

Vital Surveillances

Genomic Characterization of Human Adenovirus Type 21 Strains — 7 PLADs, China, 2023–2024

Yali Jin¹; Naiying Mao¹; Xueping Ma²; Linqing Zhao³; Liwei Sun⁴; Jikui Deng⁵; Shu Liang⁶; Hongmei Xu⁷; Xin Li⁸; Chunyu Zhu¹; Baicheng Xia¹; Aili Cui¹; Yan Zhang¹; Zhen Zhu^{1,8}

ABSTRACT

Introduction: Recent sentinel surveillance has revealed a rising prevalence of human adenovirus type 21 (HAdV-21) among HAdV infections in China. This study aimed to elucidate the molecular features of currently circulating HAdV-21 strains in China.

Methods: Whole-genome sequencing (WGS) was performed on 23 HAdV-21 strains isolated from acute respiratory infection cases, 56.5% involving lower respiratory tract infections, across 7 Chinese sentinel surveillance provincial-level administrative divisions (PLADs) (2023–2024). These sequences, along with 50 previously reported HAdV-21 genomes from 6 countries (1956–2019), were integrated into a WGS dataset for comprehensive phylogenetic, genetic variation, and recombination analyses.

Results: WGS categorized the HAdV-21 strains into 3 subtypes: HAdV-21a, HAdV-21b, and historical HAdV-21p (isolated in the 1950s). HAdV-21a (1956–2024, involving 5 of the 6 countries) and HAdV-21b (2005–2024, involving 3 of the 6 countries) exhibited extensive spatiotemporal distributions. Recent Chinese strains (2023–2024) belonged to HAdV-21a and HAdV-21b (HAdV-21a/b), showing extremely high genetic homology with Chinese 2019 strains (genetic distance: 0.00007) and global strains (distance: <0.00040). Phylogenetic analysis confirmed that HAdV-21a/b shared a common ancestor and maintained a highly conserved genome despite decades of circulation. Sequence variation analysis identified shared and subtype-specific mutations in these two subtypes. Recombination pattern analysis further revealed that HAdV-21a/b acquired an HAdV-3-derived fragment in the E4 region (breakpoint: nt32,843).

Conclusions: Recombinant HAdV-21a/b subtypes have co-circulated in China in recent years with remarkable genetic conservation. Enhanced surveillance is essential to quantify associated disease

burden and guide targeted prevention and control strategies.

Human adenoviruses (HAdVs) are non-enveloped, double-stranded DNA viruses (genome size 34–36 kb) in the genus *Mastadenovirus* (family *Adenoviridae*), classified into 7 species (A–G) with 116 recognized types (<http://hadvwg.gmu.edu/>) (1). Among these, HAdV-3, HAdV-7, HAdV-55, HAdV-4, and species C (HAdV-C) are the major epidemiologically significant causes of respiratory infections (2–6).

HAdV-21 (species B), first identified in Saudi Arabia in 1956, is increasingly recognized as a significant pathogen in military and civilian populations across multiple countries (7). Although a meta-analysis of Chinese surveillance data (2009–2021) revealed a low prevalence of HAdV-21 (0.87%), recent data from 14 sentinel surveillance sites across 11 provincial-level administrative divisions (PLADs) (2023–2024) indicated a marked increase: the proportion of HAdV-21 infections rose from 1.44% (2/139) in 2023 to 8.97% (21/234) in 2024 (Fisher's exact test: $P=0.003$) (unpublished data) (6). However, molecular epidemiological data on HAdV-21 in China remain limited, with only 3 isolates reported before 2023 (8–9).

To investigate the potential genetic correlations among the increasingly detected HAdV-21, this study performed whole-genome sequencing (WGS) of 23 HAdV-21 strains isolated from acute respiratory infections across 7 surveillance PLADs (2023–2024) and conducted phylogenetic, genetic variation, and recombination analyses by integrating these data with previously reported HAdV-21 genomes obtained from GenBank.

METHODS

Between 2023 and 2024, 23 HAdV-21 strains were

isolated from 7 Chinese sentinel surveillance PLADs (Gansu, Jilin, Ningxia, Liaoning, Guangdong, Chongqing, and Beijing) and confirmed by real-time polymerase chain reaction and amplification/analysis of 3 genes (penton base, hexon, and fiber). The corresponding 23 clinical cases ranged from 5 months to 68 years (median: 3 years). Among these cases, 14 (60.87%) required hospitalization. Ten cases presented with upper respiratory tract infections, while the remaining 13 had lower respiratory tract infections, including 10 with pneumonia. Co-infections occurred in 13 cases, most commonly with *Haemophilus influenzae* (5/13). One fatal case (Ningxia2024-669) of acute respiratory distress syndrome exhibited *Mycoplasma pneumoniae* co-infection (Table 1).

