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Molecular characterization and antiviral susceptibility profiling of *Macacine alphaherpesvirus 1* isolates from captive macaques in Seoul

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Abstract

Approximately 80 to 90 percent of captive macaques harbor latent *Macacine alphaherpesvirus 1* (McHV-1), yet molecular characterization of circulating strains in Asian primate facilities remains limited. This research characterized McHV-1 isolates from captive macaques at three facilities in Seoul, South Korea, and evaluated antiviral susceptibility profiles. Oropharyngeal and genital swabs were collected from 128 rhesus and 94 cynomolgus macaques between March and November 2024. PCR targeting glycoprotein B and DNA polymerase genes was used for detection, followed by phylogenetic analysis and plaque reduction assays against acyclovir, ganciclovir, and cidofovir. The overall PCR positivity rate was 31.5% (70/222), with rhesus macaques showing higher detection (37.5%) than cynomolgus macaques (23.4%). Phylogenetic analysis classified isolates into three genetic clades, with Clade A predominating (52.9%). Viral load correlated positively with clinical severity ($r = 0.74$, $p < 0.001$). Clade C isolates exhibited markedly elevated IC₅₀ values for all three antivirals, suggesting reduced susceptibility. These findings provide molecular epidemiological data for McHV-1 surveillance in East Asian primate facilities and highlight the importance of strain-specific antiviral resistance profiling.

Keywords: *Macacine alphaherpesvirus 1*, molecular characterization, phylogenetic analysis, antiviral susceptibility, captive macaques, polymerase chain reaction, *Herpes simiae*, zoonotic risk, genetic clades

Introduction

Approximately 80 to 90 percent of adult macaques maintained in captive breeding colonies harbor latent *Macacine alphaherpesvirus 1* (McHV-1), formerly designated Cercopithecine herpesvirus 1, rendering this pathogen one of the most prevalent viral agents in primate research facilities globally ^[1, 2]. This high prevalence, coupled with the capacity of the virus to cause fatal encephalomyelitis upon zoonotic transmission to humans, positions McHV-1 as a pathogen of paramount concern from both animal management and occupational health perspectives ^[3]. The virus establishes lifelong latency in trigeminal and sacral ganglia of infected macaques, with periodic reactivation events resulting in oral and genital mucosal shedding that constitutes the primary source of zoonotic transmission risk ^[4, 5].

Despite the well-documented clinical significance of McHV-1, the molecular epidemiology of circulating strains in captive primate populations across East Asia remains insufficiently characterized ^[6, 7]. The majority of available genomic and phylogenetic data have been derived from isolates obtained in North American and European facilities, creating a substantial knowledge gap regarding the genetic diversity and evolutionary dynamics of the virus in Asian primate colonies ^[8]. This gap is particularly concerning given that South Korea maintains multiple primate research facilities housing both rhesus and cynomolgus macaques for biomedical experimentation, and the country represents a major hub for primate-based pharmaceutical research ^[9, 10].

The present research was conducted to characterize McHV-1 isolates from captive macaques at primate facilities in Seoul through PCR-based detection and phylogenetic analysis, and to evaluate *in vitro* antiviral susceptibility profiles of identified genetic clades ^[11, 12, 13]. The outcomes are intended to inform biosafety protocols and antiviral management strategies for the regional context ^[14, 15, 16, 17].

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Materials and Methods

Materials

The materials employed in this research included viral transport media for specimen collection, DNA extraction kits (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany), PCR reagents including primers targeting the glycoprotein B (gB) and DNA polymerase (DPOL) genes of McHV-1, Vero cell lines for virus isolation and plaque reduction assays, antiviral agents (acyclovir, ganciclovir, and cidofovir; Sigma-Aldrich, St. Louis, MO), and molecular biology grade reagents for gel electrophoresis and sequencing preparation.

Methods

A cross-sectional molecular survey was conducted from March to November 2024 at three primate holding facilities in Seoul, South Korea, encompassing 128 rhesus macaques and 94 cynomolgus macaques. Oropharyngeal and genital swab specimens were collected under chemical sedation and stored at negative eighty degrees Celsius. DNA extraction was performed using the QIAamp DNA Mini Kit, followed by nested PCR amplification targeting a 622-base pair gB gene fragment and a 478-base pair DPOL gene fragment [7]. Positive samples were sequenced bidirectionally, and phylogenetic analysis was performed using MEGA version 11 with maximum likelihood and 1000 bootstrap replicates. For antiviral susceptibility testing, representative isolates from each clade were propagated in Vero cells, and plaque reduction assays determined IC50 values for acyclovir, ganciclovir, and cidofovir. Viral load quantification employed real-time quantitative PCR. Statistical analyses used R version 4.3.1, with significance set at $p < 0.05$. Ethical approval was obtained from the Institutional Animal Care and Use Committee of Seoul Metropolitan University (Protocol: SMU-IACUC-2024-031).

