Yang et al. *Zool. Res.* 2025, 46(6): 1549−1564
https://doi.org/10.24272/j.issn.2095-8137.2025.122
https://cstr.cn/32248.14.zr.2025.122

ZR | Zoological Research

Review Open Access

Updated practice for detection of viral infections in breeding macaques

Ling-Yan Yang[1,2], Zhi-Liang Lu[1,2], David Fernig[3], Chang-Jun Shi[4], Rong Rong[1,2,*]

[1] *Department of Biosciences and Bioinformatics*, *School of Science*, *Xi'an Jiaotong-Liverpool University*, *Suzhou*, *Jiangsu* 215123, *China*
[2] *Suzhou Municipal Key Lab of Cancer Biology and Chronic Disease*, *School of Science*, *Xi'an Jiaotong-Liverpool University*, *Suzhou*, *Jiangsu* 215123, *China*
[3] *Department of Biochemistry*, *Cell and Systems Biology*, *Institute of Molecular and Integrative Biology*, *University of Liverpool*, *Liverpool L69 7ZB*, *UK*
[4] *Suzhou Xishan Biotechnology Co., Ltd. (VRL-Asia)*, *Suzhou*, *Jiangsu* 215123, *China*

ABSTRACT

Macaques, particularly rhesus (*Macaca mulatta*) and cynomolgus (*M. fascicularis*) monkeys, are widely utilized in biomedical and toxicological research. Despite their critical role, effective vaccines against many viral pathogens affecting these primates remain limited, and vaccination is generally avoided in experimental populations to prevent potential confounding outcomes. Consequently, maintaining viral control within macaque colonies depends on stringent surveillance, systematic diagnostic testing, and rigorously enforced quarantine protocols. Accurate and early detection of viral infections is therefore essential for colony management and research integrity. This review synthesizes current knowledge on 10 major viruses affecting macaque colonies, outlining their biological characteristics and diagnostic methodologies, and further assesses recent technological advances in viral detection. Drawing upon five years of surveillance data collected by VRL-Asia from breeding facilities across China, this review highlights the prevalence patterns of viral infections among breeding macaques and identifies critical epidemiological trends. These insights provide a valuable reference for researchers, veterinarians, and laboratory personnel seeking to strengthen biosecurity frameworks and ensure the reliability of research involving macaques.

Keywords: Macaques; Specific pathogen-free (SPF); Viruses; Viral infections; Viral testing

INTRODUCTION

Macaques, particularly rhesus (*Macaca mulatta*) and cynomolgus (*M. fascicularis*) monkeys, serve as invaluable models in biomedical and toxicological research due to their close physiological, immunological, and genetic affinity to humans. Like humans, macaques are highly susceptible to a wide range of pathogens, including viruses, bacteria, parasites, and fungi (Liu et al., 2021b; Ohta, 2023). Among them, viral infections represent the most critical threat to animal welfare, research integrity, and biosafety. Notably, many macaque viruses establish chronic or latent infections that can persist asymptomatically, leading to undetected transmission within colonies or to human handlers (Yee et al., 2016).

To minimize these risks, specific pathogen-free (SPF) macaque colonies have been established to exclude high-priority viral agents. Standard SPF colonies focus on eliminating four key viral pathogens, including monkey B virus (BV), simian retrovirus D (SRV), simian immunodeficiency virus (SIV), and simian T-lymphotropic virus (STLV) (Bailey et al., 2016; Balansard et al., 2019; Bibollet-Ruche et al., 2004; Morton et al., 2008; Yee et al., 2016). More stringent SPF super (SPF+) colonies extend this exclusion list to encompass additional viruses such as simian varicella virus (SVV), simian foamy virus (SFV), measles virus (MeV), canine distemper virus (CDV), monkeypox virus (MPV), and simian virus 40 (SV40), thereby achieving broader pathogen control (Olivier et al., 2010; Sariol et al., 2005; Stavisky et al., 2018; Tanaka et al., 2013; Ward & Hilliard, 1994; Yasutomi, 2010). Establishing and maintaining SPF status relies on two key strategies: comprehensive diagnostic screening to identify and remove infected individuals and controlled breeding of certified SPF monkeys to ensure pathogen-free progeny. The effectiveness of these strategies relies fundamentally on precise and reproducible diagnostic technologies that enable continuous surveillance and are essential for sustaining colony health, ensuring biosecurity, and preserving the validity of

Received: 08 July 2025; Accepted: 04 September 2025; Online: 05 September 2025
Foundation items: This work was supported by the Postgraduate Research Scholarship fund of Xi'an Jiaotong-Liverpool University (FOSA2212023 to R.R.) and Research Development Fund of Xi'an Jiaotong-Liverpool University (RDF-22-01-045) to Z.L.L.)
*Corresponding author, E-mail: Rong.Rong@xjtlu.edu.cn

research outcomes.

This review synthesizes current understanding of 10 major viruses that infect macaques, some of which also carry zoonotic potential, including viruses targeted for exclusion in SPF colonies and others not yet formally listed but posing substantial epidemiological or experimental concern. Drawing on nearly five years of diagnostic data collected by VRL-Asia from macaque breeding facilities across China, this analysis further characterizes infection prevalence, temporal patterns, and cross-colony transmission dynamics. This review also discusses technological progress in viral detection, highlighting next-generation platforms with potential for rapid, field-based screening in non-human primate populations. Together, these findings refine pathogen surveillance strategies, reduce transmission risk, and strengthen the health and reliability of macaque colonies essential for biomedical research. Subsequent sections provide a detailed discussion of each virus, including biological properties, zoonotic relevance, and current diagnostic strategies.

Monkey B virus (BV)

BV, officially known as *Macacine alphaherpesvirus* 1, belongs to the family *Herpesviridae*, subfamily *Alphaherpesviridae*, genus *Simplexvirus* (Gatherer et al., 2021). Comparative genomic and phylogenetic studies have identified at least five BV genotypes corresponding to distinct macaque hosts: BVrh from rhesus macaques, BVcy from cynomolgus macaques, BVpt from pig-tail macaques, BVlt from lion-tail macaques, and BVjp from Japanese macaques (Hu et al., 2022; Lu et al., 2023). Whole-genome analyses show approximately 90% identity between BVrh and BVcy and 82% between BVpt and BVlt, suggesting that distinct macaque species may harbor different BV genotypes (Eberle et al., 2017).

Macaques serve as the natural reservoirs for BV. Infections in these macaques resemble human infections with herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), typically remaining mild or subclinical in immunocompetent hosts (Arvin et al., 2007; Eberle & Jones-Engel, 2018). Following primary replication in epithelial cells, BV migrates along sensory neuron and establishes lifelong latency within sensory ganglia, with potential reactivation triggered by immunosuppression, stress, or experimental manipulation (Weigler, 1992). Viral shedding can accompany symptomatic episodes or occur intermittently without clinical signs (Arvin et al., 2007). Infectious particles are excreted in saliva, tears, urine, feces, and genital secretions and transmitted via bites, grooming, sexual contact, or exposure to contaminated fomites (Eberle & Jones-Engel, 2018; Lu et al., 2023). Human infection typically follows direct contact with infected macaques or contaminated materials and can progress to severe neurological complications with a high case-fatality rate if left untreated (Linz et al., 2025). Documented human cases across Asia and a rare report of viral reactivation after 54 years of latency underscore the strong zoonotic potential of BV (Arvin et al., 2007; Ponzetto et al., 2023; Wang et al., 2021; Yamada et al., 2024; Zhang et al., 2022). Although no effective vaccine currently exists, early antiviral therapy using acyclovir, valacyclovir, or famciclovir remains crucial for mitigating severe outcomes in suspected or confirmed cases, with topical cidofovir demonstrating complete efficacy in preventing BV neuroinvasion when administered promptly, including against resistant strains (Cohen et al., 2002; Jerome & Morrow, 2015; Maxwell et al., 2020). Given the risk of asymptomatic viral shedding and the severe consequences of BV infections in humans, regular and frequent testing for BV in macaques is essential for managing SPF colonies and mitigating occupational exposure risks.

BV diagnosis in macaques integrates molecular, serological, and culture-based approaches. Among these, polymerase chain reaction (PCR) assays provide the highest analytical sensitivity for detecting BV DNA in oral, conjunctival, and genital swabs and in blood and cerebrospinal fluid (CSF). Diagnostic sensitivity and specificity depend on selecting appropriate genetic targets. Commonly amplified loci include *US6* (glycoprotein D, gD), *US8* (glycoprotein G, gG), *UL27* (glycoprotein B, gB), and *UL30* (DNA polymerase). Because *UL27* is highly conserved among alphaherpesviruses (Huff et al., 2003), quantitative real-time PCR (qPCR) assays targeting this gene lack BV specificity and may cross-detect closely related viruses. To improve discrimination, a qPCR assay targeting *US8* was developed to differentiate BV from closely related alphaherpesviruses, including HSV-1, HSV-2, *Papiine alphaherpesvirus 2* (Herpesvirus simian 2, HVP-2), and *Cercopithecine alphaherpesvirus 2* (Simian Agent 8, SA8) (Perelygina et al., 2003). Primers and probes designed to target non-conserved regions further enable genotype-level differentiation (Eberle et al., 2017; Smith et al., 1998). Notably, a nested PCR assay targeting the intergenic region between *US5* (encoding glycoprotein L, gL) and *US6* was established to precisely identify and distinguish the BVcy genotype from the BVrh genotype (Slomka et al., 1993). More recently, loop-mediated isothermal amplification (LAMP) techniques have accelerated field diagnostics, enabling rapid identification of BV DNA without thermal cycling (Amano et al., 2023) and the simultaneous detection of BV and MPV with high sensitivity and specificity (Zeng et al., 2023). Despite these advances, molecular assays detect only actively replicating virus at the time of sampling. Therefore, serological testing remains indispensable for comprehensive colony screening, as it identifies antibodies indicative of latent or prior infections, providing a broader epidemiological assessment of BV exposure in macaque populations.

Serological diagnosis of BV infection primarily relies on the detection of anti-BV antibodies using platforms such as enzyme-linked immunosorbent assay (ELISA), dot immunobinding assay (DIA), and enzyme immunoassay (EIA), among which ELISA remains the most widely employed. Western blot (WB) analysis, although less frequently used due to its lower reactivity and the occurrence of nonspecific bands, continues to serve as a valuable confirmatory reference technique (Yee et al., 2016). To reduce biohazard risks associated with preparing BV antigens from infected cell cultures, serological assays often incorporate antigenically related herpesviruses, including HVP-2 and SA8, which provide cross-reactive antigenic profiles without requiring high-risk viral propagation (Pöhlmann et al., 2017; Tanaka et al., 2004). Additionally, advances in recombinant antigen technology have further enhanced assay safety and performance, with recombinant BV glycoproteins gB, gC, gD, and gG exhibiting high sensitivity and specificity for serological detection (Katz et al., 2012; Perelygina et al., 2005).

Antibody responses typically emerge within 7–10 days following infection. IgM antibodies can be detected by day 6, followed by IgG antibodies around day 12, with peak titers between days 30 and 40 (Arvin et al., 2007; Weigler, 1992). Due to the predominantly latent nature of BV infection,

seroconversion often occurs upon viral reactivation. As a result, infection status cannot be determined from a single test, with at least two consecutive negative results required to classify an individual as BV-negative (Ward & Hilliard, 2002; Yee et al., 2016). Continuous surveillance remains necessary, with testing frequency adjusted based on colony conditions ranging from quarterly to annual screening (Yee et al., 2016).

Virus isolation remains the definitive method for confirming viral infections , includin BV. The virus has been recovered from the oral, conjunctival, genital, and trigeminal ganglia samples (Boulter, 1975; Weigler et al., 1993; Zwartouw & Boulter, 1984). BV demonstrates broad cellular tropism, replicating in fibroblasts, epithelial cells, and neurons (Perelygina et al., 2015), and is most commonly propagated using Vero cells (Zwartouw & Boulter, 1984). However, isolation procedures require biosafety level 3 (BSL-3) containment due to the high risk of severe disease in humans. In addition, virus isolation is labor-intensive, time-consuming, and relatively insensitive, limiting its utility in routine diagnostic applications.

Recent epidemiological studies have revealed considerable variation in BV prevalence across macaque populations. In wild macaques from Thailand, BV antibody prevalence ranges from 25% to 100%, with viral shedding detected in 1.04% of oral swabs (Sapkanarak et al., 2025). In a separate serosurvey of 400 breeding macaques from the Anhui Experimental Primate Centre, 36.5% tested positive for BV antibodies based on ELISA and immunoenzyme assays (Cai et al., 2018), indicating widespread exposure within domestic colonies. In contrast, a large-scale serosurvey of 289 549 blood samples tested by VRL-Asia found BV antibodies in 9.4% of cases, with 1 126 samples (0.4%) producing borderline values near the detection threshold. The lower prevalence likely reflects the younger age distribution of animals intended for biomedical research or export, as BV seropositivity correlates with age (Cai et al., 2018). Complementary qPCR screening of 5 978 samples identified only eight BV DNA-positive cases, with two additional indeterminate results, suggesting that most infections were latent at the time of sampling.