Viral DNA from the 23 strains was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden,

Germany). WGS was performed by iGeneTech Biotechnology Co., Ltd. using probe-based hybrid capture combined with next-generation sequencing. All strains achieved 100% genome coverage, each yielding >1 Gb of data at >8,000× depth. Suboptimal regions were validated by Sanger sequencing, and two strains were randomly resequenced for quality assurance. Genome annotation was performed in Geneious Prime (version 2023.2.2, Biomatters Ltd., Auckland, New Zealand) using the HAdV-21 prototype strain (AY601633) as a reference.

Fifty HAdV-21 genomes from 6 countries (1956–2019), including 3 from China (2019) and 47 from other countries (1956–2018), were retrieved from GenBank (Supplementary Table S1, available at <https://weekly.chinacdc.cn/>). Integration of the 23 newly sequenced strains yielded a comprehensive

TABLE 1. Detailed information on the 23 HAdV-21 strains analyzed in this study.

Strain	PLADs	Gender	Age	Case type	Year of collection	Co-infection pathogens	Clinical diagnosis	Subtype
Jilin2023-296	Jilin	Female	3 years	Inpatient	2023/5/21	/	Bronchopneumonia	HAdV-21a
Jilin2023-595	Jilin	Female	3 years	Inpatient	2023/6/19	/	Upper respiratory infection	HAdV-21a
Ningxia2024-533	Ningxia	Male	3 years	Outpatient	2024/2/10	SARS-CoV-2	Fever	HAdV-21a
Ningxia2024-595	Ningxia	Female	5 months	Inpatient	2024/3/4	Hi	Severe lung infection/Lung consolidation	HAdV-21a
Ningxia2024-608	Ningxia	Female	5 years	Outpatient	2024/3/1	MP	Acute tonsillitis	HAdV-21a
Ningxia2024-618	Ningxia	Male	13 years	Outpatient	2024/3/5	Hi	Upper respiratory infection	HAdV-21a
Ningxia2024-669*	Ningxia	Male	9 months	Inpatient	2024/3/14	MP	Severe lung infection/ARDS	HAdV-21a
Ningxia2024-671	Ningxia	Female	5 years	Inpatient	2024/3/18	/	Severe lung infection/Lung consolidation	HAdV-21a
Ningxia2024-725	Ningxia	Male	13 years	Outpatient	2024/3/27	MP/Flu B	Respiratory infection	HAdV-21a
Ningxia2024-747	Ningxia	Male	6 years	Inpatient	2024/4/1	MP	Lung infection/Acute suppurative tonsillitis	HAdV-21a
Ningxia2024-754	Ningxia	Female	3 years	Outpatient	2024/3/31	Hi	Acute bronchitis	HAdV-21a
Ningxia2024-868	Ningxia	Female	4 years	Outpatient	2024/4/29	SP/Hi	Respiratory infection	HAdV-21a
Ningxia2024-907	Ningxia	Female	7 months	Outpatient	2024/5/8	Hi	Respiratory infection	HAdV-21a
Ningxia2024-944	Ningxia	Male	2 years	Outpatient	2024/5/21	SP	Fever	HAdV-21a
Beijing2024-330	Beijing	Female	3 years	Inpatient	2024/3/28	HRV	Bronchopneumonia	HAdV-21a
Beijing2024-364	Beijing	Male	1 year	Inpatient	2024/5/6	/	Pneumonia	HAdV-21a
Beijing2024-378	Beijing	Male	2 years	Inpatient	2024/5/21	Flu B	Fever	HAdV-21a
Shenzhen2024-077	Guangdong	Male	8 months	Inpatient	2024/4/27	/	Pneumonia	HAdV-21a
Chongqing2024-069	Chongqing	Female	2 years	Inpatient	2024/4/2	/	Febrile convulsions/Bronchitis	HAdV-21a
Shenyang2024-163	Liaoning	Male	57 years	Outpatient	2024/3/12	/	Respiratory infection	HAdV-21a
Gansu2024-215	Gansu	Male	68 years	Inpatient	2024/2/17	/	Community-acquired pneumonia	HAdV-21a
Ningxia2024-674	Ningxia	Male	1 year	Inpatient	2024/3/18	/	Severe lung infection	HAdV-21b
Shenzhen2024-201	Guangdong	Male	3 years	Inpatient	2024/6/11	/	Acute laryngotracheobronchitis	HAdV-21b

* Indicates fatalities.

Note: / means no co-infecting pathogens detected.

Abbreviation: ARDS=acute respiratory distress syndrome; PLAD=provincial-level administrative division; Hi=*Haemophilus influenzae*; MP=*Mycoplasma pneumoniae*; Flu B=Influenza B virus; SP=*Streptococcus pneumoniae*.

database of 73 WGSs for phylogenetic and genetic variation analyses.

Sequences were aligned using ClustalW, and sequence similarity was assessed using BioEdit. A maximum likelihood phylogenetic tree was constructed using MEGA Version 7.0, with bootstrap support values (>80%) indicated at the tree nodes. Genetic mutations were identified using Snipit (<https://github.com/aineniamh/snipit>), focusing on variations with frequencies >80%. Recombination was analyzed using SimPlot (window size: 1,000 bp; step size: 100 bp) and Recombination Detection Program v4 (RDP4).

