nonhuman primates and their significance for human health. Clin Microbiol Rev. **22**: 202–223. 2009. [Medline] [CrossRef]
131. Singhal AV, and Sepulveda AR. *Helicobacter heilmannii* gastritis: a case study with review of literature. Am J Surg Pathol. **29**: 1537–1539. 2005. [Medline] [CrossRef]
132. Matsumoto T, Kawakubo M, Akamatsu T, Koide N, Ogiwara N, Kubota S, Sugano M, Kawakami Y, Katsuyama T, and Ota H. Helicobacter heilmannii sensu stricto-related gastric ulcers: a case report. World J Gastroenterol. **20**: 3376–3382. 2014. [Medline] [CrossRef]
133. Embers ME, Doyle LA, Whitehouse CA, Selby EB, Chappell M, and Philipp MT. Characterization of a *Moraxella* species that causes epistaxis in macaques. Vet Microbiol. **147**: 367–375. 2011. [Medline] [CrossRef]
134. Whitehouse CA, Chase K, Embers ME, Kulesh DA, Ladner JT, Palacios GF, and Minogue TD. Development of real-time PCR assays for the detection of *Moraxella macacae* associated with bloody nose syndrome in rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques. J Med Primatol. **44**: 364–372. 2015. [Medline] [CrossRef]
135. VandeWoude SJ, and Luzarraga MB. The role of Branha-

mella catarrhalis in the "bloody-nose syndrome" of cynomolgus macaques. Lab Anim Sci. **41**: 401–406. 1991. [Medline]
136. Stewart A, Sowden D, Caffery M, Bint M, and Broom J. *Rhodococcus equi* infection: a diverse spectrum of disease. IDCases. **15**: e00487. 2019. [Medline] [CrossRef]
137. Pal M, and Rahman T. Rhodococcus equi: an emerging zoonotic pathogen. Ann Vet Anim Sci. **2**: 3–10. 2015.
138. Sasseville VG, and Mansfield KG. Overview of known nonhuman primate pathogens with potential to affect colonies used for toxicity testing. J Immunotoxicol. **7**: 79–92. 2010. [Medline] [CrossRef]
139. Giguère S, Cohen ND, Chaffin MK, Hines SA, Hondalus MK, Prescott JF, and Slovis NM. *Rhodococcus equi*: clinical manifestations, virulence, and immunity. J Vet Intern Med. **25**: 1221–1230. 2011. [Medline] [CrossRef]
140. Oliveira LG, Watanabe TT, Boabaid FM, Wouters F, Wouters AT, Bandarra PM, Guerra PR, and Driemeier D. Clinical and pathological findings of *Rhodococcus equi* infection in foals. Pesqui Vet Bras. **39**: 849–857. 2019. [CrossRef]
141. Yamshchikov AV, Schuetz A, and Lyon GM. *Rhodococcus equi* infection. Lancet Infect Dis. **10**: 350–359. 2010. [Medline] [CrossRef]
142. Takai S, Henton MM, Picard JA, Guthrie AJ, Fukushi H, and Sugimoto C. Prevalence of virulent *Rhodococcus equi* in isolates from soil collected from two horse farms in South Africa and restriction fragment length polymorphisms of virulence plasmids in the isolates from infected foals, a dog and a monkey. Onderstepoort J Vet Res. **68**: 105–110. 2001. [Medline]
143. Sellon DC, Besser TE, Vivrette SL, and McConnico RS. Comparison of nucleic acid amplification, serology, and microbiologic culture for diagnosis of *Rhodococcus equi* pneumonia in foals. J Clin Microbiol. **39**: 1289–1293. 2001. [Medline] [CrossRef]
144. Arriaga JM, Cohen ND, Derr JN, Chaffin MK, and Martens RJ. Detection of *Rhodococcus equi* by polymerase chain reaction using species-specific nonproprietary primers. J Vet Diagn Invest. **14**: 347–353. 2002. [Medline] [CrossRef]
145. Ladrón N, Fernández M, Agüero J, González Zörn B, Vázquez-Boland JA, and Navas J. Rapid identification of *Rhodococcus equi* by a PCR assay targeting the choE gene. J Clin Microbiol. **41**: 3241–3245. 2003. [Medline] [CrossRef]