Simian varicella virus (SVV)

SVV, also known as *Cercopithecine alphaherpesvirus* 9, is a member of the subfamily *Alphaherpesvirinae* within the family *Herpesviridae* (Gatherer et al., 2021). It exhibits 70%–75% genomic homology with human varicella-zoster virus (VZV), the causative agent of chickenpox and shingles (Mahalingam et al., 2022). The SVV genome spans approximately 124.8 kb and encodes 74 proteins. Although multiple strains have been isolated, only one complete genome sequence is currently available, and no distinct serotypes or genotypes have been documented across macaque species (Gray, 2022; Gray et al., 2001).

SVV infection in macaques mirrors the clinical course of VZV in humans, producing a biphasic disease consisting of a primary varicella-like eruption (with a chickenpox-like rash) followed by latency and potential reactivation manifesting as zoster (shingles-like symptoms) (Gray, 2008). Initial replication occurs in the upper respiratory epithelium before systemic dissemination via T cells to the skin, mucosal surfaces, and visceral organs, including the liver and spleen. The virus also establishes latency within dorsal root ganglia, where reactivation may be triggered by stress or immunosuppression. Transmission occurs via respiratory droplets or direct contact (Dueland et al., 1992; Gray, 2003). Recurrent outbreaks in primate research facilities across the United States have demonstrated the high transmissibility and broad host susceptibility of SVV across monkey species (Clarkson et al., 1967; Allen et al., 1974; Ouwendijk & Verjans, 2015). However, no evidence of zoonotic transmission has been reported (Messaoudi et al., 2009).

Precise detection of SVV in macaques is essential for identifying both active and latent infections, supporting timely intervention, maintaining colony health, and preventing viral transmission. qPCR is the primary molecular technique used for SVV detection, offering high sensitivity and specificity across a range of clinical samples, including blood, saliva, vesicular fluid, and tissue biopsies. Diagnostic accuracy depends on careful selection of gene targets and optimization of primer and probe design. Key open reading frames (ORFs) commonly targeted to detect actively replicating virus include *ORF68* (glycoprotein E, gE), *ORF31* (glycoprotein B, gB), *ORF29* (DNA polymerase), *ORF62* (a major regulatory gene), and *ORF61* (an immediate early gene). These genes play central roles in viral replication and pathogenesis (Gray et al., 2002; Messaoudi et al., 2009). In addition, *ORF61* encodes a latency-associated transcript (LAT) that is particularly valuable for detecting latent infection in ganglionic tissue due to its involvement in the maintenance of viral latency (Jankeel et al., 2021; Meyer et al., 2011; Traina-Dorge et al., 2019). Diagnostic target selection should be guided by both infection stage and sample type to ensure reliable SVV detection. During the early phase of infection, SVV can be detected in both lesion and blood samples. Under outbreak conditions, SVV DNA concentrations in blood may exceed levels observed during varicella infection (Mahalingam et al., 2022; Messaoudi et al., 2009). Oral swabs serve as a practical, noninvasive diagnostic option for detecting acute infection (Traina-Dorge et al., 2019).

Serological assays, including ELISA, DIA, and WB, are used to detect SVV-specific antibodies and monitor prior exposure within macaque populations. These tests support routine screening and epidemiological surveillance, helping determine previous virus exposure or ongoing infections, but cannot distinguish active infection from past exposure due to persistence of antibody responses. SVV-specific IgG becomes detectable by day 14 post-infection and remains elevated for at least 84 days (Jankeel et al., 2021). For serological detection, SVV antigens are typically prepared from SVV-infected Vero cell lysates or through recombinant expression of glycoproteins such as gB and gE in bacterial or mammalian systems to improve assay specificity and reduce cross-reactivity (Gray et al., 2022).

SVV can be isolated *in vitro* from lesions or infected tissues using susceptible cell lines, such as Vero cells, thereby confirming active infection (Messaoudi et al., 2009). However, viral culture is time-consuming, labor-intensive, and requires biosafety level 2 (BSL-2) containment, making it unsuitable for large-scale surveillance. Despite these challenges, culture remains a valuable tool when serological or molecular results are inconclusive.

Over a five year surveillance period, data from VRL-Asia revealed an SVV IgG antibody positivity rate of 9.5% among 5 836 macaque blood samples, with 1.7% yielding borderline ELISA optical density (OD) values near the cutoff. Notably, most samples submitted for qPCR were collected for

diagnostic purposes rather than routine screening. Among 320 samples tested, 14.7% tested SVV DNA-positive and 1.9% produced indeterminate results. In addition to blood, SVV DNA was also identified in skin lesions, oral swabs, and tissue samples from the liver, lung, and lymph nodes, indicating systemic viral dissemination. These findings suggest that SVV may exhibit a higher propensity for reactivation and broader tissue tropism compared to BV, emphasizing the importance of molecular diagnostics and sustained surveillance in macaque colony management.

Simian retrovirus type D (SRV)

SRV is a member of the genus *Betaretrovirus* within the family *Retroviridae*. Its genome consists of a dimeric, linear, positive-sense, single-stranded RNA approximately 8 kb in length, flanked by long terminal repeats (LTRs) and encoding four major genes: *gag* (group-specific antigen), *prt* (protease), *pol* (polymerase), and *env* (envelope) (Coffin et al., 2021). First identified in the 1970s during fatal immunosuppressive outbreaks in primate research facilities in the United States, SRV was recognized as the etiological agent of a progressive and often fatal immunodeficiency syndrome in macaques—simian acquired immune deficiency syndrome (SAIDS)—due to its clinical similarity to human AIDS (Henrickson et al., 1983; Marx et al., 1985). Serological and genomic studies have since delineated at least eight SRV serotypes (SRV-1 to SRV-8) (Nandi et al., 2003, 2006; Takano et al., 2013; Zao et al., 2010).

Transmission occurs through exposure to infected blood, saliva, urine, feces, or vertical transfer from dam to offspring. SRV displays broad cellular tropism, infecting both lymphocytes, including T cells, and non-lymphocytes, including monocytes and dendritic cells (Henrickson et al., 1983; Stromberg et al., 1984). Serotypes exhibit species-specific host preferences (Montiel, 2010): SRV-1, 3, and 5 are more frequently detected in rhesus macaques (Jensen et al., 1970), while SRV-2, 4, and 8 are more common in cynomolgus macaques (Zao et al., 2011, 2016). Following cell entry, viral RNA undergoes reverse transcription into double-stranded DNA, which integrates into the host genome as a provirus, establishing persistent infections characterized by prolonged clinical latency with continuous or intermittent viral shedding before the development of SAIDS. Notably, SRV-1, 2, and 3 are associated with SAIDS following extended latent periods (Marx et al., 1984, 1985). SRV-2 has also been implicated in fibroid development, while SRV-4 causes disease in Japanese but not in cynomolgus macaques (Hara et al., 2007; Zao et al., 2011). Emerging evidence links SRV-5 to hemorrhagic syndrome and SRV-8 to broad immunosuppressive effects through disruption of both innate and adaptive immune signaling (Koide et al., 2019; Xu et al., 2023; Yang et al., 2025; Zhu et al., 2020). Although cross-species transmission to humans is theoretically possible, no human infections have been documented (Zao et al., 2016).

Diagnostic approaches for SRV include both serological and molecular methods. DIA and ELISA are widely employed for high-throughput screening of antibodies against SRV-1–5 and the latterly identified SRV-8 (Montiel, 2010). WB analysis is employed to confirm positive or ambiguous results. Antigen preparation for these assays typically involves viral lysates from SRV-infected Raji (human Burkitt's lymphoma) or A549 (human lung carcinoma) cells (Thouless et al., 1996), or recombinant Gag or Env proteins expressed in heterologous systems (Kwang et al., 1988).

Molecular detection using PCR enables direct identification of viral RNA or proviral DNA in blood, tissue, or secretions. Reverse transcription PCR (RT-PCR) and RT-qPCR are particularly useful for identifying active viral replication in seronegative animals, while proviral DNA detection in peripheral blood mononuclear cells (PBMCs) remains crucial for diagnosing latent infections (Hara et al., 2007). High viral loads in saliva also support its use as a diagnostic sample for PCR testing (Rosenblum et al., 2000). Selection of appropriate gene targets is essential for achieving high diagnostic accuracy in SRV detection. The most frequently targeted genes include *gag*, *pol*, and *env*. For routine screening, a qPCR assay targeting the *env* gene enables simultaneous detection of proviral DNA from SRV-1–5 and SRV-8 (White et al., 2009; Zao et al., 2016). Amplification of variable regions within *env* also allows differentiation among SRV-1 to SRV-5 (White et al., 2009). A separate qPCR platform targeting the *gag* gene has been optimized to detect SRV-1, SRV-2, SRV-3, and SRV-5 concurrently (Chung et al., 2008). Further genetic characterization and phylogenetic analysis of SRV strains can be achieved through viral genome sequencing. Careful primer design is essential to avoid cross-reactivity with other retroviruses or endogenous sequences (Morton et al., 2008). Detection of SRV is complicated by delayed antibody response and the presence of asymptomatic latent carriers, highlighting the necessity of combining antibody and nucleic acid detection to minimize false negatives (Zao et al., 2011). Furthermore, dynamic changes in both antibody titers and viral loads have been observed in SRV-infected macaques, leading to fluctuations in detection rates using both methods (Lerche et al., 1994). Consequently, parallel testing using both antibody and nucleic acid assays is recommended, particularly for long-term colony residents and newly acquired individuals, to ensure early identification of infected animals and to prevent onward transmission.

SRV can be isolated using permissive cell lines, such as Raji cells (Takano et al., 2013). Although viral culture can confirm infection, it is laborious, time-intensive, and requires BSL-2 containment, limiting its utility in routine surveillance.

Over the past five years, VRL-Asia has tested 295 772 serum samples, revealing an SRV antibody-positive rate of 2.9%. In contrast, a nationwide serosurvey of Chinese breeding facilities (n=752) reported higher prevalence, with a 5.2% sample positivity rate and a 16.2% breeding unit positivity rate, accompanied by significant regional disparities (Liu et al., 2021a). These discrepancies in seropositivity may reflect differences in sample populations and testing methodologies. VRL-Asia primarily screened young monkeys intended for research or export, employing ELISA or DIA for initial screening, followed by WB confirmation. In contrast, the Chinese survey relied solely on ELISA, increasing the risk of false-positive results. Additionally, a separate analysis of 231 captive macaques detected SRV antibodies in 5%, exclusively among older individuals (Kaul et al., 2019), suggesting an age-related rise in seroprevalence. Notably, in a study of 411 Chinese rhesus macaques from a single Chinese facility, SRV nucleic acids were detected in 19.7% of samples by PCR (Zhu et al., 2012). In contrast, qPCR screening of 95 281 whole-blood samples by VRL-Asia yielded a lower positive rate of 5.7%, likely due to demographic and procedural differences between cohorts. Of the 2 682 samples tested for both SRV antibodies and nucleic acids, 32 were positive or

indeterminate (P+I). Among these, 34.4% were positive for both markers, 34.4% for antibodies only, and 31.2% for nucleic acids only. These results emphasize the diagnostic limitations of relying on a single method and highlight the necessity of integrating serological and molecular tools to ensure accurate SRV detection (Yee et al., 2016).

Simian T-lymphotropic virus (STLV)

STLV, a member of the genus *Deltaretrovirus* within the family *Retroviridae*, possesses a single-stranded, positive-sense RNA genome approximately 9 kb in length. Upon cellular entry, the RNA genome undergoes reverse transcription into double-stranded DNA and integrates into host chromatin as a provirus. STLV shares a close evolutionary and genomic relationship with *human T-lymphotropic virus* (HTLV), together forming the *primate T-cell lymphotropic virus* (PTLV) group (Hussein et al., 2025). Based on phylogenetic and serological analyses, STLV is classified into four subtypes (STLV-1, STLV-2, STLV-3, and STLV-4), each corresponding to an HTLV counterpart. STLV-1 is widely distributed across Asian and African primates, STLV-4 occurs exclusively in Cameroonian gorillas, STLV-2 has only been detected in captive bonobos in Europe and the United States, and STLV-3 has been identified exclusively in African nonhuman primates (NHPs) (Jiang et al., 2023; Sintasath et al., 2009).

STLV is primarily transmitted through exposure to infected bodily fluids, including blood, saliva, and sexual secretions, as well as through vertical routes such as breastfeeding. Transmission is commonly associated with biting, grooming, or sexual contact (Jégado et al., 2019). The virus preferentially targets CD4+ T cells through cell-to-cell transmission (Jégado et al., 2019). Following integration, proviral latency is established, with periodic reactivation facilitating continued transmission while evading immune clearance. Recent evidence suggests that maternal transmission can occur without inducing seroconversion for extended periods (Grover et al., 2025). Although many infections remain subclinical, chronic STLV infection may lead to T-cell leukemia/lymphoma (ATL) or immune-mediated inflammatory disease in macaques, resembling the pathogenic outcomes of HTLV-1 in humans (Brignolo et al., 2004; Roussel et al., 2015).

STLV detection requires a combination of serological, molecular, and virological methods to ensure diagnostic reliability. Serological assays, including DIA, ELISA, and WB, detect STLV-specific antibodies directed primarily against Env and Gag proteins (Morton et al., 2008). Due to the strong antigenic homology between STLV and HTLV, commercially available HTLV I/II ELISA and WB kits can be used for STLV screening (Ibuki et al., 1997; Rudolph et al., 1992). Antibody development in STLV-1 infection may be significantly delayed, requiring anywhere from 43 weeks to five years to reach detectable levels in some animals (Kidiga et al., 2025; Liška et al., 1997). Therefore, combining serological assays with nucleic acid testing is recommended to enhance diagnostic accuracy and reduce the risk of missed infections.