RESULTS

Genomic Characterization

All 23 HAdV-21 strains were fully assembled (genome sizes: 35,364–35,393 bp; GC: approximately 51.2%), consistent with previous reports (8–9). Annotation using the HAdV-21 prototype strain as a reference identified 48 conserved protein-coding regions (Supplementary Table S2, available at <https://weekly.chinacdc.cn/>). Sequence identity analysis among the 23 HAdV-21 strains was $\geq 99.7\%$. Strains with identical sequences were detected across different PLADs and years, e.g., the 2023 Jilin strains (Jilin2023-296 and Jilin2023-595) matched 2024 strains from 5 PLADs (Beijing2024-364, Gansu2024-215, Ningxia2024-907, Shenyang2024-163, and Shenzhen2024-077). Identical sequences were also detected within the same PLAD, such as Ningxia2024-595 and Ningxia2024-725.

The strains shared 98.8% nucleotide identity with the HAdV-21 prototype strain. Analysis of 12 functional domains, including major capsid proteins (penton base, hexon, fiber), core proteins (pV, pVII, pTP, pIVa2), minor proteins (pIX, pIIIa, pVI, pVIII), and the non-structural protein pX, showed relatively high similarity (nt: 98.4%–99.8%, aa: 97.9%–100.0%) across 11 regions, with the penton base showing lower conservation (nt: 96.3%, aa: 95.7%–95.9%).

Phylogenetic Analysis

To determine the genetic relationships between the 23 HAdV-21 strains (2023–2024) and previously circulating Chinese and globally prevalent strains, a phylogenetic analysis was performed using the constructed WGS dataset (Figure 1). The results showed that the 73 HAdV-21 strains were categorized

into 3 distinct evolutionary lineages: HAdV-21a, HAdV-21b, and HAdV-21p (bootstrap support >80%). HAdV-21a and HAdV-21b (HAdV-21a/b) exhibited closer phylogenetic relationships to each other (genetic distance=0.001) than to HAdV-21p (genetic distance=0.007 for both subtypes), indicating a shared ancestry for HAdV-21a/b.

Further analyses revealed distinct temporal and geographical distribution patterns among the 3 HAdV-21 subtypes. HAdV-21p primarily comprised a prototype strain from Saudi Arabia and a historical strain from Germany (1950s). Conversely, HAdV-21a comprised 21 strains from 7 Chinese PLADs (2023–2024), 3 Chinese strains from 2 PLADs (2019), and 24 strains from the United States, Malaysia, Germany, and Switzerland (1956–2016). HAdV-21b included 2 strains from 2 Chinese PLADs (2024) and 21 strains from the United States and Germany (2005–2018). All 3 subtypes demonstrated minimal intra-subtype genetic variation (distance: 0.00014–0.00031). HAdV-21a/b demonstrated high genetic consistency between 2023–2024 Chinese strains and both the 2019 Chinese strains (distance: 0.00007) and global strains (distance <0.00040), strongly suggesting close genetic relationships. No significant correlation was observed between clinical severity and 2 subtypes (HAdV-21a and HAdV-21b).

Genetic Variation

Genetic variation analysis of HAdV-21a/b was performed using WGS with the HAdV-21p prototype strain as a reference. The results showed that the 2 subtypes shared 352 specific nucleotide variants, including 64 insertions and 49 deletions, with HAdV-21a exhibiting 27 unique variants (10 deletions) and HAdV-21b displaying 36 unique variants (3 insertions and 18 deletions). The variants were distributed across the genome, with insertions and deletions concentrated in the L2 (46.90%) and E3 (23.01%) regions, respectively. Non-coding region variants were also detected in the Chinese HAdV-21a (4 sites, 1 insertion) and HAdV-21b (7 sites).

Amino acid variation analysis across the 12 functional domains identified 70 shared HAdV-21a/b substitutions in 11 proteins, excluding pX (Figure 2). 9 of 10 shared hexon gene variants were localized to hypervariable regions (HVRs), with HVR7 exhibiting the highest mutation frequency (4 sites). In the fiber gene, 2 of 3 variants occurred in the shaft region and one in the knob region. The penton base gene exhibited 23 variations, primarily clustered within the