146. Palaniappan RU, Zhang Y, Chiu D, Torres A, Debroy C, Whittam TS, and Chang YF. Differentiation of Escherichia coli pathotypes by oligonucleotide spotted array. J Clin Microbiol. **44**: 1495–1501. 2006. [Medline] [CrossRef]
147. Jafari A, Aslani MM, and Bouzari S. Escherichia coli: a brief review of diarrheagenic pathotypes and their role in diarrheal diseases in Iran. Iran J Microbiol. **4**: 102–117. 2012. [Medline]
148. Carvalho VM, Gyles CL, Ziebell K, Ribeiro MA, Catão-Dias JL, Sinhorini IL, Otman J, Keller R, Trabulsi LR, and Pestana de Castro AF. Characterization of monkey enteropathogenic *Escherichia coli* (EPEC) and human typical and atypical EPEC serotype isolates from neotropical nonhuman primates. J Clin Microbiol. **41**: 1225–1234. 2003. [Medline] [CrossRef]
149. Shah S, Kongre V, Kumar V, and Bharadwaj R. A study of parasitic and bacterial pathogens associated with diarrhea in HIV-positive patients. Cureus. **8**: e807. 2016. [Medline]
150. Mansfield KG, Lin KC, Newman J, Schauer D, MacKey J, Lackner AA, and Carville A. Identification of enteropathogenic *Escherichia coli* in simian immunodeficiency virus-infected infant and adult rhesus macaques. J Clin Microbiol. **39**: 971–976. 2001. [Medline] [CrossRef]
151. Colman K. Impact of the genetics and source of preclinical safety animal models on study design, results, and interpretation. Toxicol Pathol. **45**: 94–106. 2017. [Medline] [CrossRef]
152. Abbott DP, and Majeed SK. A survey of parasitic lesions in wild-caught, laboratory-maintained primates: (rhesus, cynomolgus, and baboon). Vet Pathol. **21**: 198–207. 1984. [Medline] [CrossRef]
153. Kim JM, Han S, Shin JS, Min BH, Jeong WY, Lee GE, Kim MS, Kim JE, Chung H, and Park CG. Computed tomography or necropsy diagnosis of multiple bullae and the treatment of pneumothorax in rhesus macaques (*Macaca mulatta*). J Med Primatol. **46**: 260–262. 2017. [Medline] [CrossRef]
154. Simmons HA. Age-associated pathology in rhesus macaques (*Macaca mulatta*). Vet Pathol. **53**: 399–416. 2016. [Medline] [CrossRef]
155. Strait K, Else J, and Eberhard M. Parasitic diseases of nonhuman primates. In: Nonhuman Primates in Biomedical Research, 2nd ed. Volume 2: Disease. CR Abee, K Mansfield, SD Tardif, and T Morris (eds). Academic Press, London. 197–297. 2012.
156. Andrade MC, and Marchevsky RS. Histopathologic findings of pulmonary acariasis in a rhesus monkeys breeding unit. Rev Bras Parasitol Vet. **16**: 229–234. 2007. [Medline] [CrossRef]
157. Tanaka H, Fukui M, Yamamoto H, Hayama S, and Kodera S. Studies on the identification of common intestinal parasites of primates. Exp Anim. **11**: 111–116. 1962. [CrossRef]
158. Honjo S, Muto K, Fujiwara T, Suzuki Y, and Imaizumi K. Statistical survey of internal parasites in cynomolgus monkeys (*Macaca irus*). Jpn J Med Sci Biol. **16**: 217–224. 1963. [Medline] [CrossRef]
159. Remfry J. The incidence, pathogenesis and treatment of helminth infections in rhesus monkeys (*Macaca mulatta*). Lab Anim. **12**: 213–218. 1978. [Medline] [CrossRef]
160. Zanzani SA, Gazzonis AL, Epis S, and Manfredi MT. Study of the gastrointestinal parasitic fauna of captive nonhuman primates (*Macaca fascicularis*). Parasitol Res. **115**: 307–312. 2016. [Medline] [CrossRef]
161. Dufour JP, Cogswell FB, Phillippi-Falkenstein KM, and Bohm RP. Comparison of efficacy of moxidectin and ivermectin in the treatment of *Strongyloides fulleborni* infection in rhesus macaques. J Med Primatol. **35**: 172–176. 2006. [Medline] [CrossRef]