Detection of STLV by nucleic acid testing primarily involves amplification of proviral DNA from PBMCs, most commonly using qPCR. Selection of gene targets depends on the specific diagnostic objective—whether broad detection, subtype differentiation, or phylogenetic analysis is required. To ensure both sensitivity and specificity, assays are designed to amplify a combination of conserved and variable genomic regions. The tax gene, which encodes a regulatory protein critical for viral replication and pathogenesis, is frequently targeted due to its high sequence conservation across all known STLV subtypes (Alais et al., 2018; Naderi et al., 2012). The *pol* gene, which encodes viral enzymes required for reverse transcription and integration, is also commonly used in diagnostic assays (Dube et al., 2013). For subtyping and evolutionary analysis, the env gene is preferred, as it contains more variable regions that allow for discrimination among STLV subtypes and facilitate phylogenetic reconstruction (Mahieux et al., 1997).

Virus isolation remains technically challenging due to its low *in vitro* replication rate. However, STLV can be cultured by co-incubating PBMCs with mitogen-stimulated human umbilical cord blood lymphocytes for several weeks under BSL-2 conditions (Ibuki et al., 1997).

Based on surveillance data over the past five years, VRL-Asia reported an STLV antibody prevalence of 1.4% from 257 304 serum samples. In contrast, an independent seroprevalence study identified a 10% positivity rate in adult and aged animals, suggesting age-related increases in cumulative exposure (Kaul et al., 2019). qPCR screening by VRL-Asia detected STLV proviral DNA in 7.5% of 306 samples, substantially exceeding the seropositivity rate. This disparity likely reflects the delayed or absent antibody responses observed in a subset of infected individuals. Nucleic acid testing provides superior sensitivity, enabling detection of early or latent infections even in the absence of measurable antibody titers. Because integrated proviral DNA persists throughout the host lifespan, qPCR remains effective for long-term surveillance, while antibody levels may decline or fluctuate, especially during chronic infection. These findings underscore the diagnostic limitations of serology alone and support the implementation of combined testing strategies to enhance early detection and reduce the risk of undetected transmission within macaque colonies.

Simian immunodeficiency virus (SIV)

SIV is a lentivirus within the family *Retroviridae*. Its positive-sense, single-stranded RNA genome, approximately 9.5 kb in length, is reverse-transcribed into double-stranded DNA and integrated into the host genome upon infection (Coffin et al., 2021). SIV has been identified in over 40 species of NHPs and exhibits extensive genetic diversity. At least 10 distinct phylogenetic lineages have been defined, sharing about 60% sequence identity (Aghokeng et al., 2010; Wilde et al., 2023).

Natural hosts include various African monkey species and chimpanzees, in which SIV typically establishes lifelong, asymptomatic infections without marked immunopathology (Diop et al., 2002). In contrast, Asian primates, particularly macaques, are not natural hosts but can be experimentally infected (Flynn, 2024; Locatelli et al., 2014). SIV is primarily transmitted through sexual contact, blood exchange, and mother-to-offspring transmission via breastfeeding or *in utero* exposure. SIV infects $CD4^+$ T cells, macrophages, and dendritic cells, establishing latent reservoirs and persistent viremia in natural hosts. In macaques, however, it causes AIDS-like disease characterized by CD4+ depletion, hematologic abnormalities, opportunistic infections, and lymphoproliferative disorders (He et al., 2022; Silvestri et al., 2007). Consequently, SIV-infected rhesus macaques serve as the principal animal model for studying HIV pathogenesis and evaluating therapeutic strategies (Sambaturu et al., 2025).

SIV is included in the standard diagnostic panel for SPF

macaques, necessitating highly sensitive and specific diagnostic methods (Morton et al., 2008; Yee et al., 2016). The primary diagnostic approach involves serological detection of virus-specific antibodies in the host using techniques such as DIA, ELISA, and WB. Antigen preparations for these assays typically include lysates from virus-infected cells or recombinant viral proteins such as envelope glycoprotein gp120 and capsid protein p27 (Lehner et al., 1994; Li et al., 2017; Rychert & Amedee, 2005). Updated ELISA platforms incorporating synthetic gp41 peptides have demonstrated 96% sensitivity and 97.5% specificity for antibody detection (Aghokeng et al., 2010). WB remains the confirmatory standard, with specimens showing reactivity to both Env and Gag proteins classified as seropositive, while those showing partial reactivity are considered indeterminate (Li et al., 2017). Given the possibility of delayed seroconversion, follow-up testing at 3–6 month intervals is recommended for newly acquired animals or those with indeterminate results (Murphy et al., 2006).

Molecular diagnostics complement serological screening by directly detecting viral nucleic acids. Notably, RT-PCR and RT-qPCR target *pol*, *gag*, or LTR sequences and can detect SIV RNA in plasma, saliva, and tissue biopsies (Long & Berkemeier, 2020; Monjure et al., 2014). Proviral DNA PCR is used to identify integrated SIV DNA in PBMCs and lymphoid tissues, offering particular utility in detecting latent infections. Recent applications of digital droplet PCR (ddPCR) have improved sensitivity for detecting low-level viral genomes in clinical samples (Long & Berkemeier, 2020, 2021, 2022). With the increasing use of lentiviral vectors in research and gene therapy, careful design of primers and probes is crucial to avoid non-specific amplification of viral-derived sequences, which may lead to false-positive results. Alternatively, simultaneously targeting multiple viral genes can enhance diagnostic reliability, ensuring accurate detection of SIV infection.

Virus isolation may be performed by co-culturing PBMCs with CEMx174 cells (Plummer, 1962). However, this method requires BSL-3 laboratory conditions and extended incubation, making it unsuitable for routine detection.

Over the past five years, large-scale screening by VRL-Asia has detected no SIV-positive macaques. More than 234 000 serum samples have been screened using DIA, ELISA, and WB to detect SIV-specific antibodies, and over 300 PBMC samples have been analyzed by qPCR for proviral DNA. Notably, the absence of positive cases confirms that Asian macaques do not serve as natural reservoirs for SIV, consistent with global epidemiological findings.

Simian foamy virus (SFV)

SFV belongs to the genus *Spumavirus* within the family *Retroviridae*. It possesses a positive-sense, single-stranded RNA genome approximately 13 kb in length, which is reverse-transcribed into double-stranded DNA upon infection (Coffin et al., 2021). First isolated in 1954 from monkey kidney cells, SFV was named for the distinctive “foamy” cytopathic effect observed in infected cells (Plummer, 1962). The virus exhibits marked species specificity, although occasional cross-species transmission to humans has been documented following direct exposure to infected primates (Liu, 2016; Switzer & Heneine, 2011; Switzer et al., 2025).

SFV naturally infects a wide range of NHPs, spreading primarily through bites, saliva exchange, and potentially via grooming or maternal transmission during nursing (Murray & Linial, 2019). Initial replication occurs in fibroblasts and epithelial cells before the virus disseminates to immune cells, establishing systemic infection (Stenbak et al., 2020). Unlike other retroviruses, SFV maintains a lifelong, nonpathogenic infection in its hosts (Jones-Engel et al., 2007). The proviral genome integrates into host DNA, permitting persistent, low-level replication in immune cells—particularly PBMCs—while forming latent reservoirs in lymphoid tissues. High salivary viral loads facilitate efficient horizontal transmission (Stenbak et al., 2020). Human infection occurs primarily through primate bites, scratches, or exposure to contaminated biological materials, with high seroprevalence reported among individuals in occupations involving frequent primate contact (Switzer et al., 2025; Virdana et al., 2024). Although infection appears lifelong, no pathogenic outcomes have been confirmed; nevertheless, its zoonotic potential warrants ongoing surveillance (Boneva et al., 2007; Rua & Gessain, 2015).

Diagnosis of SFV infection incorporates serological, molecular, and virological approaches. Serological assays, such as ELISA and WB, are routinely used to detect SFV-specific antibodies in clinical samples, with WB providing superior specificity for confirmatory testing. Antigens for SFV serological assays are typically derived from whole-viral lysates or recombinant Gag and Env proteins (Murray et al., 2006).

Molecular detection by PCR or RT-qPCR targeting *pol*, *gag*, or LTR sequences allows identification of SFV proviral DNA or RNA in saliva, blood, or tissue samples (Khan et al., 1999; Muniz et al., 2017; Murray et al., 2006). For colony surveillance, detection of proviral DNA remains the preferred approach as it reliably identifies latent infection. Virus isolation can be achieved by co-culturing PBMCs with fibroblast or epithelial cell lines under BSL-2 conditions, producing the characteristic foamy cytopathic effect; however, this method is rarely used in routine diagnostics due to its technical demands (Khan et al., 1999).

Reported SFV prevalence among adult primates often exceeds 50%, and in some wild populations approaches 100%, indicating widespread endemic infection (Kaewchot et al., 2022; Kaul et al., 2019). Data from VRL-Asia over the past five years show an antibody-positive rate of 21.2% among 2 865 macaque serum samples, representing one of the highest rates among retroviral pathogens. In contrast, qPCR testing of 2 850 blood samples yielded a much lower positivity rate of 1.0%. Among 2 682 samples analyzed by both methods, 20.2% were antibody-positive or indeterminate, whereas only 4.0% showed nucleic acid detection. Of all samples with positive or indeterminate results, 16.5% were positive by both methods, 81.0% by antibody testing alone, and 2.5% by qPCR alone. The marked difference between serological and molecular results suggests that most infections are latent or nonreplicative, with persistent antibody responses but minimal detectable viral DNA. Latency and transcriptional silencing may account for undetectable proviral sequences despite continued seropositivity. As most samples originated from two semi-free-ranging institutions, broader surveys are required to determine SFV prevalence across diverse macaque breeding populations and environmental settings.

Measles virus (MeV)

MeV, a member of the genus *Morbillivirus* within the family *Paramyxoviridae*, contains a 15.9 kb negative-sense single-

stranded RNA genome (Rima et al., 2019). The original prototype strain was isolated in 1954 from a human throat swab, establishing MeV as a distinct human pathogen (Enders & Peebles, 1954). The World Health Organization (WHO) currently recognizes 24 MeV genotypes based on sequence variability in the nucleocapsid (*N*) and hemagglutinin (*H*) genes. Of these, only genotypes B3 and D8 remain in global circulation, responsible for the majority of recent outbreaks worldwide (Rubalskaia et al., 2023).

MeV is a highly contagious virus that causes measles, an acute febrile illness characterized by high fever, cough, coryza, conjunctivitis, and a generalized maculopapular rash (Ohta, 2023). While humans are the only natural reservoir of MeV, NHPs exhibit high susceptibility, with transmission from infected humans leading to outbreaks and mortality rates as high as 23% in captive primate populations (Choi et al., 1999; Ortiz-Cam et al., 2023). Primary infection begins in the respiratory epithelium, followed by systemic dissemination through the lymphatic and circulatory systems. The virus targets epithelial and immune tissues, producing rash, pneumonia, immunosuppression, and encephalitis. Transmission occurs via aerosolized respiratory droplets (Jones-Engel et al., 2006).

RT-PCR remains the diagnostic gold standard, as outlined in comprehensive WHO guidelines (World Health Organization, 2007). RT-qPCR can detect MeV RNA in nasopharyngeal swabs, blood, urine, and pulmonary tissue with high sensitivity and specificity, targeting conserved regions of the *N* and *H* genes. In addition, an *N* gene-based RT-qPCR assay has been developed to distinguish wild-type strains from vaccine-derived variants (Roy et al., 2017). Optimal diagnostic sensitivity is achieved when samples are collected within 3–4 days after rash onset (World Health Organization, 2007). Although infectious virus is typically cleared by day 14, MeV RNA can persist in PBMCs for 30–90 days and in lymphoid tissues for up to six months (Nelson et al., 2020).

Serological testing complements molecular diagnostics and is routinely used to assess exposure within macaque colonies. Enzyme immunoassays, including EIA, ELISA, and DIA, detect antibodies in clinical samples. Diagnostic antigens are typically derived from virus-infected cell lysates (Boteler et al., 1983) or produced recombinantly, with the N protein being the most commonly used (Samuel et al., 2003). In MeV-infected animals, MeV-specific IgM antibodies can be detected as early as 9–13 days post-infection, while IgG antibodies become detectable from day 13, reaching peak levels by day 24 (El Mubarak et al., 2007). Neutralizing antibodies appear in conjunction with rash onset and undergo avidity maturation over 3–4 months (Nelson et al., 2020). In breeding populations, serological screening is widely employed to monitor prior exposure or vaccination status and to exclude ongoing MeV circulation.

Virus isolation from throat, nasal, or urine samples can be performed using Vero-SLAM cells under BSL-2 conditions (World Health Organization, 2007). Although effective for confirming infection, isolation is rarely used for routine diagnostics in macaque colonies due to its labor-intensive nature and the limited viability of clinical samples.