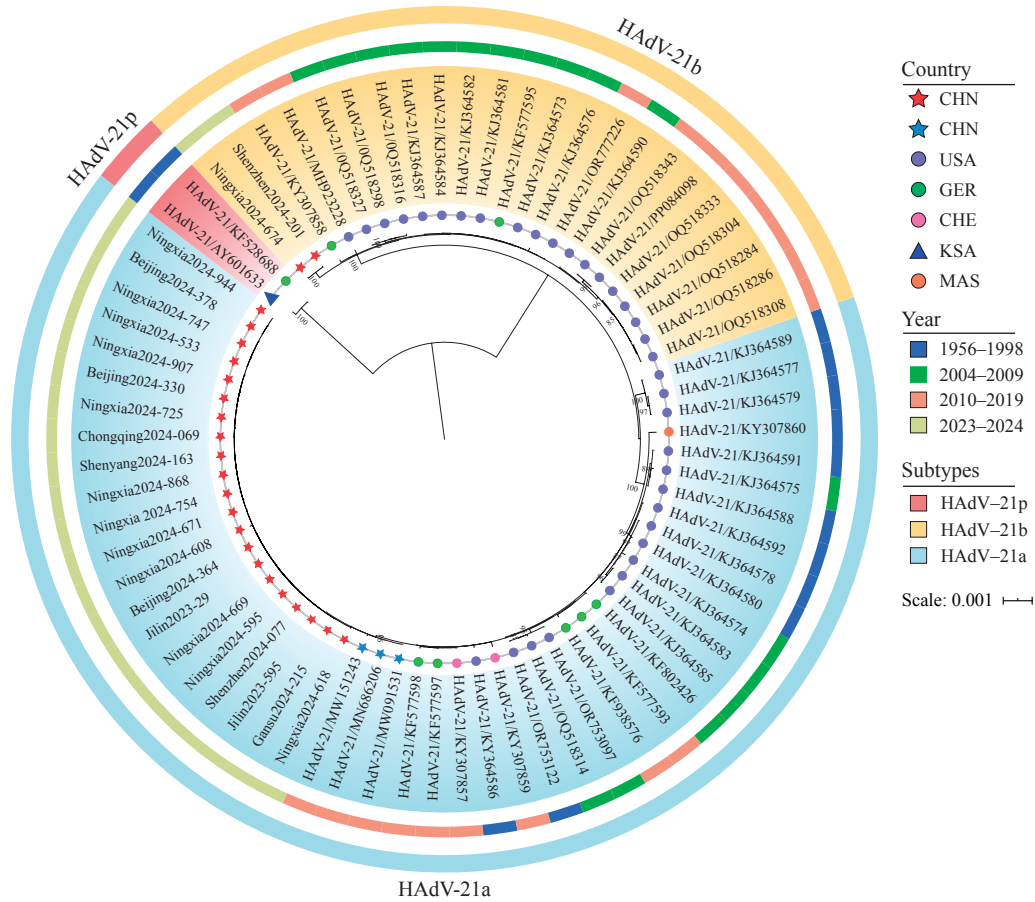


FIGURE 1. Maximum-likelihood phylogenetic trees of HAAdV-21 were constructed from a dataset of 73 strains (23 from this study and 50 from the GenBank database). Trees derived from nine individual gene fragments of HAAdV-21 are presented in panels A-I, and the tree based on the WGS is presented in panel J. The HAAdV-21 and HAAdV-3 prototype strains are marked in red and dark blue, respectively, across all panels
Abbreviation: WGS=whole-genome sequencing; HAAdV-21=human adenovirus type 21.

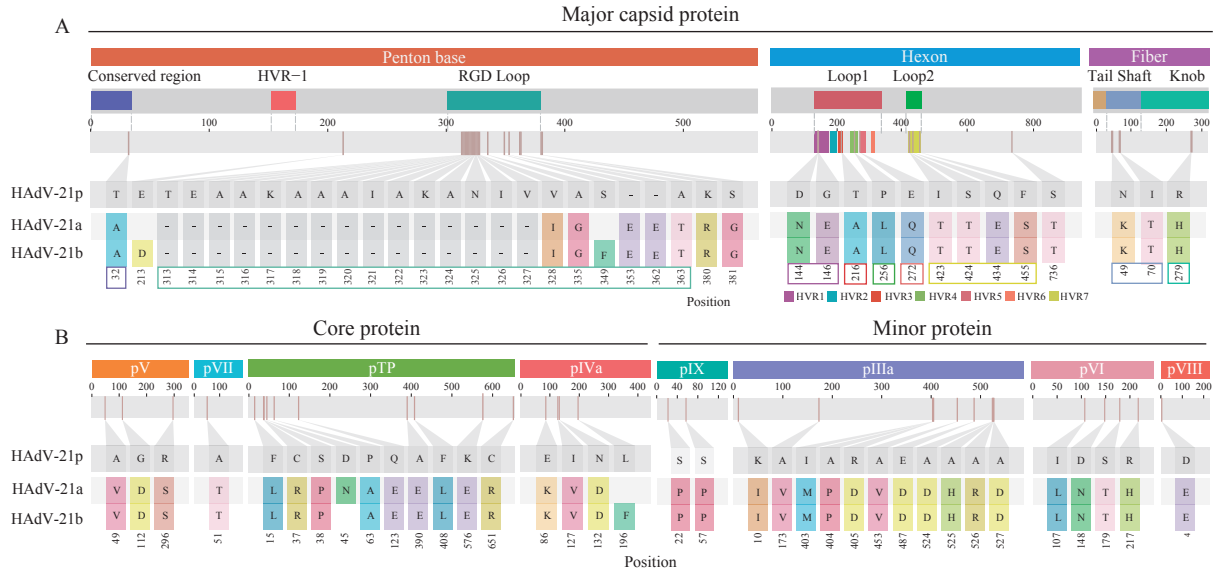


FIGURE 2. Amino acid variations in 11 key proteins of (A) HAAdV-21a and (B) HAAdV-21b subtypes compared to the prototype HAAdV-21 strain (designated HAAdV-21p).
Abbreviation: HAAdV-21=human adenovirus type 21.