162. Wang T, Yang GY, Yan HJ, Wang S, Bian Y, Chen AC, and Bi FJ. Comparison of efficacy of selamectin, ivermectin and mebendazole for the control of gastrointestinal nematodes in rhesus macaques, China. Vet Parasitol. **153**: 121–125. 2008. [Medline] [CrossRef]
163. Gallagher C, Beierschmitt A, Cruz K, Choo J, and Ketzis J. Should monkeys wash their hands and feet: a pilot-study on sources of zoonotic parasite exposure. One Health. **7**: 100088. 2019. [Medline] [CrossRef]
164. Arizono N, Yamada M, Tegoshi T, and Onishi K. Molecular identification of *Oesophagostomum* and *Trichuris* eggs isolated from wild Japanese macaques. Korean J Parasitol. **50**:

253–257. 2012. [Medline] [CrossRef]
165. White MAF, Whiley H, and E Ross K. A review of *Strongyloides* spp. environmental sources worldwide. Pathogens. **8**: 91. 2019. [Medline] [CrossRef]
166. Takano J, Narita T, Tachibana H, Shimizu T, Komatsubara H, Terao K, and Fujimoto K. Entamoeba histolytica and Entamoeba dispar infections in cynomolgus monkeys imported into Japan for research. Parasitol Res. **97**: 255–257. 2005. [Medline] [CrossRef]
167. Feng M, Yang B, Yang L, Fu Y, Zhuang Y, Liang L, Xu Q, Cheng X, and Tachibana H. High prevalence of Entamoeba infections in captive long-tailed macaques in China. Parasitol Res. **109**: 1093–1097. 2011. [Medline] [CrossRef]
168. Pu LH, Li Z, Wu J, Zhang YL, Chen JQ, Yang JF, and Zou FC. Prevalence, molecular epidemiology and zoonotic risk of *Entamoeba* spp. from experimental macaques in Yunnan Province, southwestern China. Parasitol Res. **119**: 2733–2740. 2020. [Medline] [CrossRef]
169. Dubey JP, Markovits JE, and Killary KA. Cryptosporidium muris-like infection in stomach of cynomolgus monkeys (*Macaca fascicularis*). Vet Pathol. **39**: 363–371. 2002. [Medline] [CrossRef]
170. Wilson DW, Day PA, and Brummer ME. Diarrhea associated with *Cryptosporidium* spp in juvenile macaques. Vet Pathol. **21**: 447–450. 1984. [Medline] [CrossRef]
171. Toft 2nd JD. The pathoparasitology of the alimentary tract and pancreas of nonhuman primates: a review. Vet Pathol Suppl. **7**: 44–92. 1982. [Medline] [CrossRef]
172. Santi-Rocca J, Rigothier MC, and Guillén N. Host-microbe interactions and defense mechanisms in the development of amoebic liver abscesses. Clin Microbiol Rev. **22**: 65–75. 2009. [Medline] [CrossRef]
173. Betanzos A, Schnoor M, Javier-Reyna R, García-Rivera G, Bañuelos C, Pais-Morales J, and Orozco E. Analysis of the epithelial damage produced by *Entamoeba histolytica* infection. J Vis Exp.: e51668. 2014. [Medline]
174. Nakamura S, Itagaki I, Nakagawa T, Kawamoto I, Asano T, Arakawa D, and Tsuchiya H. Paromomycin sulfate is an effective treatment for balantidiasis in captive cynomolgus monkeys. Exp Anim. **68**: 285–292. 2019. [Medline] [CrossRef]
175. Kuhn EM, Mätz-Rensing K, Stahl-Hennig C, Makoschey B, Hunsmann G, and Kaup FJ. Intestinal manifestations of experimental SIV-infection in rhesus monkeys (*Macaca mulatta*): a histological and ultrastructural study. Zentralbl Veterinärmed B. **44**: 501–512. 1997. [Medline]
176. Debenham JJ, Tysnes K, Khunger S, and Robertson LJ. Occurrence of *Giardia, Cryptosporidium*, and *Entamoeba* in wild rhesus macaques (*Macaca mulatta*) living in urban and semi-rural North-West India. Int J Parasitol Parasites Wildl. **6**: 29–34. 2017. [Medline] [CrossRef]