Over the past five years, VRL-Asia has conducted ELISA-based serological testing on 9 189 macaque serum samples, yielding a 48.4% antibody-positive rate for MeV. In contrast, RT-qPCR screening of 1 055 specimens revealed no active MeV infection. Several factors may explain this disparity. Vaccination with live attenuated measles virus is occasionally administered as a preventive measure in breeding facilities (Nederlof et al., 2025), resulting in seropositivity without active infection. Cross-reactivity with CDV, a related morbillivirus, may also lead to false positives in serological assays. Additionally, prior MeV infections may have been resolved, leaving persistent antibody titers in the absence of detectable viral RNA. These findings highlight the importance of interpreting serological and molecular results in parallel, particularly in settings with mixed exposure histories or vaccination practices.

Canine distemper virus (CDV)

CDV, a negative-sense, single-stranded RNA virus approximately 15.7 kb in length, is classified within the genus *Morbillivirus* of the family *Paramyxoviridae*. Although CDV primarily infects domestic dogs, it also causes disease in a wide range of carnivores and primates, including wolves, foxes, raccoons, bears, tigers, and NHPs (Beineke et al., 2015). No evidence currently supports zoonotic transmission to humans (Karki et al., 2022). The earliest confirmed CDV infection in macaques was reported in 1989, involving a Japanese macaque with severe viral encephalitis (Yoshikawa et al., 1989). Subsequent outbreaks have been documented in China, including rhesus macaque colonies in Guangxi in 2006 and Beijing in 2008, as well as cynomolgus macaques exported to Japan from China (Sakai et al., 2013; Sun et al., 2010) and hand-reared cynomolgus monkeys in southern China (Wang et al., 2025). Despite having only one recognized serotype, CDV strains exhibit considerable geographic diversity, with *H* gene sequencing delineating multiple genotypes from America, Asia, Europe, Africa, and Australia, suggesting potential variations in host adaptation (Wipf et al., 2025).

CDV initiates infection in the respiratory epithelium and disseminates via lymphatic and hematogenous routes to multiple organ systems, often producing severe pneumonia, immunosuppression, and neurological sequelae (Sakai et al., 2013; Shin et al., 2022). Clinical manifestations include fever, ocular and nasal discharge, coughing, diarrhea, and generalized rash (Ohta, 2023). Transmission occurs predominantly through aerosolized respiratory droplets or direct contact with infected bodily fluids or contaminated fomites. Recent evidence has confirmed prolonged environmental persistence of infectious particles, complicating outbreak control (Allen et al., 2023; Wilkes, 2022).

Laboratory confirmation of CDV relies on molecular, serological, and virological methods. RT-qPCR remains the preferred method for detecting CDV RNA in blood, cerebrospinal fluid, nasal swabs, and tissue samples from the respiratory or nervous systems (Sakai et al., 2013). Conserved regions of the *N*, *P*, and *H* genes serve as primary targets for amplification (Rivera-Martínez et al., 2024). To enhance diagnostic precision, assays targeting the *M* gene and *M-F* intergenic regions have been developed to differentiate between vaccine-derived and wild-type strains (Wilkes et al., 2014). A dual-target RT-qPCR assay incorporating the *H* gene further improves detection of low viral loads and enables genotype differentiation (Sui et al., 2023).

Serological detection of CDV-specific antibodies is routinely conducted using ELISA, with antigens typically derived from

lysates of cells infected with either wild-type or vaccine strains. The temporal profiles of IgM and IgG responses are comparable to those observed during MeV infection. Cross-reactivity between CDV and MeV should be carefully considered when interpreting serological results.

Although CDV can be isolated by culturing clinical samples in Vero or MDCK cells, where syncytia and other cytopathic effects confirm viral presence (Sakai et al., 2013), isolation is infrequently used due to the extended culture time required and the need for BSL-2 facilities (Rendon-Marin et al., 2019).

Between 2019 and 2024, VRL-Asia screened 7 172 serum samples for CDV-specific antibodies and reported a seropositivity rate of 54.6%. In contrast, RT-qPCR testing of 12 518 samples yielded a positivity rate of only 1.8%. This marked disparity likely reflects multiple factors, including prior vaccination, serological cross-reactivity with related morbilliviruses, and the transient nature of viremia. Because CDV RNA is typically detectable only during periods of active viral shedding, animals with resolved infections or in latent phases may not be identified by nucleic acid testing.

Monkeypox virus (MPV)

MPV, a member of the genus *Orthopoxvirus* within the subfamily *Chordopoxvirinae* of the family *Poxviridae*, possesses a large double-stranded DNA genome of approximately 197 kb in length, encoding around 190 ORFs (McInnes et al., 2023; Nakhaie et al., 2023). Two major phylogenetic clades have been identified, including the Congo Basin (Central African) clade, which exhibits higher virulence and mortality, and the West African clade, which causes a less severe clinical course with substantially lower case fatality rates (Moss, 2024).

Although first identified in 1958 during an outbreak in captive monkeys, MPV is believed to circulate naturally among African rodents and other small mammals. Zoonotic pathogen transmission to humans and NHPs occurs through direct contact with infected animals, their secretions, or contaminated materials (von Magnus et al., 1959), while human-to-human transmission is primarily via respiratory droplets or close contact with lesion material. As of July 2024, 102 997 laboratory-confirmed human infections and 223 associated deaths were reported across 121 countries (Jadhav et al., 2025), highlighting MPV as a significant re-emerging threat with expanding global distribution. In macaques, MPV infection results in a disease resembling smallpox, characterized by fever, lethargy, lymphadenopathy, and a generalized vesiculopustular rash typically involving the face, limbs, palms, and soles. Following initial replication in epithelial and immune cells at the entry site, the virus disseminates through the lymphatic and hematogenous routes to visceral organs including the spleen, liver, and lungs. In severe cases, particularly in immunocompromised hosts, systemic disease may progress to pneumonia, gastrointestinal pathology, or multi-organ failure (Johnson et al., 2011). MPV is shed through cutaneous lesions, respiratory secretions, and various bodily fluids, including blood, urine, and feces. Notably, viral DNA can persist for extended periods in skin crusts, prolonging the window for potential transmission (Mitjà et al., 2023).

qPCR enables rapid and sensitive detection of MPV DNA in clinical specimens, including lesion exudates, blood, and respiratory secretions. Diagnostic assays frequently target the *E9L* gene, which encodes DNA polymerase and supports high-sensitivity detection across orthopoxviruses, and the *B6R* gene, encoding an extracellular envelope protein, which permits specific identification of MPV when paired with fluorescent probes (Nakhaie et al., 2023). Additional targets include the RNA polymerase subunit 18 (*RPO18*) gene for broad *Orthopoxvirus* screening and the complement-binding protein (*C3L*) gene for MPV-specific confirmation (Nakhaie et al., 2023). Recently, qPCR assays targeting the *J2L* and *B7R* genes have further improved diagnostic precision (Li et al., 2006; Marennikova et al., 1971).

Serological detection for MPV exposure involves detecting virus-specific antibodies, with ELISA widely applied for population-level screening and WB used for confirmation. Assays typically employ antigens derived from vaccinia virus preparations or recombinant MPV proteins (Alakunle et al., 2024; Chauhan et al., 2023). However, this approach is suboptimal for early-stage diagnosis due to the delayed humoral response following infection.

MPV can be isolated from lesion material using chicken embryo chorioallantoic membranes, which produce characteristic hemorrhagic lesions, or through propagation in cultured cell lines such as Vero, A-1, and HEP-2. Unlike variola and vaccinia viruses, MPV does not replicate efficiently in pig embryonic kidney (PEK) cells, serving as a useful differential marker (Chauhan et al., 2023). It is important to note that all MPV isolation procedures must be conducted in a BSL-3 laboratory.

Over the past five years, VRL-Asia has analyzed 12 982 serum samples for MPV-specific antibodies and 475 samples by qPCR, with no evidence of infection detected. These findings are consistent with previous reports indicating that MPV infections are primarily endemic in West and Central Africa, despite recent global outbreaks (Jadhav et al., 2025).

Simian virus (SV40)

SV40 is a non-enveloped, icosahedral polyomavirus with a circular double-stranded DNA genome of approximately 5.2 kb classified within the genus *Betapolyomavirus* of the family *Polyomaviridae* (Moens et al., 2017). Originally discovered as a contaminant in early polio vaccines administered between 1955 and 1963, SV40 is now recognized as an endemic virus in NHPs, particularly macaques (Sweet & Hilleman, 1960; Wilson, 2022), and has been identified in human tissues, prompting extensive research into its potential oncogenicity and zoonotic relevance (Carbone et al., 2020; Limam et al., 2020; Liu et al., 2024).

In its natural hosts, SV40 predominantly causes asymptomatic infections, suggesting a well-adapted virus-host relationship. Transmission within macaque populations occurs via exposure to infected secretions, particularly saliva and urine, or through contact with contaminated surfaces in communal enclosures (Ilyinskii et al., 1992). Following primary infection of the respiratory epithelium, the virus disseminates to lymphoid tissues and kidneys, where it can establish latent reservoirs, and occasionally to the central nervous system, with urinary shedding facilitating transmission among susceptible hosts (Butel & Lednicky, 1999; Ohta, 2023). In immunocompromised hosts, particularly SIV-infected rhesus macaques, SV40 reactivation can occur in renal and neural tissues (Ohta, 2023).

The virus exhibits oncogenic potential through its large T-antigen (LTAg), which inactivates tumor suppressors p53 and Rb to drive cellular transformation (An et al., 2012; Googins

et al., 2025), although direct evidence of oncogenesis in macaques remains limited. In humans, SV40 is associated with mesotheliomas, brain tumors, osteosarcomas, and lymphomas (Carbone et al., 2020; Limam et al., 2020; Rotondo et al., 2019), highlighting its cross-species pathological significance.

Molecular diagnostics focus on PCR amplification of conserved viral genes such as *LTag*, *VP1*, and *VP2* (Kiasari et al., 2022), enabling sensitive and specific amplification of SV40 sequences in blood, urine, tissues (e.g., kidney and brain), and environmental samples (Bofill-Mas et al., 2004; Newman et al., 1998). Serological testing complements nucleic acid detection by assessing virus-specific immune responses. ELISA, the most common method, employs whole-virus preparations or recombinant proteins, such as VP1 and LTAg, as antigens for seroprevalence studies. Although rarely implemented in routine diagnostics, SV40 isolation using permissive cell lines such as BSC-1, Vero, or MRC-5 remains feasible under BSL-2 containment (Bofill-Mas et al., 2004; Motamedi et al., 2020), albeit with limited practical utility due to its low efficiency and extended culture times.

National diagnostic standards in China do not currently mandate SV40 screening and its inclusion in routine surveillance remains uncommon. Nevertheless, to assess SV40 prevalence, VRL-Asia analyzed 457 macaque serum samples from 23 facilities and revealed an antibody-positive rate of 82.5%, aligning with previous estimates of 92.1% in Chinese rhesus macaques and 95% in cynomolgus macaques (Verschoor et al., 2008). In contrast, PCR screening of 383 blood samples from 16 farms identified SV40 DNA in only 2.9%, consistent with the known tropism of the virus for renal, brain, and lymphoid tissues rather than peripheral blood (Fagrouch et al., 2011; Newman et al., 1998). Given its high seroprevalence, it is worth considering the incorporation of SV40 surveillance in current screening guidelines, particularly in primate colonies involved in vaccine production.

Other viruses

Beyond the 10 principal viruses discussed, other viral agents, such as Lymphocryptovirus (LCV), rhesus cytomegalovirus (RhCMV), rhesus rhadinovirus (RRV), and simian adenoviruses (SAdVs) (O'Sullivan et al., 1994; Wevers et al., 2011; Yee et al., 2023), warrant attention due to their potential impact on macaque health and research outcomes (Ohta, 2023; Wachtman & Mansfield, 2012). While not included in routine SPF screening, these viruses should be considered in facilities housing immunocompromised animals or observing unexplained clinical syndromes.

SUMMARY AND FUTURE PROSPECTS

This review provides a comprehensive overview of current knowledge on 10 viral agents targeted for exclusion from macaque breeding populations, with implications for biomedical research validity and animal welfare. By delineating the virological features and host infection patterns of each pathogen, the discussion provides an evidence-based framework for selecting diagnostic modalities and interpreting surveillance results to strengthen and inform colony management and biosecurity strategies.

Routine detection protocols rely on standardized serological and molecular assays. For alphaherpesviruses such as BV and SVV, which establish lifelong latency in sensory ganglia with potential for subclinical reactivation, serological assays (e.g., ELISA) remain the cornerstone of routine monitoring. Nucleic acid-based testing (e.g., qPCR) serves as a complementary approach for confirming active infection during reactivation episodes. In contrast, retroviruses, including SRV, STLV, SIV, and SFV, require integrated testing approaches combining serology and detection of proviral DNA to capture both active and latent infections and minimize the risk of undetected carriers. A similar dual-modality strategy is critical for paramyxoviruses, such as MeV and CDV, which pose diagnostic challenges due to antibody cross-reactivity, particularly when interpreting positive antibody results in animals vaccinated against MeV.