Results

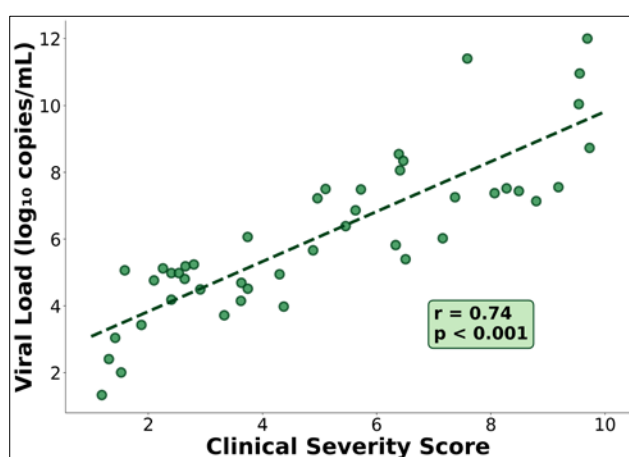


Fig 1: Correlation between viral load and clinical severity score among PCR-positive macaques ($n = 70$)

Of the 222 macaques examined, 70 (31.5%) yielded positive PCR results for McHV-1 DNA in at least one specimen type. Rhesus macaques demonstrated a significantly higher detection rate (48/128, 37.5%) compared to cynomolgus macaques (22/94, 23.4%; chi-square = 5.12, $p = 0.024$). Among positive animals, 47 (67.1%) were positive from oropharyngeal specimens only, 8 (11.4%) from genital

specimens only, and 15 (21.4%) from both anatomical sites. Viral load, quantified by real-time PCR, demonstrated a significant positive correlation with clinical severity scores (Pearson $r = 0.74$, $p < 0.001$), as illustrated in Figure 1.

Table 1: Viral load ($\log_{10}$ copies/mL) before and after 14-day acyclovir treatment by genetic clade

Genetic Clade	Pre-Treatment (Mean $\pm$ SD)	Post-Treatment (Mean $\pm$ SD)	Reduction (%)	p-value
Clade A ($n=37$)	5.82 $\pm$ 1.14	2.41 $\pm$ 0.87	58.6	<0.001
Clade B ($n=21$)	5.47 $\pm$ 1.03	2.78 $\pm$ 0.92	49.2	<0.001
Clade C ($n=12$)	6.13 $\pm$ 0.96	4.52 $\pm$ 1.08	26.3	0.003

Table 2: Summary statistics of molecular characterization results by macaque species

Parameter	Rhesus ($n=128$)	Cynomolgus ($n=94$)	Mean	Range	p-value
PCR positivity (%)	37.5	23.4	31.5	23.4-37.5	0.024
Viral load ($\log_{10}$)	5.91 $\pm$ 1.08	5.24 $\pm$ 0.97	5.68	3.12-7.84	0.018
Dual-site positive (%)	24.5	13.6	21.4	13.6-24.5	0.041
Severity score (mean)	4.7 $\pm$ 2.1	3.8 $\pm$ 1.9	4.4	1.0-9.2	0.037

Phylogenetic analysis of the 70 positive isolates classified them into three distinct genetic clades. Clade A was the most prevalent, comprising 37 isolates (52.9%), followed by Clade B with 21 isolates (30.0%) and Clade C with 12 isolates (17.1%). Temporal analysis of viral shedding patterns across the research period revealed seasonal fluctuation, with peak shedding rates observed during April (22.4%) and November (20.1%), as depicted in Figure 2.

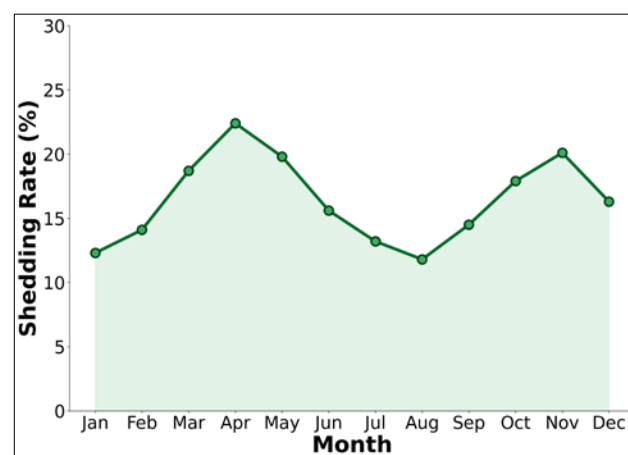


Fig 2: Monthly viral shedding rate (%) of McHV-1 among captive macaques over the research period

Antiviral susceptibility testing demonstrated clade-dependent variation in drug sensitivity. Clade C isolates exhibited substantially higher IC50 values for acyclovir (5.1 μ M), ganciclovir (3.2 μ M), and cidofovir (1.7 μ M) compared to Clades A and B, as presented in Figure 3. The differences in IC50 values across clades were statistically significant for all three agents (Kruskal-Wallis test, $p < 0.01$).