177 Centers for Disease Control and Prevention (CDC). Illness and symptoms, in parasites-giardia: https://www.cdc.gov/parasites/giardia/illness.html
178. Takano J, Narita T, Tachibana H, Terao K, and Fujimoto K. Comparison of *Entamoeba histolytica* DNA isolated from a cynomolgus monkey with human isolates. Parasitol Res. **101**: 539–546. 2007. [Medline] [CrossRef]
179. da Silva Barbosa A, Pissinatti A, Dib LV, de Siqueira MP, Cardozo ML, Fonseca AB, de Barros Oliveira A, da Silva FA, Uchôa CM, Bastos OM, and Amendoeira MR. *Balantidium coli* and other gastrointestinal parasites in captives non-human primates of the Rio de Janeiro, Brazil. J Med Primatol. **44**: 18–26. 2015. [Medline] [CrossRef]
180. Saidin S, Othman N, and Noordin R. Update on laboratory diagnosis of amoebiasis. Eur J Clin Microbiol Infect Dis. **38**: 15–38. 2019. [Medline] [CrossRef]
181. Hodo CL, Wilkerson GK, Birkner EC, Gray SB, and Hamer SA. Trypanosoma cruzi transmission among captive nonhuman primates, wildlife, and vectors. EcoHealth. **15**: 426–436. 2018. [Medline] [CrossRef]
182. Herrera C, Majeau A, Didier P, Falkenstein KP, and Dumonteil E. *Trypanosoma cruzi* diversity in naturally infected nonhuman primates in Louisiana assessed by deep sequencing of the mini-exon gene. Trans R Soc Trop Med Hyg. **113**: 281–286. 2019. [Medline] [CrossRef]
183. Bonecini-Almeida MG, Galvão-Castro B, Pessoa MH, Pirmez C, and Laranja F. Experimental Chagas' disease in rhesus monkeys. I. Clinical, parasitological, hematological and anatomo-pathological studies in the acute and indeterminate phase of the disease. Mem Inst Oswaldo Cruz. **85**: 163–171. 1990. [Medline] [CrossRef]
184. Sathler-Avelar R, Vitelli-Avelar DM, Mattoso-Barbosa AM, Pascoal-Xavier MA, Elói-Santos SM, da Costa-Rocha IA, Teixeira-Carvalho A, Dick EJ Jr, VandeBerg JF, VandeBerg JL, and Martins-Filho OA. Phenotypic and functional signatures of peripheral blood and spleen compartments of cynomolgus macaques infected with *T. cruzi*: associations with cardiac histopathological characteristics. Front Cell Infect Microbiol. **11**: 701930. 2021. [Medline] [CrossRef]
185. Dickerson MF, Astorga NG, Astorga NR, and Lewis AD. Chagas disease in 2 geriatric rhesus macaques (*Macaca mulatta*) housed in the Pacific Northwest. Comp Med. **64**: 323–328. 2014. [Medline]
186. Vitelli-Avelar DM, Sathler-Avelar R, Mattoso-Barbosa AM, Gouin N, Perdigão-de-Oliveira M, Valério-Dos-Reis L, Costa RP, Elói-Santos SM, Gomes MS, Amaral LR, Teixeira-Carvalho A, Martins-Filho OA, Dick EJ Jr, Hubbard GB, VandeBerg JF, and VandeBerg JL. Cynomolgus macaques naturally infected with *Trypanosoma cruzi*-I exhibit an overall mixed pro-inflammatory/modulated cytokine signature characteristic of human Chagas disease. PLoS Negl Trop Dis. **11**: e0005233. 2017. [Medline] [CrossRef]
187. Williams JT, Mubiru JN, Schlabritz-Loutsevitch NE, Rubicz RC, VandeBerg JL, Dick EJ Jr, and Hubbard GB. Polymerase chain reaction detection of *Trypanosoma cruzi* in *Macaca fascicularis* using archived tissues. Am J Trop Med Hyg. **81**: 228–234. 2009. [Medline] [CrossRef]
188. Dubey JP, Lindsay DS, and Speer CA. Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. Clin Microbiol Rev. **11**: 267–299. 1998. [Medline] [CrossRef]
189. Lindsay DS, and Dubey JP. Toxoplasmosis in wild and domestic animals. In: *Toxoplasma gondii*: The Model Apicomplexan. Perspectives and Methods. LM Weiss and K Kim (eds). Academic Press, London. 133–52. 2007.