VRL-Asia has established and rigorously validated qPCR and ELISA platforms under China National Accreditation Service for Conformity Assessment (CNAS)-accredited quality frameworks. Assay performance was verified through comprehensive internal benchmarking, inter-laboratory comparisons, and ongoing proficiency assessments. The qPCR system demonstrated high analytical sensitivity (1.3–20.0 copies/μL), strong target specificity, and robust precision, with intra- and inter-assay coefficients of variation (CVs) maintained below 2.0% and 7.0%, respectively. Similarly, the ELISA platform exhibited robust analytical sensitivity, reliably detecting positive controls at dilutions up to 1:512, with minimal cross-reactivity limited to CDV and MeV antigens, and consistent precision across assays (intra-assay CVs <17.0%, inter-assay CVs <19.0%) (Supplementary Tables S1, S2). Application of these standardized assays across 2020–2024 surveillance data from a broad spectrum of macaque populations—including open breeding facilities and high-containment research environments such as academic institutions, contract research organizations, and biomedical laboratories—revealed distinct epidemiological patterns. SIV and MPV were undetectable in all samples, indicating successful exclusion from these populations. In contrast, seropositivity for SVV, SFV, and SV40 remained elevated, indicating persistent circulation despite existing controls (Table 1). These findings signal the effectiveness of current biosecurity protocols for SIV and MPV, while highlighting the need for revised containment and eradication strategies for SFV and SV40. CDV also remains a concern, as specific viral strains are capable of inducing acute systemic disease in macaques, with reported case fatality rates approaching 10% (Sakai et al., 2013). These results reinforce the importance of targeted, data-driven surveillance strategies in SPF colonies. Routine screening protocols should be guided by pathogen prevalence and facility-specific risk assessments rather than uniform, inflexible panels. Adoption of an adaptive testing framework enables efficient deployment of resources, maintains high diagnostic coverage, and minimizes unnecessary testing burdens while enhancing overall colony health management.

Recent advances in viral detection technologies have significantly enhanced diagnostic speed, accuracy, and accessibility. Novel platforms such as isothermal nucleic acid amplification techniques (INAATs), ddPCR, and clustered regularly interspaced short palindromic repeat (CRISPR)-associated (Cas) detection systems have overcome key limitations of conventional diagnostics, including reliance on thermal cycling, high-cost instrumentation, and specialized technical expertise. As summarized in Table 2, these methods provide streamlined, rapid, and cost-effective solutions suitable for both laboratory and field-based applications.

Table 1 Virological characteristics and regional prevalence of common viruses in macaques

Virus	Family	Genome	Infection in macaques	Zoonotic	Transmission	Infection in humans	References	Prevalence (%) *
BV	*Herpesviridae*	dsDNA	Highly prevalent; Asymptomatic; Latent infection	Yes	Bites, scratches	Fatal encephalitis (80% mortality)	Hu et al., 2022; Lu et al., 2023	9.4 (*n*=289 549) 0.1 (*n*=5 978)
SRV	*Retroviridae*	ssRNA-RT	Regional; Asymptomatic or Immunodeficiency, lymphoma	Yes	Bodily fluids, vertical	Asymptomatic	Montiel, 2010; Nham et al., 2024	2.9 (*n*=295 772) 5.7 (*n*=95 281)
STLV	*Retroviridae*	ssRNA-RT	Low prevalence; Asymptomatic or T-cell leukemia	Potential	Vertical, sexual	No evidence	Jégado et al., 2019; Ohta, 2023	1.4 (*n*=257 304) 7.5 (*n*=306)
SIV	*Retroviridae*	ssRNA-RT	Low prevalence; AIDS-like disease	No evidence	Mucosal, blood	No evidence	Jasinska et al., 2023; Locatelli et al., 2014	0 (*n*=234 786) 0 (*n*=322)
MPV	*Poxviridae*	dsDNA	Low prevalence; Pustular rash, systemic illness	Yes	Contact, respiratory	Severe rash, fever, lymphadenopathy	Alakunle et al., 2024; Moss, 2024	0 (*n*=12 982) 0 (*n*=475)
MeV	*Paramyxoviridae*	ssRNA(-)	Low prevalence; Rash, immunosuppression	Yes	Respiratory droplets	Classic measles	Dogadov et al., 2023; El Mubarak et al., 2007	48.4** (*n*=9 189) 0 (*n*=1 055)
SFV	*Retroviridae*	ssRNA-RT	Highly prevalent; Asymptomatic; Persistent infection	Yes	Bites, scratches	Asymptomatic or mild seroconversion	Couteaudier et al., 2022; Stenbak et al., 2020	21.2 (*n*=2 865) 1.0 (*n*=2 850)
SV40	*Polyomaviridae*	dsDNA	Highly prevalent; Nephropathy, tumors	Yes	Contaminated vaccines	Mesothelioma (debated link)	Bofill-Mas et al., 2004; Milavetz & Balakrishnan, 2025	83.8 (*n*=495) 2.9 (*n*=383)
SVV	*Herpesviridae*	dsDNA	Highly prevalent; Varicella-like rash	No evidence	Aerosols	No evidence	Jankeel et al., 2021; Mahalingam et al., 2022	9.5 (*n*=5 836) 14.7 (*n*=320)
CDV	*Paramyxoviridae*	ssRNA(-)	Regional; Neurological signs	No evidence	Respiratory	No evidence	Karki et al., 2022; Sakai et al., 2013	54.6** (*n*=7 172) 1.8 (*n*=12 518)

*: Detection data for monkey viruses were derived from samples submitted to the VRL-Asia testing facility between 1 January 2020 and 31 December 2024. For each virus, the first percentage represents the virus antibody-positive rate, while the second indicates the nucleic acid-positive rate. Sample sizes are provided in parentheses. **: To prevent measles outbreaks, some farms vaccinate macaques against measles, resulting in the presence of measles antibodies in vaccinated animals. Additionally, as MeV and CDV belong to the same genus (*Morbillivirus*), cross-reactivity in serological assays may occur, causing animals infected with CDV to test positive for measles antibodies, and vice versa. BV: Monkey B virus; SRV: Simian retrovirus type D; STLV: Simian T-lymphotropic virus; SIV: Simian immunodeficiency virus; MPV: Monkeypox virus; MeV: Measles virus; SFV: Simian foamy virus; SV40: Simian virus 40; SVV: Simian varicella virus; CDV: Canine distemper virus. dsDNA: Double-stranded DNA; ssRNA-RT: Single-stranded RNA with reverse transcriptase; ssRNA(-): Negative-sense single-stranded RNA.

Table 2 Comparative analysis of viral detection methods in macaques

Parameter	DIA	ELISA	WB	qPCR	ddPCR	LAMP, RPA-CRISPR
Detection target	Viral antibodies	Viral antibodies	Viral antibodies	Viral nucleic acids	Viral nucleic acids	Viral nucleic acids
Typical viruses detected	BV, SRV, STLV, SIV, MPV	BV, SRV, STLV, SIV, MeV, CDV, SV40, SVV	SRV, SIV, STLV	SRV, SFV, STLV	BV, SRV, STLV	BV, MPV, MeV
Sensitivity	Medium	High	Low	Very high	Very high	High
Specificity	Medium	Medium	High	Very high	Very high	High
Sample type	Serum, plasma	Serum, plasma	Serum, plasma	Whole blood, tissue, lesional swab	Whole blood, tissue, lesional swab	Lesional swab, body fluid
Turnaround time (hour)	2–4	2–4	6–24	2–3	3–4	0.5–2
Equipment needs	Water bath	Microplate reader, incubator	Water bath	Nucleic acid extraction and qPCR system	Nucleic acid extraction and ddPCR system	Portable reader
Automation potential	Low	High	None	Medium	Low	Medium
Best application scenario	Quarantine screening	Large-scale surveillance	Confirmatory testing	Quarantine screening and confirmation	Confirmatory testing	POCT

BV: Monkey B virus; SRV: Simian retrovirus type D; STLV: Simian T-lymphotropic virus; SIV: Simian immunodeficiency virus; MPV: Monkeypox virus; MeV: Measles virus; SFV: Simian foamy virus; SV40: Simian virus 40; SVV: Simian varicella virus; CDV: Canine distemper virus.

INAATs, such as recombinase polymerase amplification (RPA), can be integrated with CRISPR-Cas detection systems and visualized through fluorescence or lateral-flow readouts. This configuration has achieved high analytical sensitivity and specificity for MPV detection in clinical samples, enabling point-of-care testing (POCT) in resource-limited settings without the need for thermal cycling (Chen et al., 2025; Guo et al., 2025; Low et al., 2023). Similarly, a multiplex LAMP assay has been developed for the simultaneous detection of BV and MPV, with a detection threshold as low as 3 copies/μL for BV DNA (Zeng et al., 2023). Notably, ddPCR offers precise, absolute quantification of viral nucleic acids without dependence on external calibration curves (Kojabad et al., 2021). This technique has proven effective in detecting low-level SIV replication in animals under antiretroviral suppression (Long & Berkemeier, 2020) and has

demonstrated high sensitivity for CDV, with detection limits as low as 3 copies/μL (Iribarnegaray et al., 2024).

These emerging platforms represent a major advance in molecular diagnostics, offering high sensitivity, rapid turnaround, and broad scalability for infectious disease surveillance. While qPCR remains the standard in diagnostic laboratories due to its reliability and compatibility with high-throughput workflows, INAATs offer critical advantages for rapid on-site testing to mitigate risks to humans and animals, particularly during zoonotic emergencies involving BV, MPV, or MeV outbreaks. In addition, ddPCR extends diagnostic capabilities by enabling precise quantification of low-copy or inhibitor-rich viral targets, including retroviruses such as SRV, SFV, and STLV, which are frequently harbored at low levels in chronically infected monkeys. Although these newer technologies gained prominence during the COVID-19 pandemic, their broader integration into routine diagnostics for breeding colonies remains constrained by high reagent costs and equipment complexity. Nonetheless, improvements in automation, miniaturization, and reagent stability are expected to accelerate their adoption. Ongoing development should prioritize reducing costs, simplifying workflows, and improving accessibility to enable broad implementation of these technologies across research and surveillance platforms.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

L.Y.Y. contributed to manuscript writing and final editing. R.R. contributed to the critical revision of the article and funding acquisition. Z.L.L., C.J.S., and D.F. contributed to the conception and revision of this manuscript. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

We sincerely appreciate VRL-Asia for providing testing data on the viruses discussed in this manuscript. We also extend our gratitude to Ying-Juan Huang and Ting Zhu for collecting diagnostic method validation data, Shu-Ting Zhu, Jue Wang, and Shen-Lu Hang for contributing supplementary test data, and Li-Peng Wang and Xin-Ru Sun for sample collection.

REFERENCES

Aghokeng AF, Ayouba A, Mpoudi-Ngole E, et al. 2010. Extensive survey on the prevalence and genetic diversity of SIVs in primate bushmeat provides insights into risks for potential new cross-species transmissions. *Infection, Genetics and Evolution*, **10**(3): 386–396.

Alais S, Pasquier A, Jegado B, et al. 2018. STLV-1 co-infection is correlated with an increased SFV proviral load in the peripheral blood of SFV/STLV-1 naturally infected non-human primates. *PLoS Neglected Tropical Diseases*, **12**(10): e0006812.

Alakunle E, Kolawole D, Diaz-Cánova D, et al. 2024. A comprehensive review of monkeypox virus and mpox characteristics. *Frontiers in Cellular and Infection Microbiology*, **14**: 1360586.

Allen C, Ellis A, Liang RB, et al. 2023. Prolonged persistence of canine distemper virus RNA, and virus isolation in naturally infected shelter dogs. *PLoS One*, **18**(1): e0280186.

Allen WP, Felsenfeld AD, Wolf RH, et al. 1974. Recent studies on the isolation and characterization of Delta herpesvirus. *Laboratory Animal Science*, **24**(1): 222–228.

Amano M, Sapkanarak K, Thbthimthong W, et al. 2023. Development of quantitative real-time PCR and loop-mediated isothermal amplification assays for the surveillance and diagnosis of herpes B virus infection. *Viruses*, **15**(10): 2086.

An P, Sáenz Robles MT, Pipas JM. 2012. Large T antigens of polyomaviruses: amazing molecular machines. *Annual Review of Microbiology*, **66**: 213–236.

Arvin A, Campadelli-Fiume G, Mocarski E, et al. 2007. Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis. Cambridge: Cambridge University Press.

Bailey AL, Lauck M, Sibley SD, et al. 2016. Zoonotic potential of simian Arteriviruses. *Journal of Virology*, **90**(2): 630–635.

Balansard I, Cleverley L, Cutler KL, et al. 2019. Revised recommendations for health monitoring of non-human primate colonies (2018): FELASA Working Group Report. *Laboratory Animals*, **53**(5): 429–446.

Beineke A, Baumgärtner W, Wohlsein P. 2015. Cross-species transmission of canine distemper virus-an update. *One Health*, **1**: 49–59.

Bibollet-Ruche F, Bailes E, Gao F, et al. 2004. New simian immunodeficiency virus infecting De Brazza's monkeys (*Cercopithecus neglectus*): evidence for a *Cercopithecus* monkey virus clade. *Journal of Virology*, **78**(14): 7748–7762.

Bofill-Mas S, Albiñana-Giménez N, Pipkin PA, et al. 2004. Isolation of SV40 from the environment of a colony of cynomolgus monkeys naturally infected with the virus. *Virology*, **330**(1): 1–7.

Boneva RS, Switzer WM, Spira TJ, et al. 2007. Clinical and virological characterization of persistent human infection with simian foamy viruses. *AIDS Research and Human Retroviruses*, **23**(11): 1330–1337.