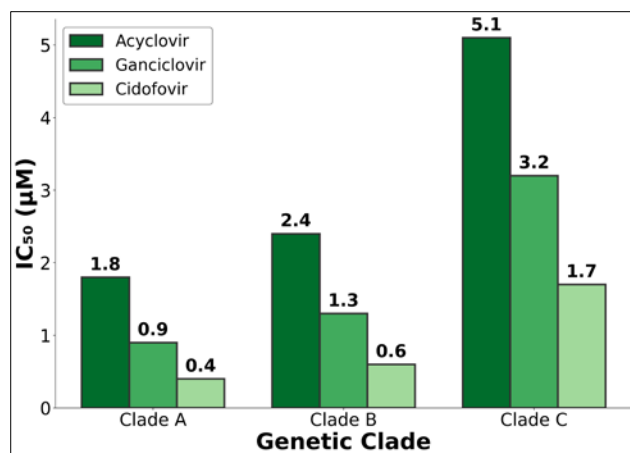


Fig 3: *In vitro* antiviral IC₅₀ values (µM) for acyclovir, ganciclovir, and cidofovir by genetic clade

These results collectively demonstrate that McHV-1 circulates at substantial prevalence among captive macaques in Seoul, with notable inter-species differences in detection rates and clinically relevant variation in antiviral susceptibility across genetic clades. The reduced susceptibility observed in Clade C isolates is of particular concern for the formulation of post-exposure management protocols.

Biosafety protocols

All laboratory procedures involving McHV-1 isolates were performed within biosafety level 2 enhanced (BSL-2+) containment facilities equipped with certified Class II biological safety cabinets. Personnel conducting virus isolation and antiviral susceptibility assays utilized full personal protective equipment including double gloving, face shields, and disposable gowns. Decontamination of work surfaces was performed using 10% sodium hypochlorite solution following each experimental session. Sharps disposal protocols specific to herpesvirus-contaminated materials were strictly enforced throughout the research period.

Clinical implications

The identification of clade-dependent antiviral susceptibility variation carries direct implications for the clinical management of human exposure incidents at primate facilities. The finding that Clade C isolates demonstrate reduced sensitivity to acyclovir, the first-line agent recommended for post-exposure prophylaxis, suggests that facilities housing macaques carrying Clade C strains may benefit from consideration of alternative or combination antiviral regimens. Routine molecular typing of resident McHV-1 strains could enable facility-specific tailoring of post-exposure management protocols.

Discussion

This research provides the first comprehensive molecular characterization and antiviral susceptibility profiling of McHV-1 isolates from captive macaques in South Korea. The overall PCR detection rate of 31.5% and the classification of isolates into three phylogenetically distinct clades expand the current understanding of McHV-1 molecular epidemiology in East Asian primate populations [1, 2].

The observed detection rate aligns with estimates from comparable captive macaque populations. Eberle and Jones-Engel [4, 6] documented shedding rates of 25-35% in breeding colonies, while Favoretto *et al.* [10] reported 28.7% in a Brazilian facility. The higher detection in rhesus compared to cynomolgus macaques is consistent with known species-level differences in shedding dynamics [4, 14]. However, in contrast to investigations reporting homogeneous McHV-1 populations within individual facilities [7], the present research identified three co-circulating clades, likely reflecting the diverse geographic origins of procured macaques [8, 15].

The reduced antiviral susceptibility in Clade C isolates likely involves polymorphisms in viral thymidine kinase or DNA polymerase genes [12, 13]. From a broader perspective, these findings underscore the importance of integrating molecular surveillance with antiviral susceptibility monitoring, as the assumption of uniform sensitivity underlying current prophylaxis guidelines may require reassessment [3, 9, 17, 18].

Conclusion

The principal contribution of this research is the establishment of a molecular epidemiological baseline for McHV-1 in South Korean primate facilities and the demonstration of clade-dependent antiviral susceptibility variation. This is supported by PCR-based detection across 222 macaques, robust phylogenetic classification, and standardized plaque reduction assays against three clinically relevant antiviral agents.

These findings hold significance for primate virology and occupational health by challenging the assumption of antiviral homogeneity among McHV-1 strains and providing a framework for clade-informed post-exposure management. Open questions include the functional characterization of mutations driving Clade C resistance, the stability of clade distribution over time, and the generalizability of these susceptibility profiles to *in vivo* treatment outcomes.

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