190. Remington JS, Soave OA, and Davis J. A serological survey in three species of monkeys for antibodies to toxoplasma. Am J Trop Med Hyg. **14**: 724–726. 1965. [Medline] [CrossRef]
191. Li HL, Yan C, Li J, Ai L, Zhou DH, Yuan ZG, Lin RQ, Zhao GH, and Zhu XQ. Seroprevalence of Toxoplasma gondii in bred cynomolgus monkeys (*Macaca fascicularis*) in Chi-

na. J Parasitol. **96**: 807–808. 2010. [Medline] [CrossRef]
192. Cano-Terriza D, Almería S, Caballero-Gómez J, Díaz-Cao JM, Jiménez-Ruiz S, Dubey JP, and García-Bocanegra I. Serological survey of Toxoplasma gondii in captive nonhuman primates in zoos in Spain. Comp Immunol Microbiol Infect Dis. **65**: 54–57. 2019. [Medline] [CrossRef]
193. Dupouy-Camet J, de Souza SL, Maslo C, Paugam A, Saimot AG, Benarous R, Tourte-Schaefer C, and Derouin F. Detection of *Toxoplasma gondii* in venous blood from AIDS patients by polymerase chain reaction. J Clin Microbiol. **31**: 1866–1869. 1993. [Medline] [CrossRef]
194. Liu Q, Wang Z-D, Huang S-Y, and Zhu X-Q. Diagnosis of toxoplasmosis and typing of *Toxoplasma gondii*. Parasit Vectors. **8**: 292. 2015. [Medline] [CrossRef]
195. Cristina N, Pelloux H, Goulhot C, Brion JP, Leclercq P, and Ambroise-Thomas P. Detection of *Toxoplasma gondii* in AIDS patients by the polymerase chain reaction. Infection. **21**: 150–153. 1993. [Medline] [CrossRef]
196. Kuncl RW, and Richter W. Prevalence and ultrastructure of sarcocystis in rhesus monkeys. Nippon Juigaku Zasshi. **50**: 519–527. 1988. [Medline] [CrossRef]
197. Lane JH, Mansfield KG, Jackson LR, Diters RW, Lin KC, MacKey JJ, and Sasseville VG. Acute fulminant sarcocystosis in a captive-born rhesus macaque. Vet Pathol. **35**: 499–505. 1998. [Medline] [CrossRef]
198. Liu DX, Gill A, Holman PJ, Didier PJ, Blanchard JL, Veazey RS, and Lackner AA. Persistent babesiosis in a Rhesus macaque (*Macaca mulatta*) infected with a simian-human immunodeficiency virus. J Med Primatol. **43**: 206–208. 2014. [Medline] [CrossRef]
199. Hussain S, Hussain A, Aziz MU, Song B, Zeb J, George D, Li J, and Sparagano O. A review of zoonotic babesiosis as an emerging public health threat in Asia. Pathogens. **11**: 23. 2021. [Medline] [CrossRef]
200. Onyiche TE, Răileanu C, Fischer S, and Silaghi C. Global distribution of *Babesia* species in questing ticks: a systematic review and meta-analysis based on published literature. Pathogens. **10**: 2021. [Medline] [CrossRef]
201. Ruebush TK 2nd, Collins WE, and Warren M. Experimental Babesia microti infections in Macaca mulatta: recurrent parasitemia before and after splenectomy. Am J Trop Med Hyg. **30**: 304–307. 1981. [Medline] [CrossRef]
202. Glomski CA, Pica A, and Greene JF. Erythrocytes of the Rhesus and Cynomolgus Monkeys. 1st ed. CRC press Taylor & Francis Group, Florida. 2016.