Boteler WL, Luipersbeck PM, Fuccillo DA, et al. 1983. Enzyme-linked immunosorbent assay for detection of measles antibody. *Journal of Clinical Microbiology*, **17**(5): 814–818.

Boulter EA. 1975. The isolation of Monkey B virus (*Herpesvirus simiae*) from the trigeminal ganglia of a healthy seropositive rhesus monkey. *Journal of Biological Standardization*, **3**(3): 279–280.

Brignolo L, Spinner A, Yee JL, et al. 2004. Subsets of T cells in healthy rhesus macaques (*Macaca mulatta*) infected with simian T-lymphotropic virus type 1. *Comparative Medicine*, **54**(3): 271–274.

Butel JS, Lednicky JA. 1999. Cell and molecular biology of simian virus 40: implications for human infections and disease. *JNCI: Journal of the National Cancer Institute*, **91**(2): 119–134.

Cai B, Dai CW, Li S, et al. 2018. Analysis on antibody testing of B virus (corcopithecine herpesvirus 1). *Anhui Medical Journal*, **39**(3): 261–263. (In Chinese)

Carbone M, Gazdar A, Butel JS. 2020. SV40 and human mesothelioma. *Translational Lung Cancer Research*, **9**(Suppl 1): S47–S59.

Chauhan RP, Fogel R, Limson J. 2023. Overview of diagnostic methods, disease prevalence and transmission of Mpox (formerly monkeypox) in humans and animal reservoirs. *Microorganisms*, **11**(5): 1186.

Chen H, Cheng XD, Wang YX, et al. 2025. Two-dimensional nanozyme-catalyzed colorimetric CRISPR assay for the microfluidic detection of Monkeypox Virus. *Analytical Chemistry*, **97**(8): 4407–4415.

Choi YK, Simon MA, Kim DY, et al. 1999. Fatal measles virus infection in Japanese macaques (Macaca fuscata). *Veterinary Pathology*, **36**(6): 594–600.

Chung HK, Unangst T, Treece J, et al. 2008. Development of real-time PCR assays for quantitation of simian betaretrovirus serotype-1, -2, -3, and -5 viral DNA in Asian monkeys. *Journal of Virological Methods*, **152**(1-2): 91–97.

Clarkson MJ, Thorpe E, McCarthy K. 1967. A virus disease of captive vervet monkeys (Cercopithecus aethiops) caused by a new herpesvirus. *Archiv für die Gesamte Virusforschung*, **22**(1): 219–234.

Coffin J, Blomberg J, Fan H, et al. 2021. ICTV virus taxonomy profile: *Retroviridae* 2021. *The Journal of General Virology*, **102**(12): 001712.

Cohen JI, Davenport DS, Stewart JA, et al. 2002. Recommendations for prevention of and therapy for exposure to B virus (*Cercopithecine herpesvirus* 1). *Clinical Infectious Diseases*, **35**(10): 1191–1203.

Couteaudier M, Montange T, Njouom R, et al. 2022. Plasma antibodies from humans infected with zoonotic simian foamy virus do not inhibit cell-to-cell transmission of the virus despite binding to the surface of infected cells. *PLoS Pathogens*, **18**(5): e1010470.

Diop OM, Guèye A, Ayouba A, et al. 2002. Simian immunodeficiency viruses and the origin of HIVs. *In*: Essex M, Mboup S, Kanki PJ, et al. AIDS in Africa. 2nd ed. Boston: Springer, 104–120.

Dogadov DI, Kyuregyan KK, Goncharenko AM, et al. 2023. Measles in non-human primates. *Journal of Medical Primatology*, **52**(2): 135–143.

Dube S, Saksena N, Spicer T, et al. 2013. Delayed seroconversion to STLV-1 infection is associated with mutations in the pol and rex genes. *Virology Journal*, **10**: 282.

Dueland AN, Martin JR, Devlin ME, et al. 1992. Acute simian varicella infection. Clinical, laboratory, pathologic, and virologic features. *Laboratory Investigation*, **66**(6): 762–773.

Eberle R, Jones-Engel L. 2018. Questioning the extreme neurovirulence of monkey B virus (*Macacine alphaherpesvirus 1*). *Advances in Virology*, **2018**: 5248420.

Eberle R, Maxwell LK, Nicholson S, et al. 2017. Genome sequence variation among isolates of monkey B virus (*Macacine alphaherpesvirus* 1) from captive macaques. *Virology*, **508**: 26–35.

El Mubarak HS, Yüksel S, van Amerongen G, et al. 2007. Infection of cynomolgus macaques (*Macaca fascicularis*) and rhesus macaques (*Macaca mulatta*) with different wild-type measles viruses. *The Journal of General Virology*, **88**(Pt 7): 2028–2034.

Enders JF, Peebles TC. 1954. Propagation in tissue cultures of cytopathogenic agents from patients with measles. *Proceedings of the Society for Experimental Biology and Medicine*, **86**(2): 277–286.

Fagrouch Z, Karremans K, Deuzing I, et al. 2011. Molecular analysis of a novel simian virus 40 (SV40) type in rhesus macaques and evidence for double infections with the classical SV40 type. *Journal of Clinical Microbiology*, **49**(4): 1280–1286.

Flynn JK. 2024. Investigating Mechanisms of Microbial Translocation in SIV Infection of Rhesus Macaques. Ph. D. dissertation, Georgetown University, Washington.

Gatherer D, Depledge DP, Hartley CA, et al. 2021. ICTV virus taxonomy profile: *Herpesviridae* 2021. *The Journal of General Virology*, **102**(10): 001673.

Googins MR, An P, Gauthier CH, et al. 2025. Polyomavirus large T antigens: unraveling a complex interactome. *Tumour Virus Research*, **19**: 200306.

Gray WL. 2003. Pathogenesis of simian varicella virus. *Journal of Medical Virology*, **70 Suppl 1**(S1): S4–S8.

Gray WL. 2008. Simian varicella in old world monkeys. *Comparative Medicine*, **58**(1): 22–30.

Gray WL. 2022. Comparative analysis of the simian varicella virus and varicella zoster virus genomes. *Viruses*, **14**(5): 844.

Gray WL, Mullis L, Soike KF. 2002. Viral gene expression during acute simian varicella virus infection. *The Journal of General Virology*, **83**(Pt 4): 841–846.

Gray WL, Starnes B, White MW, et al. 2001. The DNA sequence of the simian varicella virus genome. *Virology*, **284**(1): 123–130.

Gray WL, Wichman G, Das A, et al. 2022. An enzyme-linked immunosorbent assay (ELISA) to determine Simian Varicella Virus antibody titers in infected rhesus monkeys (*Macaca mulatta*). *Journal of Medical Primatology*, **51**(1): 20–26.

Grover P, Murata M, Kidiga M, et al. 2025. Identification of natural remission of mother-to-child retroviral transmission. *The Journal of Infectious Diseases*, **232**(1): 203–211.

Guo JL, Shan YF, Hu G, et al. 2025. Rapid visual detection of Monkeypox virus by one-step LAMP-CRISPR/Cas12b assay. *Virology Journal*, **22**(1): 151.

Hara M, Kikuchi T, Sata T, et al. 2007. 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**(2): 281–288.

He X, Aid M, Ventura JD, et al. 2022. Rapid loss of CD4 T cells by pyroptosis during acute SIV infection in Rhesus macaques. *Journal of Virology*, **96**(17): e00808–22.

Henrickson RV, Osborn KG, Madden DL, et al. 1983. Epidemic of acquired immunodeficiency in rhesus monkeys. *The Lancet*, **321**(8321): 388–390.

Hu G, Du HJ, Liu YN, et al. 2022. Herpes B virus: history, zoonotic potential, and public health implications. *Biosafety and Health*, **4**(4): 213–219.

Huff JL, Eberle R, Capitanio J, et al. 2003. Differential detection of B virus and rhesus cytomegalovirus in rhesus macaques. *The Journal of General Virology*, **84**(Pt 1): 83–92.

Hussein O, Mahgoub M, Shichijo T, et al. 2025. Evolution of primate T-cell leukemia virus type 1 accessory genes and functional divergence of its antisense proteins. *PLoS Pathogens*, **21**(5): e1013158.

Ibuki K, Ido E, Setiyaningsih S, et al. 1997. Isolation of STLV-I from orangutan, a great ape species in Southeast Asia, and its relation to other HTLV-Is/STLV-Is. *Japanese Journal of Cancer Research: Gann*, **88**(1): 1–4.

Ilyinskii PO, Daniel MD, Horvath CJ, et al. 1992. Genetic analysis of simian virus 40 from brains and kidneys of macaque monkeys. *Journal of Virology*, **66**(11): 6353–6360.

Iribarnegaray V, Godiño G, Larrañaga C, et al. 2024. Droplet digital PCR enhances sensitivity of canine distemper virus detection. *Viruses*, **16**(11): 1720.

Jadhav V, Paul A, Trivedi V, et al. 2025. Global epidemiology, viral evolution, and public health responses: a systematic review on Mpox (1958–2024). *Journal of Global Health*, **15**: 04061.

Jankeel A, Coimbra-Ibraim I, Messaoudi I. 2021. Simian varicella virus: molecular virology and mechanisms of pathogenesis. *In*: Arvin AM, Moffat JF, Abendroth A, et al. Varicella-Zoster Virus: Genetics, Pathogenesis and Immunity. Cham: Springer, 163–188.

Jasinska AJ, Apetrei C, Pandrea I. 2023. Walk on the wild side: SIV infection in African non-human primate hosts-from the field to the laboratory. *Frontiers in Immunology*, **13**: 1060985.

Jégado B, Kashanchi F, Dutartre H, et al. 2019. STLV-1 as a model for studying HTLV-1 infection. *Retrovirology*, **16**(1): 41.

Jensen EM, Zelljadt I, Chopra HC, et al. 1970. Isolation and propagation of a virus from a spontaneous mammary carcinoma of a rhesus monkey. *Cancer Research*, **30**(9): 2388–2393.

Jerome KR, Morrow RA. 2015. Herpes simplex viruses and herpes B virus. *In*: Jorgensen JH, Carroll KC, Funke G, et al. Manual of Clinical Microbiology. 11th ed. Washington: ASM Press, 1687–1703.

Jiang XJ, Fan ZY, Li SJ, et al. 2023. A review on zoonotic pathogens associated with non-human primates: Understanding the potential threats to humans. *Microorganisms*, **11**(2): 246.

Johnson RF, Dyall J, Ragland DR, et al. 2011. Comparative analysis of monkeypox virus infection of cynomolgus macaques by the intravenous or intrabronchial inoculation route. *Journal of Virology*, **85**(5): 2112–2125.

Jones-Engel L, Engel GA, Schillaci MA, et al. 2006. Considering human-primate transmission of measles virus through the prism of risk analysis. *American Journal of Primatology*, **68**(9): 868–879.

Jones-Engel L, Steinkraus KA, Murray SM, et al. 2007. Sensitive assays for simian foamy viruses reveal a high prevalence of infection in commensal, free-ranging Asian monkeys. *Journal of Virology*, **81**(14): 7330–7337.

Kaewchot S, Tangsudjai S, Sariya L, et al. 2022. Zoonotic pathogens

survey in free-living long-tailed macaques in Thailand. *International Journal of Veterinary Science and Medicine*, **10**(1): 11–18.

Karki M, Rajak KK, Singh RP. 2022. Canine morbillivirus (CDV): a review on current status, emergence and the diagnostics. *VirusDisease*, **33**(3): 309–321.

Katz D, Shi W, Patrusheva I, et al. 2012. An automated ELISA using recombinant antigens for serologic diagnosis of B virus infections in macaques. *Comparative Medicine*, **62**(6): 527–534.

Kaul A, Schönmann U, Pöhlmann S. 2019. Seroprevalence of viral infections in captive rhesus and cynomolgus macaques. *Primate Biology*, **6**(1): 1–6.

Khan AS, Sears JF, Muller J, et al. 1999. Sensitive assays for isolation and detection of simian foamy retroviruses. *Journal of Clinical Microbiology*, **37**(8): 2678–2686.

Kiasari BA, Fallah FH, Koohi MK, et al. 2022. Simian virus 40 DNA in immunocompetent children with respiratory disease. *Journal of Medical Virology*, **94**(11): 5507–5511.

Kidiga M, Murata M, Grover P, et al. 2025. Identification of occult simian T-cell Leukemia virus type 1 infection in Japanese macaques. *The Journal of Infectious Diseases*, **232**(2): 510–518.

Koide R, Yoshikawa R, Okamoto M, et al. 2019. Experimental infection of Japanese macaques with simian retrovirus 5. *Journal of General Virology*, **100**(2): 266–277.

Kojabad AA, Farzanehpour M, Galeh HEG, et al. 2021. Droplet digital PCR of viral DNA/RNA, current progress, challenges, and future perspectives. *Journal of Medical Virology*, **93**(7): 4182–4197.

Kwang HS, Barr PJ, Sabin EA, et al. 1988. Simian retrovirus-D serotype 1 (SRV-1) envelope glycoproteins gp70 and gp20: expression in yeast cells and identification of specific antibodies in sera from monkeys that recovered from SRV-1 infection. *Journal of Virology*, **62**(5): 1774–1780.