RGD loop, including 15 amino acid deletions (313TEAAKAAAIKANIV327) and 2 insertions (362EE363). Subtype-specific mutations were also detected, including pIVa2 (HAdV-21b: L196F), pTP (HAdV-21a: D45N), and penton base (HAdV-21b: E213D and S349F).

Genetic Recombination

To assess recombination, phylogenetic analysis was performed with the HAdV-21 strains and 18 species B prototype strains (Supplementary Table S1) using 9 consecutive genomic fragments (nt1–7,000; nt7,001–13,877; penton base; nt15,564–18,453; hexon; nt21,304–26,000; nt26,001–31,405; fiber; nt32,378–end) and WGS (Supplementary Figure S1, available at <https://weekly.chinacdc.cn/>). The results demonstrated that subtype classification using the nt32,378–end fragment matched the WGS-based classification, whereas the other 8 fragments failed to distinguish HAdV-21a/b. In the nt32,378–end region, HAdV-21a/b exhibited closer phylogenetic relationships with the HAdV-3 prototype strain, suggesting potential recombination in the E4 region. SimPlot and RDP4 analyses (supported by four

algorithms) further confirmed identical recombination patterns in both subtypes, with a breakpoint at nt32,843 in the E4 gene (Figure 3).

DISCUSSION

This study analyzed 23 HAdV-21 strains (2023–2024) isolated from patients with acute respiratory infections. Among these, 56.5% (13/23) had lower respiratory tract infections, including 9 pneumonia cases and 1 fatal infant case. To elucidate the genetic basis of these emerging strains, this study performed a comprehensive genomic characterization.

Phylogenetic analyses identified viral genetic subtypes and spatiotemporal transmission patterns. Globally, HAdV-21 strains are classified into 3 subtypes: HAdV-21a, HAdV-21b, and HAdV-21p. HAdV-21p, comprising historical strains from the 1950s, is no longer detected. Conversely, HAdV-21a (1956–2024, 5 countries) and HAdV-21b (2005–2024, 3 countries) demonstrated extensive spatiotemporal distribution, reflecting stable epidemic trends and highlighting these strains as the dominant circulating subtypes in multiple countries, including

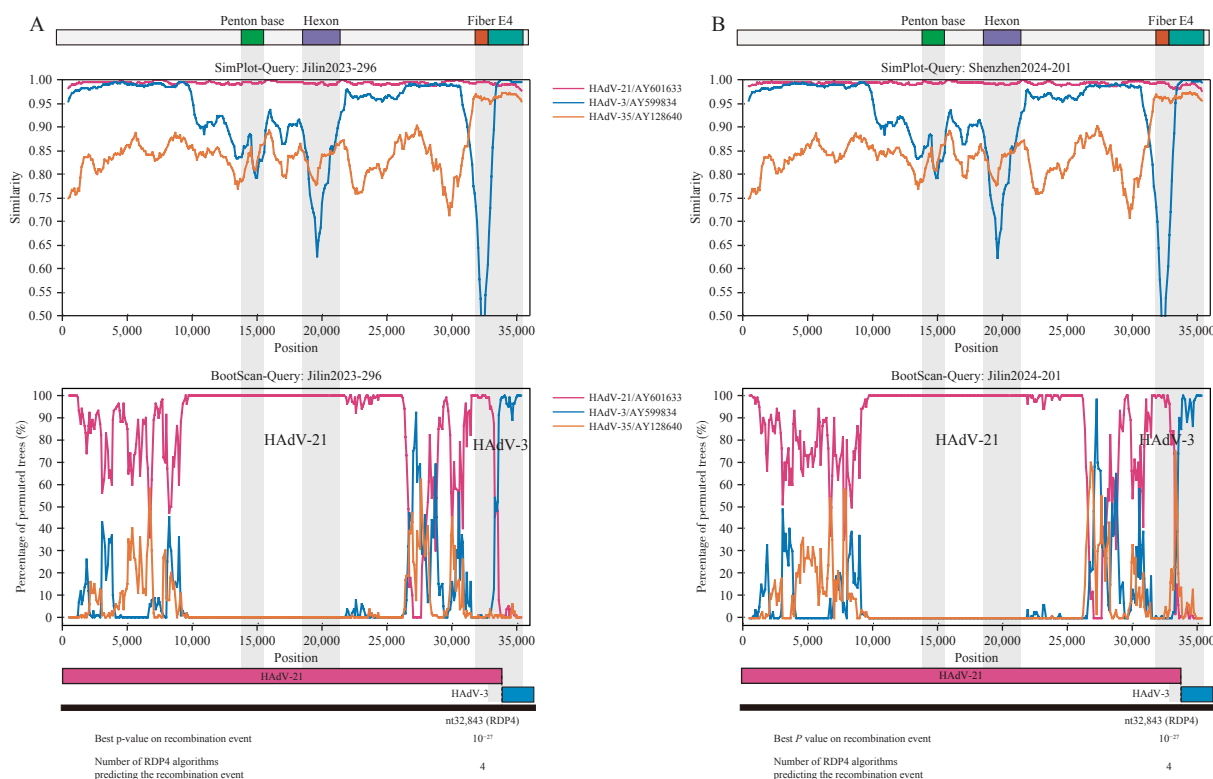


FIGURE 3. Recombinant analysis of (A) HAdV-21a and (B) HAdV-21b strains.