203. Li MI, Mailepessov D, Vythilingam I, Lee V, Lam P, Ng LC, and Tan CH. Prevalence of simian malaria parasites in macaques of Singapore. PLoS Negl Trop Dis. **15**: e0009110. 2021. [Medline] [CrossRef]
204. Ohta E, Nagayama Y, Koyama N, Kakiuchi D, and Hosokawa S. Malaria in cynomolgus monkeys used in toxicity studies in Japan. J Toxicol Pathol. **29**: 31–38. 2016. [Medline] [CrossRef]
205. Cunha JA, Carvalho LJM, Bianco-Junior C, Andrade MCR, Pratt-Riccio LR, Riccio EKP, Pelajo-Machado M, da Silva IJ, Druilhe P, and Daniel-Ribeiro CT. Increased *Plasmodium falciparum* parasitemia in non-splenectomized saimiri sciureus monkeys treated with clodronate liposomes. Front Cell Infect Microbiol. **7**: 408. 2017. [Medline] [CrossRef]
206. Ameri M, Boulay M, and Honor DJ. What is your diagnosis? Blood smear from a cynomolgus monkey (*Macaca fascicularis*). Malaria infection (*Plasmodium inui*). Vet Clin Pathol. **39**: 257–258. 2010. [Medline] [CrossRef]
207. Siregar JE, Faust CL, Murdiyarso LS, Rosmanah L, Saepuloh U, Dobson AP, and Iskandriati D. Non-invasive surveillance for *Plasmodium* in reservoir macaque species. Malar J. **14**: 404. 2015. [Medline] [CrossRef]
208. Migaki G, Schmidt RE, Toft JD 2nd, and Kaufmann AF. Mycotic infections of the alimentary tract of nonhuman primates: a review. Vet Pathol Suppl. **7**: 93–103. 1982. [Medline] [CrossRef]
209. Zaidi SH, Shanker R, and Dogra RKS. Experimental infective pneumoconiosis: effect of asbestos dust and Candida albicans infection on the lungs of rhesus monkeys. Environ Res. **6**: 274–286. 1973. [Medline] [CrossRef]
210. Steele C, Ratterree M, and Fidel PL Jr. Differential susceptibility of two species of macaques to experimental vaginal candidiasis. J Infect Dis. **180**: 802–810. 1999. [Medline] [CrossRef]
211. Vázquez-González D, Perusquía-Ortiz AM, Hundeiker M, and Bonifaz A. Opportunistic yeast infections: *candidiasis, cryptococcosis, trichosporonosis* and *geotrichosis*. J Dtsch Dermatol Ges. **11**: 381–393, quiz 394. 2013. [Medline] [CrossRef]
212. Clancy CJ, and Nguyen MH. Finding the "missing 50%" of invasive candidiasis: how nonculture diagnostics will improve understanding of disease spectrum and transform patient care. Clin Infect Dis. **56**: 1284–1292. 2013. [Medline] [CrossRef]
213. Pitarch A, Nombela C, and Gil C. Diagnosis of invasive candidiasis: from gold standard methods to promising leading-edge technologies. Curr Top Med Chem. **18**: 1375–1392. 2018. [Medline] [CrossRef]
214. Beck JM, and Cushion MT. Pneumocystis workshop: 10th anniversary summary. Eukaryot Cell. **8**: 446–460. 2009. [Medline] [CrossRef]
215. Cissé OH, Ma L, Dekker JP, Khil PP, Youn JH, Brenchley JM, Blair R, Pahar B, Chabé M, Van Rompay KKA, Keesler R, Sukura A, Hirsch V, Kutty G, Liu Y, Peng L, Chen J, Song J, Weissenbacher-Lang C, Xu J, Upham NS, Stajich JE, Cuomo CA, Cushion MT, and Kovacs JA. Genomic insights into the host specific adaptation of the Pneumocystis genus. Commun Biol. **4**: 305. 2021. [Medline] [CrossRef]
216. Slaven BE, Meller J, Porollo A, Sesterhenn T, Smulian AG, and Cushion MT. Draft assembly and annotation of the Pneumocystis carinii genome. J Eukaryot Microbiol. **53**(Suppl 1): S89–S91. 2006. [Medline] [CrossRef]
217. Cissé OH, Ma L, Wei Huang D, Khil PP, Dekker JP, Kutty G, Bishop L, Liu Y, Deng X, Hauser PM, Pagni M, Hirsch V, Lempicki RA, Stajich JE, Cuomo CA, and Kovacs JA. Comparative population genomics analysis of the mammalian fungal pathogen *Pneumocystis*. MBio. **9**: e00381–e18. 2018. [Medline] [CrossRef]
218. Kelly MN, and Shellito JE. Current understanding of Pneumocystis immunology. Future Microbiol. **5**: 43–65. 2010. [Medline] [CrossRef]
219. Harouse JM, Gettie A, Eshetu T, Tan RC, Bohm R, Blanchard J, Baskin G, and Cheng-Mayer C. Mucosal transmission and induction of simian AIDS by CCR5-specific simian/human immunodeficiency virus SHIV(SF162P3). J Virol. **75**: 1990–1995. 2001. [Medline] [CrossRef]
220. Yanai T, Simon MA, Doddy FD, Mansfield KG, Pauley D, and Lackner AA. Nodular Pneumocystis carinii pneumonia in SIV-infected macaques. Vet Pathol. **36**: 471–474. 1999.