Lehner T, Bergmeier LA, Tao L, et al. 1994. Targeted lymph node immunization with simian immunodeficiency virus p27 antigen to elicit genital, rectal, and urinary immune responses in nonhuman primates. *Journal of Immunology*, **153**(4): 1858–1868.

Lerche NW, Yee JL, Jennings MB. 1994. Establishing specific retrovirus-free breeding colonies of macaques: an approach to primary screening and surveillance. *Laboratory Animal Science*, **44**(3): 217–221.

Li HZ, Nykoluk M, Li L, et al. 2017. Natural and cross-inducible anti-SIV antibodies in Mauritian cynomolgus macaques. *PLoS One*, **12**(10): e0186079.

Li Y, Olson VA, Laue T, et al. 2006. Detection of *monkeypox virus* with real-time PCR assays. *Journal of Clinical Virology*, **36**(3): 194–203.

Limam S, Missaoui N, Bdioui A, et al. 2020. Investigation of simian virus 40 (SV40) and human JC, BK, MC, KI, and WU polyomaviruses in glioma. *Journal of Neurovirology*, **26**(3): 347–357.

Linz B, Muchohi S, Barton Nielsen R. 2025. Herpes B virus: occupational exposures and diagnostics. *Clinics in Laboratory Medicine*, **45**(1): 63–71.

Liška V, Fultz PN, Su LY, et al. 1997. Detection of simian T cell leukemia virus type I infection in seronegative macaques. *AIDS Research and Human Retroviruses*, **13**(13): 1147–1153.

Liu DY. 2016. Molecular Detection of Human Viral Pathogens. Boca Raton: CRC Press.

Liu RA, Wang BY, Chen X, et al. 2024. Association study of pleural mesothelioma and oncogenic simian virus 40 in the crocidolite-contaminated area of Dayao county, Yunnan province, southwest China. *Genetic Testing and Molecular Biomarkers*, **28**(5): 189–198.

Liu YQ, Yu H, Wu RK, et al. 2021a. Serological investigation on simian type D Retrovirus in monkey farms in some areas of China. *China Animal Health Inspection*, **38**(11): 27–30. (In Chinese)

Liu ZJ, Qian XK, Hong MH, et al. 2021b. Global view on virus infection in non-human primates and implications for public health and wildlife conservation. *Zoological Research*, **42**(5): 626–632.

Locatelli S, McKean KA, Sesink Clee PR, et al. 2014. The evolution of resistance to simian immunodeficiency virus (SIV): a review. *International Journal of Primatology*, **35**(2): 349–375.

Long S, Berkemeier B. 2020. Development and optimization of a simian immunodeficiency virus (SIV) droplet digital PCR (ddPCR) assay. *PLoS One*, **15**(10): e0240447.

Long S, Berkemeier B. 2021. Development of a reverse transcription droplet digital PCR (RT-ddPCR) assay for sensitive detection of simian immunodeficiency virus (SIV). *Virology Journal*, **18**(1): 35.

Long S, Berkemeier B. 2022. Ultrasensitive detection and quantification of viral nucleic acids with Raindance droplet digital PCR (ddPCR). *Methods*, **201**: 49–64.

Low SJ, O'neill MT, Kerry WJ, et al. 2023. Rapid detection of monkeypox virus using a CRISPR-Cas12a mediated assay: a laboratory validation and evaluation study. *The Lancet Microbe*, **4**(10): e800–e810.

Lu JL, Long YR, Sun JH, et al. 2023. Towards a comprehensive view of the herpes B virus. *Frontiers in Immunology*, **14**: 1281384.

Mahalingam R, Feia B, Coleman C, et al. 2022. Simian varicella virus pathogenesis in skin during varicella and zoster. *Viruses*, **14**(6): 1167.

Mahieux R, Pecon-Slattery J, Gessain A. 1997. Molecular characterization and phylogenetic analyses of a new, highly divergent simian T-cell lymphotropic virus type 1 (STLV-1marc1) in Macaca arctoides. *Journal of Virology*, **71**(8): 6253–6258.

Marennikova SS, Gurvich EB, Shelukhina EM. 1971. Comparison of the properties of five pox virus strains isolated from monkeys. *Archiv für die Gesamte Virusforschung*, **33**(3): 201–210.

Marx PA, Bryant ML, Osborn KG, et al. 1985. Isolation of a new serotype of simian acquired immune deficiency syndrome type D retrovirus from Celebes black macaques (Macaca nigra) with immune deficiency and retroperitoneal fibromatosis. *Journal of Virology*, **56**(2): 571–578.

Marx PA, Maul DH, Osborn KG, et al. 1984. Simian AIDS: isolation of a type D retrovirus and transmission of the disease. *Science*, **223**(4640): 1083–1086.

Maxwell LK, Black DH, Wright GE, et al. 2020. Effective prophylactic therapy for exposure to monkey B virus (*Macacine alphaherpesvirus* 1). *Comparative Medicine*, **70**(1): 56–66.

McInnes CJ, Damon IK, Smith GL, et al. 2023. ICTV virus taxonomy profile: *Poxviridae* 2023. *The Journal of General Virology*, **104**(5): 001849.

Messaoudi I, Barron A, Wellish M, et al. 2009. Simian varicella virus infection of rhesus macaques recapitulates essential features of varicella zoster virus infection in humans. *PLoS Pathogens*, **5**(11): e1000657.

Meyer C, Kerns A, Barron A, et al. 2011. Simian varicella virus gene expression during acute and latent infection of rhesus macaques. *Journal of Neurovirology*, **17**(6): 600–612.

Milavetz BI, Balakrishnan L. 2025. Simian virus 40 (SV40) - Fresh perspectives on a historic virus. *Virology*, **604**: 110427.

Mitjà O, Ogoina D, Titanji BK, et al. 2023. Monkeypox. *The Lancet*, **401**(10370): 60–74.

Moens U, Calvignac-Spencer S, Lauber C, et al. 2017. ICTV virus taxonomy profile: *Polyomaviridae*. *Journal of General Virology*, **98**(6): 1159–1160.

Monjure CJ, Tatum CD, Panganiban AT, et al. 2014. Optimization of PCR for quantification of simian immunodeficiency virus genomic RNA in plasma of rhesus macaques (*Macaca mulatta*) using armored RNA. *Journal of Medical Primatology*, **43**(1): 31–43.

Montiel NA. 2010. REVIEW ARTICLE: an updated review of simian *betaretrovirus* (SRV) in macaque hosts. *Journal of Medical Primatology*, **39**(5): 303–314.

Morton WR, Agy MB, Capuano SV, et al. 2008. Specific pathogen-free macaques: definition, history, and current production. *ILAR Journal*, **49**(2):

137–144.
Moss B. 2024. Understanding the biology of monkeypox virus to prevent future outbreaks. *Nature Microbiology*, **9**(6): 1408–1416.
Motamedi N, Sewald X, Luo Y, et al. 2020. SV40 polyomavirus activates the Ras-MAPK signaling pathway for vacuolization, cell death, and virus release. *Viruses*, **12**(10): 1128.
Muniz CP, Zheng HQ, Jia HW, et al. 2017. A non-invasive specimen collection method and a novel simian foamy virus (SFV) DNA quantification assay in New World primates reveal aspects of tissue tropism and improved SFV detection. *PLoS One*, **12**(9): e0184251.
Murphy HW, Miller M, Ramer J, et al. 2006. Implications of simian retroviruses for captive primate population management and the occupational safety of primate handlers. *Journal of Zoo and Wildlife Medicine*, **37**(3): 219–233.
Murray SM, Linial ML. 2019. Simian foamy virus Co-infections. *Viruses*, **11**(10): 902.
Murray SM, Picker LJ, Axthelm MK, et al. 2006. Expanded tissue targets for foamy virus replication with simian immunodeficiency virus-induced immunosuppression. *Journal of Virology*, **80**(2): 663–670.
Naderi M, Paryan M, Azadmanesh K, et al. 2012. Design and development of a quantitative real time PCR assay for monitoring of HTLV-1 provirus in whole blood. *Journal of Clinical Virology*, **53**(4): 302–307.
Nakhaie M, Arefinia N, Charostad J, et al. 2023. Monkeypox virus diagnosis and laboratory testing. *Reviews in Medical Virology*, **33**(1): e2404.
Nandi JS, Tikute SA, Chhangani AK, et al. 2003. Natural infection by simian retrovirus-6 (SRV-6) in Hanuman langurs (*Semnopithecus entellus*) from two different geographical regions of India. *Virology*, **311**(1): 192–201.
Nandi JS, Van Dooren S, Chhangani AK, et al. 2006. New simian β retroviruses from rhesus monkeys (*Macaca mulatta*) and langurs (*Semnopithecus entellus*) from Rajasthan, India. *Virus Genes*, **33**(1): 107–116.
Nederlof RA, Sainmaa S, Wissink-Argilaga N, et al. 2025. Preventative vaccination of nonhuman primates. *Journal of Zoological and Botanical Gardens*, **6**(1): 8.
Nelson AN, Lin WW, Shivakoti R, et al. 2020. Association of persistent wild-type measles virus RNA with long-term humoral immunity in rhesus macaques. *JCI Insight*, **5**(3): e134992.
Newman JS, Baskin GB, Frisque RJ. 1998. Identification of SV40 in brain, kidney and urine of healthy and SIV-infected rhesus monkeys. *Journal of Neurovirology*, **4**(4): 394–406.
Nham P, Halley B, Van Rompay KKA, et al. 2024. Simian retrovirus transmission in rhesus macaques. *Journal of Medical Primatology*, **53**(4): e12726.
Ohta E. 2023. Pathologic characteristics of infectious diseases in macaque monkeys used in biomedical and toxicologic studies. *Journal of Toxicologic Pathology*, **36**(2): 95–122.
Olivier KJ Jr, Price KD, Hutto DL, et al. 2010. Naturally occurring infections in non-human primates (NHP) and immunotoxicity implications: discussion sessions. *Journal of Immunotoxicology*, **7**(2): 138–146.
Ortiz-Cam L, Jones-Engel L, Mendoza P, et al. 2023. Association between seroprevalence of measles virus in monkeys and degree of human-monkey contact in Bangladesh. *One Health*, **17**: 100571.
O'Sullivan MG, Anderson DC, Fikes JD, et al. 1994. Identification of a novel simian parvovirus in cynomolgus monkeys with severe anemia. A paradigm of human B19 parvovirus infection. *The Journal of Clinical Investigation*, **93**(4): 1571–1576.
Ouwendijk WJD, Verjans GMGM. 2015. Pathogenesis of varicelloviruses in primates. *The Journal of Pathology*, **235**(2): 298–311.
Perelygina L, Patrusheva I, Hombaiah S, et al. 2005. Production of herpes B virus recombinant glycoproteins and evaluation of their diagnostic potential. *Journal of Clinical Microbiology*, **43**(2): 620–628.
Perelygina L, Patrusheva I, Manes N, et al. 2003. Quantitative real-time PCR for detection of monkey B virus (*Cercopithecine herpesvirus* 1) in clinical samples. *Journal of Virological Methods*, **109**(2): 245–251.
Perelygina L, Patrusheva I, Vasireddi M, et al. 2015. B virus (*Macacine herpesvirus 1*) glycoprotein D is functional but dispensable for virus entry into macaque and human skin cells. *Journal of Virology*, **89**(10): 5515–5524.
Plummer G. 1962. Foamy virus of monkeys. *Microbiology*, **29**(4): 703–709.
Pöhlmann S, Krüger A, Hafezi W, et al. 2017. Detection systems for antibody responses against herpes B virus. *Primate Biology*, **4**(1): 9–16.
Ponzetto E, Delhez Q, Hoppenbrouwers M, et al. 2023. Case report: late reactivation of herpes B virus after a monkey bite: a case of severe meningoencephalitis. *The American Journal of Tropical Medicine and Hygiene*, **109**(6): 1277–1281.
Rendon-Marin S, da Fontoura Budaszewski R, Canal CW, et al. 2019. Tropism and molecular pathogenesis of canine distemper virus. *Virology Journal*, **16**(1): 30.
Rima B, Balkema-Buschmann A, Dundon WG, et al. 2019. ICTV virus taxonomy profile: *Paramyxoviridae*. *Journal of General Virology*, **100**(12): 1593–1594.
Rivera-Martínez A, Rodríguez-Alarcón CA, Adame-Gallegos JR, et al. 2024. Canine distemper virus: origins, mutations, diagnosis, and epidemiology in Mexico. *Life*, **14**(8): 1002.
Rosenblum LL, Weiss RA, Mcclure MO. 2000. Virus load and sequence variation in simian retrovirus type 2 infection. *Journal of Virology*, **74**(8): 3449–3454.
Rotondo JC, Mazzoni E, Bononi I, et al. 2019. Association between simian virus 40 and human tumors. *Frontiers in Oncology*, **9**: 670.
Roussel M, Pontier D, Ngoubangoye B, et al. 2015. Modes of transmission of simian T-lymphotropic Virus Type 1 in semi-captive mandrills (*Mandrillus sphinx*). *Veterinary Microbiology*, **179**(3-4): 155–161.
Roy F, Mendoza L, Hiebert J, et al. 2017. Rapid identification of measles virus vaccine genotype by Real-Time PCR. *Journal of Clinical Microbiology*, **55**(3): 735–743.
Rua R, Gessain A. 2015. Origin, evolution and innate immune control of simian foamy viruses in humans. *Current Opinion in Virology*, **10**: 47–55.
Rubalskaia TS, Erokhov DV, Zherdeva PE, et al. 2023. Global genetic diversity of measles virus (Paramyxoviridae: *Morbillivirus: Morbillivirus hominis*): historical aspects and current state. *Voprosy virusologii*, **68**(5): 361–371.
Rudolph DL, Yee J, Mone J, et al. 1992. Serologic confirmation of simian T-lymphotropic virus type I infection by using immunoassays developed for human T-lymphotropic virus antibody detection. *Journal of Clinical Microbiology*, **30**(4): 858–861.
Rychert J, Amedee AM. 2005. The antibody response to SIV in lactating rhesus macaques. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, **38**(2): 135–141.
Sakai K, Nagata N, Ami Y, et al. 2013. Lethal canine distemper virus outbreak in cynomolgus monkeys in Japan in 2008. *Journal of Virology*, **87**(2): 1105–1114.
Sambaturu N, Fray EJ, Hariharan V, et al. 2025. SIV proviruses seeded later in infection are harbored in short-lived $CD4^+$ T cells. *Cell Reports*, **44**(5): 115663.
Samuel D, Sasnauskas K, Jin L, et al. 2003. Development of a measles specific IgM ELISA for use with serum and oral fluid samples using recombinant measles nucleoprotein produced in *Saccharomyces cerevisiae*. *Journal of Clinical Virology*, **28**(2): 121–129.
Sapkanarak K, Yasuda J, Amano M, et al. 2025. Prevalence of herpes B virus in wild long-tailed macaques, Thailand, 2018–2024. *Emerging Infectious Diseases*, **31**(4): 741–750.
Sariol CA, Arana T, Maldonado E, et al. 2005. Herpes B-virus seroreactivity

in a colony of *Macaca mulatta*: data from the Sabana Seca Field Station, a new specific pathogen-free program. *Journal of Medical Primatology*, **34**(1): 13–19.