Note: Jilin2023-296 and Shenzhen2024-201 were used as representative HAdV-21a and HAdV-21b strains, respectively.

Abbreviation: HAdV-21=human adenovirus type 21.

China. Furthermore, all subtypes exhibited minimal intra-subtype genetic variation (distance <0.00031), suggesting genomic stability during prolonged circulation, consistent with observations in other HAdV types, such as HAdV-4 and HAdV-55 (10–11). This stability may result from the conserved replication mechanism of HAdV or selective equilibrium under host immune pressure.

Notably, Chinese HAdV-21a/b strains (2023–2024) exhibited extremely high genetic homology and consistency with 2019 Chinese strains (distance: 0.00007) and historical global strains (distance: <0.00040), indicating that the currently circulating HAdV-21 is not a novel variant but a continuous transmission of genetically related strains. Enhanced respiratory infection surveillance following coronavirus disease 2019 (COVID-19) likely contributed to increased detection rates in recent years. Although HAdV-21 demonstrates relatively lower virulence and infectivity than other common types (e.g., HAdV-3, -7, -4, -55), it is associated with diverse clinical manifestations, including severe pneumonia, acute respiratory distress syndrome, acute flaccid paralysis, myocarditis, and fatal outcomes across age groups, necessitating urgent research on its pathogenic characteristics (8–9,12–14).

Phylogenetic and recombination analyses confirmed a common ancestor for HAdV-21a/b. Despite high overall genome conservation, sequence variation analysis identified both shared and subtype-specific mutations in HAdV-21a/b, representing key molecular markers for subtype differentiation and indicating continuous adaptive evolutionary pressure. Mutations in the hexon gene are predominantly localized in HVRs containing critical neutralizing antibody epitopes, suggesting that HVRs are primary adaptive targets for immune evasion. Consistent with previous studies, HAdV-21a/b exhibited 15 amino acid deletions and 2 insertions in the RGD loop of the penton base gene compared to the prototype strain. This shortened RGD loop closely resembles those of HAdV-3 and HAdV-7, which are associated with severe diseases (15). Because the RGD loop is a core functional domain mediating viral cell entry, its alteration may directly affect viral pathogenicity and infection efficiency; therefore, the functional consequences of the penton base warrant further investigation.

Recombination analysis revealed that HAdV-21a/b acquired an HAdV-3-derived fragment in the E4 region (breakpoint: nt32,843). As E4 regulates viral

DNA replication, transcript splicing, and late gene expression, this recombination likely enhances viral stability and replication efficiency through functional complementation, thereby conferring transmission advantages. Genetic recombination drives HAdV evolution, where adaptive recombination patterns establish stable transmission, as exemplified by HAdV-21a and HAdV-21b persisting in circulation for approximately 70 and 20 years, respectively.

The findings in this report are subject to at least three limitations. First, HAdV-21 strains were collected from only 7 Chinese PLADs, limiting the generalizability of the findings; broader sampling in future work would improve representativeness. Second, the impact of HAdV-21 genomic differences on viral infection phenotypes remains unclear, warranting further investigation. Finally, although this study identified a significant HAdV-3 fragment-containing recombination event, functional validation was lacking, necessitating additional experiments to clarify its implications for viral pathogenicity and transmission.

In conclusion, this study identified the co-circulation of HAdV-21a and HAdV-21b in China and characterized their genomic features. Given HAdV-21's increasing prevalence, persistence of similar viruses across multiple PLADs, and potential association with severe clinical outcomes, enhanced surveillance is required to quantify disease burden in China and provide targeted prevention and control strategies.

Conflicts of interest: No conflicts of interest.

Acknowledgements: The study used only viral strains and did not involve specimen collection. We sincerely thank the seven sentinel surveillance PLADs for providing the viral strains and associated data.

Funding: Supported by the National Key R&D Program of China (2022YFC2305303) and the Health and Wellness Sector Research Program of Gansu (GSWSN2021-010).

doi: 10.46234/ccdcw2025.228

* Corresponding author: Zhen Zhu, zhuzhen@ivdc.chinacdc.cn.