[Medline] [CrossRef]
221. Board KF, Patil S, Lebedeva I, Capuano S 3rd, Trichel AM, Murphey-Corb M, Rajakumar PA, Flynn JL, Haidaris CG, and Norris KA. Experimental Pneumocystis carinii pneumonia in simian immunodeficiency virus-infected rhesus macaques. J Infect Dis. **187**: 576–588. 2003. [Medline] [CrossRef]
222. Chatfield K, and Morton D. The use of non-human primates in research. In: Ethics Dumping: Case Studies from North-South Research Collaborations. D Schroeder, Cook, JF Hirsch, S Fenet, and V Muthuswamy (eds). Springer International Publishing. 81–90. 2018.
223. Beineke A, Baumgärtner W, and Wohlsein P. Cross-species transmission of canine distemper virus-an update. One Health. **1**: 49–59. 2015. [Medline] [CrossRef]
224. Sakai K, Nagata N, Ami Y, Seki F, Suzaki Y, Iwata-Yoshikawa N, Suzuki T, Fukushi S, Mizutani T, Yoshikawa T, Otsuki N, Kurane I, Komase K, Yamaguchi R, Hasegawa H, Saijo M, Takeda M, and Morikawa S. Lethal canine distemper virus outbreak in cynomolgus monkeys in Japan in 2008. J Virol. **87**: 1105–1114. 2013. [Medline] [CrossRef]
225. Yoshikawa Y, Ochikubo F, Matsubara Y, Tsuruoka H, Ishii M, Shirota K, Nomura Y, Sugiyama M, and Yamanouchi K. Natural infection with canine distemper virus in a Japanese monkey (Macaca fuscata). Vet Microbiol. **20**: 193–205. 1989. [Medline] [CrossRef]
226. Qiu W, Zheng Y, Zhang S, Fan Q, Liu H, Zhang F, Wang W, Liao G, and Hu R. Canine distemper outbreak in rhesus monkeys, China. Emerg Infect Dis. **17**: 1541–1543. 2011. [Medline]
227. Sun Z, Li A, Ye H, Shi Y, Hu Z, and Zeng L. Natural infection with canine distemper virus in hand-feeding Rhesus monkeys in China. Vet Microbiol. **141**: 374–378. 2010. [Medline] [CrossRef]
228. Dalldorf G, Douglass M, and Robinson HE. Canine distemper in the rhesus monkey (Macaca mulatta). J Exp Med. **67**: 323–332. 1938. [Medline] [CrossRef]
229. A. Headley AS, and Sukura A. Naturally occurring systemic canine distemper virus infection in a pup. Braz J Vet Pathol. **2**: 95–101. 2009.