Shin DL, Chludzinski E, Wu NH, et al. 2022. Overcoming the barrier of the respiratory epithelium during canine distemper virus infection. *mBio*, **13**(1): e03043–21.

Silvestri G, Paiardini M, Pandrea I, et al. 2007. Understanding the benign nature of SIV infection in natural hosts. *Journal of Clinical Investigation*, **117**(11): 3148–3154.

Sintasath DM, Wolfe ND, Zheng HQ, et al. 2009. Genetic characterization of the complete genome of a highly divergent simian T-lymphotropic virus (STLV) type 3 from a wild *Cercopithecus mona* monkey. *Retrovirology*, **6**: 97.

Slomka MJ, Brown DWG, Clewley JP, et al. 1993. Polymerase chain reaction for detection of herpesvirus simiae (B virus) in clinical specimens. *Archives of Virology*, **131**(1-2): 89–99.

Smith AL, Black DH, Eberle R. 1998. Molecular evidence for distinct genotypes of monkey B virus (herpesvirus simiae) which are related to the macaque host species. *Journal of Virology*, **72**(11): 9224–9232.

Stavisky RC, Ramsey JK, Meeker T, et al. 2018. Trauma rates and patterns in specific pathogen free (SPF) rhesus macaque (*Macaca mulatta*) groups. *American Journal of Primatology*, **80**(3): e22742.

Stenbak CR, Pinto-Santini DM, Murray SM, et al. 2020. Simian foamy viruses: infections in human and nonhuman primate hosts. *In*: Knauf S, Jones-Engel L. Neglected Diseases in Monkeys. Cham: Springer.

Stromberg K, Benveniste RE, Arthur LO, et al. 1984. Characterization of exogenous type D retrovirus from a fibroma of a macaque with simian AIDS and fibromatosis. *Science*, **224**(4646): 289–292.

Sui P, Sun YY, Shi YJ, et al. 2023. Establishment and evaluation of a multiplex real-time RT-PCR for quantitative and differential detection of wild-type canine distemper virus from vaccine strains. *Heliyon*, **9**(9): e19344.

Sun ZZ, Li AX, Ye HH, et al. 2010. Natural infection with canine distemper virus in hand-feeding Rhesus monkeys in China. *Veterinary Microbiology*, **141**(3-4): 374–378.

Sweet BH, Hilleman MR. 1960. The vacuolating virus, S. V. 40. *Proceedings of the Society for Experimental Biology and Medicine*, **105**: 420–427.

Switzer WM, Heneine W. 2011. Foamy virus infection of humans. *In*: Liu D. Molecular Detection of Viral Pathogens. Boca Raton: CRC Press, 131–146.

Switzer WM, Zheng HQ, Muniz CP, et al. 2025. High prevalence of simian foamy virus infection of South American Indians. *PLoS Pathogens*, **21**(6): e1013169.

Takano JI, Leon A, Kato M, et al. 2013. Isolation and DNA characterization of a simian retrovirus 5 from a Japanese monkey (*Macaca fuscata*). *Journal of General Virology*, **94**(Pt 5): 955–959.

Tanaka S, Mannen K, Sato H. 2004. Use of herpesvirus papio 2 as an alternative antigen in immunoblotting assay for B virus diagnosis. *Journal of Veterinary Medical Science*, **66**(5): 529–532.

Tanaka T, Lerche NW, Farver TB, et al. 2013. Specific-pathogen-free status is associated with lower infant mortality rate in rhesus macaque (*Macaca mulatta*) colonies at the California National Primate Research Center. *Journal of Medical Primatology*, **42**(4): 186–191.

Thouless ME, Wang Y, Welch MJ. 1996. Increased enzyme-linked immunosorbent assay specificity with solubilized simian retrovirus 2-infected cell membranes. *Laboratory Animal Science*, **46**(6): 619–622.

Traina-Dorge V, Mehta S, Rooney B, et al. 2019. Simian varicella virus DNA in saliva and buccal cells after experimental acute infection in Rhesus macaques. *Frontiers in Microbiology*, **10**: 1009.

Verschoor EJ, Niphuis H, Fagrouch Z, et al. 2008. Seroprevalence of SV40-like polyomavirus infections in captive and free-ranging macaque species. *Journal of Medical Primatology*, **37**(4): 196–201.

Virdana S, Rizaldi R, Maliza R. 2024. Potential zoonotic disease transmission from macaques to human in ecotourism areas: a systematic review. *Journal of Medical Microbiology and Infectious Diseases*, **12**(4): 243–258.

von Magnus P, Andersen EK, Petersen KB, et al. 1959. A pox-like disease in cynomolgus monkeys. *Acta Pathologica Microbiologica Scandinavica*, **46**(2): 156–176.

Wachtman L, Mansfield K. 2012. Viral diseases of nonhuman primates. *In*: Abee CR, Mansfield K, Tardif S, et al. Nonhuman Primates in Biomedical Research. 2nd ed. Amsterdam: Academic Press, 1–104.

Wang CJ, Yang LY, Wang LP, et al. 2025. Diagnosis of an outbreak of Canine Distemper in cynomolgus monkeys in an experimental monkey farm in 2019. *Laboratory Animal and Comparative Medicine*, **45**(3): 360–367. (In Chinese)

Wang WL, Qi WJ, Liu JY, et al. 2021. Notes from the field: first human infection case of monkey B Virus identified in China, 2021. *China CDC Weekly*, **3**(29): 632–633.

Ward JA, Hilliard JK. 1994. B virus-specific pathogen-free (SPF) breeding colonies of macaques: issues, surveillance, and results in 1992. *Laboratory Animal Science*, **44**(3): 222–228.

Ward JA, Hilliard JK. 2002. Herpes B virus-specific pathogen-free breeding colonies of macaques: serologic test results and the B-virus status of the macaque. *Contemporary Topics in Laboratory Animal Science*, **41**(4): 36–41.

Weigler BJ. 1992. Biology of B virus in macaque and human hosts: a review. *Clinical Infectious Diseases*, **14**(2): 555–567.

Weigler BJ, Hird DW, Hilliard JK, et al. 1993. Epidemiology of cercopithecine herpesvirus 1 (B virus) infection and shedding in a large breeding cohort of rhesus macaques. *The Journal of Infectious Diseases*, **167**(2): 257–263.

Wevers D, Metzger S, Babweteera F, et al. 2011. Novel adenoviruses in wild primates: a high level of genetic diversity and evidence of zoonotic transmissions. *Journal of Virology*, **85**(20): 10774–10784.

White JA, Todd PA, Rosenthal AN, et al. 2009. Development of a generic real-time PCR assay for simultaneous detection of proviral DNA of simian *Betaretrovirus* serotypes 1, 2, 3, 4 and 5 and secondary uniplex assays for specific serotype identification. *Journal of Virological Methods*, **162**(1-2): 148–154.

Wilde THC, Shukla RK, Madden C, et al. 2023. Simian immunodeficiency virus and storage buffer: Field-friendly preservation methods for RNA viral detection in primate feces. *Msphere*, **8**(6): e00484–23.

Wilkes RP. 2022. Canine distemper virus in endangered species: species jump, clinical variations, and vaccination. *Pathogens*, **12**(1): 57.

Wilkes RP, Sanchez E, Riley MC, et al. 2014. Real-time reverse transcription polymerase chain reaction method for detection of Canine distemper virus modified live vaccine shedding for differentiation from infection with wild-type strains. *Journal of Veterinary Diagnostic Investigation*, **26**(1): 27–34.

Wilson VG. 2022. SV40–An obscure monkey virus and the golden age of molecular biology. *In*: Wilson VG. Viruses: Intimate Invaders. Cham: Springer, 149–170.

Wipf A, Perez-Cutillas P, Ortega N, et al. 2025. Geographical distribution of carnivore hosts and genotypes of Canine Distemper Virus (CDV) worldwide: a scoping review and spatial meta-analysis. *Transboundary and Emerging Diseases*, **2025**: 6632068.

World Health Organization. 2007. Manual for the Laboratory Diagnosis of Measles and Rubella Virus Infection. 2nd ed. Geneva: World Health Organization.

Xu LB, Yang YP, Li YD, et al. 2023. Characterizing the pathogenicity and immunogenicity of simian retrovirus subtype 8 (SRV-8) using SRV-8-infected cynomolgus monkeys. *Viruses*, **15**(7): 1538.

Yamada S, Katano H, Sato Y, et al. 2024. Macacine alphaherpesvirus 1 (B Virus) infection in humans, Japan, 2019. *Emerging Infectious Diseases*, **30**(1): 177–179.

Yang YP, Xu LB, Lu Y, et al. 2025. Dynamic alterations in bacterial and fungal microbiome and inflammatory cytokines following SRV-8 infection in cynomolgus monkeys. *Zoological Research*, **46**(2): 325–338.

Yasutomi Y. 2010. Establishment of specific pathogen-free macaque colonies in Tsukuba Primate Research Center of Japan for AIDS research. *Vaccine*, **28 Suppl 2**: B75–B77.

Yee JL, Strelow LI, White JA, et al. 2023. Horizontal transmission of endemic viruses among rhesus macaques (*Macaca mulatta*): implications for human cytomegalovirus vaccine/challenge design. *Journal of Medical Primatology*, **52**(1): 53–63.

Yee JL, Vanderford TH, Didier ES, et al. 2016. Specific pathogen free macaque colonies: a review of principles and recent advances for viral testing and colony management. *Journal of Medical Primatology*, **45**(2): 55–78.

Yoshikawa Y, Ochikubo F, Matsubara Y, et al. 1989. Natural infection with canine distemper virus in a Japanese monkey (*Macaca fuscata*). *Veterinary Microbiology*, **20**(3): 193–205.

Zao CL, Armstrong K, Tomanek L, et al. 2010. The complete genome and genetic characteristics of SRV-4 isolated from cynomolgus monkeys (*Macaca fascicularis*). *Virology*, **405**(2): 390–396.

Zao CL, Tomanek L, Cooke A, et al. 2016. A novel simian retrovirus subtype discovered in cynomolgus monkeys (*Macaca fascicularis*). *Journal of General Virology*, **97**(11): 3017–3023.

Zao CL, Ward JA, Tomanek L, et al. 2011. Virological and serological characterization of SRV-4 infection in cynomolgus macaques. *Archives of Virology*, **156**(11): 2053–2056.

Zeng Y, Zhao YJ, Ren XN, et al. 2023. Rapid detection of monkeypox virus and monkey B virus by a multiplex loop-mediated isothermal amplification assay. *Journal of Infection*, **86**(4): e114–e116.

Zhang TP, Zhao Z, Sun XL, et al. 2022. Fatal progressive ascending encephalomyelitis caused by herpes B virus infection: first case from China. *World Journal of Emergency Medicine*, **13**(4): 330–333.

Zhu JT, Yang LY, Zhang QB, et al. 2020. Autophagy induced by simian retrovirus infection controls viral replication and apoptosis of Jurkat T Lymphocytes. *Viruses*, **12**(4): 381.

Zhu L, Han JB, Zhang XH, et al. 2012. Epidemiological survey of a captive Chinese rhesus macaque breeding colony in Yunnan for SRV, STLV and BV. *Zoological Research*, **33**(1): 49–54. (In Chinese)

Zwartouw HT, Boulter EA. 1984. Excretion of B virus in monkeys and evidence of genital infection. *Laboratory Animals*, **18**(1): 65–70.