¹ National Key Laboratory of Intelligent Tracking and Forecasting for Infectious Disease, NHC Key Laboratory of Medical Virology and Viral Diseases, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China; ² Testing and Inspection Institute, Ningxia Hui Autonomous Region Center for Disease Control and Prevention, Yinchuan City, Ningxia Hui Autonomous Region, China; ³ Laboratory of Virology, Beijing Key Laboratory of Etiology of Viral Diseases in Children, Capital Institute of Pediatrics, Beijing, China; ⁴ Precision Medicine Research Center, Children's Hospital of Changchun, Changchun City, Jilin Province, China; ⁵ Division of Infectious Diseases, Shenzhen Children's Hospital, Shenzhen City, Guangdong

Province, China; ⁶ Key Laboratory of Infectious Diseases of Gansu Province, Gansu Provincial Center for Disease Control and Prevention, Lanzhou City, Gansu Province, China; ⁷ Department of Infectious Diseases, Children's Hospital Affiliated to Chongqing Medical University, Chongqing, China; ⁸ Health Supervision Institute, Shenyang Center for Disease Control and Prevention, Shenyang City, Liaoning Province, China.

Copyright © 2025 by Chinese Center for Disease Control and Prevention. All content is distributed under a Creative Commons Attribution Non Commercial License 4.0 (CC BY-NC).