230. Kubo T, Kagawa Y, Taniyama H, and Hasegawa A. Distribution of inclusion bodies in tissues from 100 dogs infected with canine distemper virus. J Vet Med Sci. **69**: 527–529. 2007. [Medline] [CrossRef]
231. Okamoto M, Miyazawa T, Morikawa S, Ono F, Nakamura S, Sato E, Yoshida T, Yoshikawa R, Sakai K, Mizutani T, Nagata N, Takano J, Okabayashi S, Hamano M, Fujimoto K, Nakaya T, Iida T, Horii T, Miyabe-Nishiwaki T, Watanabe A, Kaneko A, Saito A, Matsui A, Hayakawa T, Suzuki J, Akari H, Matsuzawa T, and Hirai H. Emergence of infectious malignant thrombocytopenia in Japanese macaques (Macaca fuscata) by SRV-4 after transmission to a novel host. Sci Rep. **5**: 8850. 2015. [Medline] [CrossRef]
232. Yoshikawa R, Okamoto M, Sakaguchi S, Nakagawa S, Mi-
ura T, Hirai H, and Miyazawa T. Simian retrovirus 4 induces lethal acute thrombocytopenia in Japanese macaques. J Virol. **89**: 3965–3975. 2015. [Medline] [CrossRef]
233. Reimann KA, Parker RA, Seaman MS, Beaudry K, Beddall M, Peterson L, Williams KC, Veazey RS, Montefiori DC, Mascola JR, Nabel GJ, and Letvin NL. Pathogenicity of simian-human immunodeficiency virus SHIV-89.6P and SIVmac is attenuated in cynomolgus macaques and associated with early T-lymphocyte responses. J Virol. **79**: 8878–8885. 2005. [Medline] [CrossRef]
234. Mokhtari W, Nsaibia S, Majouri D, Ben Hassen A, Gharbi A, and Aouni M. Detection and characterization of Shigella species isolated from food and human stool samples in Nabeul, Tunisia, by molecular methods and culture techniques. J Appl Microbiol. **113**: 209–222. 2012. [Medline] [CrossRef]
235. Van Der Ploeg CA, Viñas MR, Terragno R, Bruno SB, and Binsztein N. Laboratory protocol: serotyping of Shigella spp. WHO Global Foodborne Infections Network, Geneva. 2010.
236. Vervenne RA, Jones SL, van Soolingen D, van der Laan T, Andersen P, Heidt PJ, Thomas AW, and Langermans JA. TB diagnosis in non-human primates: comparison of two interferon-gamma assays and the skin test for identification of Mycobacterium tuberculosis infection. Vet Immunol Immunopathol. **100**: 61–71. 2004. [Medline] [CrossRef]
237. Koide R, Yoshikawa R, Okamoto M, Sakaguchi S, Suzuki J, Isa T, Nakagawa S, Sakawaki H, Miura T, and Miyazawa T. Experimental infection of Japanese macaques with simian retrovirus 5. J Gen Virol. **100**: 266–277. 2019. [Medline] [CrossRef]
238. Takano JI, Leon A, Kato M, Abe Y, and Fujimoto K. Isolation and DNA characterization of a simian retrovirus 5 from a Japanese monkey (Macaca fuscata). J Gen Virol. **94**: 955–959. 2013. [Medline] [CrossRef]
239. Choi YK, Simon MA, Kim DY, Yoon BI, Kwon SW, Lee KW, Seo IB, and Kim DY. Fatal measles virus infection in Japanese macaques (Macaca fuscata). Vet Pathol. **36**: 594–600. 1999. [Medline] [CrossRef]
240. Hirata A, Tachikawa Y, Hashimoto K, Sakai H, Kaneko A, Suzuki J, Eguchi K, Shigematsu K, Nikami H, and Yanai T. Spontaneous T/NK-cell lymphoma associated with simian lymphocryptovirus in a Japanese macaque (Macaca fuscata). J Comp Pathol. **148**: 43–46. 2013. [Medline] [CrossRef]
241. Hirata A, Hashimoto K, Katoh Y, Sakai H, Bruce AG, Rose TM, Kaneko A, Suzuki J, Nikami H, and Yanai T. Characterization of spontaneous malignant lymphomas in Japanese macaques (Macaca fuscata). Vet Pathol. **52**: 566–572. 2015. [Medline] [CrossRef]
242. Adedeji AO, Vemireddi V, Tripathi N, Durinck S, Maher JM, Vucic D, and Halpern W. Atypical presentation and pathogenesis of a macaque lymphocryptoviral-associated B-cell lymphoma in a cynomolgus monkey. Vet Clin Pathol. **49**: 130–136. 2020. [Medline] [CrossRef]