Submitted: July 02, 2025

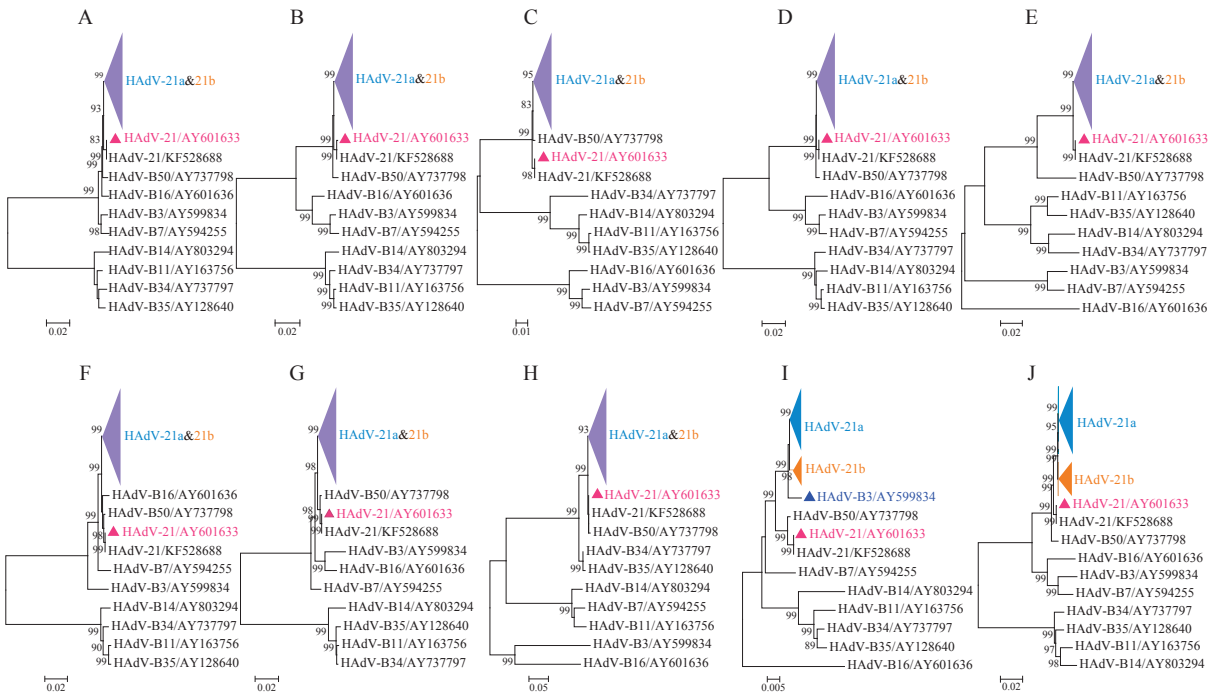
Accepted: October 14, 2025

Issued: October 24, 2025

REFERENCES

1. Davison AJ, Benkő M, Harrach B. Genetic content and evolution of adenoviruses. *J Gen Virol* 2003;84(Pt 11):2895-908. <http://dx.doi.org/10.1099/vir.0.19497-0>.
2. Dou HX, Chen CM, Song TY, Sun X, He FF, Jia YJ, et al. Epidemiological characteristics and genomic analysis of respiratory adenovirus in Jining City from February 2023 to July 2024. *BMC Genomics* 2025;26(1):369. <https://doi.org/10.1186/s12864-025-11558-1>.
3. Wu CC, Zhang YF, Liang A, Wu XX, Zhu YQ, Huang ZX, et al. Comparative analysis between genotypes of adenovirus isolates from hospitalized children with acute respiratory tract infections and clinical manifestations in Wuhan, China, from June 2022 to September 2023. *Virol Sin* 2025;40(1):50 – 60. <https://doi.org/10.1016/j.virs.2024.12.004>.
4. Mahmood K, Ahmed W, Farooq S, Habib G, Sindhu MA, Asif A, et al. Molecular characterization of human adenoviruses associated with pediatric respiratory infections in Karachi, Pakistan. *BMC Infect Dis* 2024;24(1):538. <https://doi.org/10.1186/s12879-024-09415-9>.
5. Abbasi S, Shafiei-Jandaghi NZ, Shadab A, Hassani SA, Rahimi Foroushani A, Hosseinkhan N, et al. Phylogenetic characterization of rhinovirus and adenovirus in hospitalized children aged ≤ 18 years with severe acute respiratory infection in Iran. *Iran J Microbiol* 2023;15(1):155 – 62. <https://doi.org/10.18502/ijm.v15i1.11932>.
6. Liu MC, Xu Q, Li TT, Wang T, Jiang BG, Lv CL, et al. Prevalence of human infection with respiratory adenovirus in China: a systematic review and meta-analysis. *PLoS Negl Trop Dis* 2023;17(2):e0011151. <https://doi.org/10.1371/journal.pntd.0011151>.
7. Bell SD Jr, McComb DE, Murray ES, Chang RS, Snyder JC. Adenoviruses isolated from Saudi Arabia. I. Epidemiologic features. *Am J Trop Med Hyg* 1959;8(4):492 – 500. <https://doi.org/10.4269/ajtmh.1959.8.492>.
8. Ye FQ, Han YF, Zhu JJ, Li P, Zhang Q, Lin YF, et al. First identification of human adenovirus subtype 21a in China with MinION and illumina sequencers. *Front Genet* 2020;11:285. <https://doi.org/10.3389/fgene.2020.00285>.
9. Liu WK, Zhang L, Cai Y, Zhang Q, Chen DH, Qiu SY, et al. Human adenovirus subtype 21a isolates from children with severe lower respiratory illness in China. *Front Microbiol* 2022;13:924172. <https://doi.org/10.3389/fmicb.2022.924172>.
10. Wang JJ, Feng QY, Duan YL, Ai JH, Zhu Y, Wang R, et al. Human adenovirus type 4 (HAdV-4) associated acute respiratory tract infection in children & genetic characteristics of HAdV-4 in China: a prospective multicenter study. *BMC Infect Dis* 2024;24(1):936. <https://doi.org/10.1186/s12879-024-09835-7>.
11. Hang J, Kajon AE, Graf PCF, Berry IM, Yang Y, Sanborn MA, et al. Human adenovirus type 55 distribution, regional persistence, and genetic variability. *Emerg Infect Dis* 2020;26(7):1497 – 505. <https://doi.org/10.3201/eid2607.191707>.
12. Jia XB, Wan LL, Xiao F, Huang XL, Zhou J, Wang Y, et al. Rapid and sensitive detection of human adenovirus types 3 and 7 using CRISPR-Cas12b coupled with multiple cross displacement amplification. *Infect Med* 2025;4(2):100181. <https://doi.org/10.1016/j.imj.2025.100181>.
13. Abd-Jamil J, Teoh BT, Hassan EH, Roslan N, Abubakar S. Molecular identification of adenovirus causing respiratory tract infection in pediatric patients at the University of Malaya Medical Center. *BMC Pediatr* 2010;10(1):46. <https://doi.org/10.1186/1471-2431-10-46>.
14. Pfortmueller CA, Barbani MT, Schefold JC, Hage E, Heim A, Zimmerli S. Severe acute respiratory distress syndrome (ARDS) induced by human adenovirus B21: report on 2 cases and literature review. *J Crit Care* 2019;51:99 – 104. <https://doi.org/10.1016/j.jcrc.2019.02.019>.
15. Hage E, Huzly D, Ganzenmueller T, Beck R, Schulz TF, Heim A. A human adenovirus species B subtype 21a associated with severe pneumonia. *J Infect* 2014;69(5):490 – 9. <https://doi.org/10.1016/j.jinf.2014.06.015>.

SUPPLEMENTARY MATERIAL



SUPPLEMENTARY FIGURE S1. Maximum-likelihood phylogenetic tree constructed with 23 HAdV-21 strains from this study and 50 HAdV-21 strains from the GenBank database based on (A–I) nine fragments and (J) whole-genome sequence (WGS). The HAdV-21 prototype strain is indicated in red and HAdV-3 prototype strain is indicated in dark blue. Abbreviation: WGS=whole-genome sequencing, HAdV-21=human adenovirus type 21.

SUPPLEMENTARY TABLE S1. List of 50 HAdV-21 strains and 17 prototype strains of species B from GenBank.

No.	Strain name	Country	Year	Accession No.	Type
1	AV-1645	KSA	1956	AY601633	HAdV-21p
2	Human adenovirus 21	GER	1950s	KF528688	HAdV-21p
3	HAdV-B21/USA/7K4/1956	USA	1956	OR753122	HAdV-21a
4	CDC RU8176	USA	1978	KJ364577	HAdV-21a
5	CDC V1375E	USA	1984	KJ364579	HAdV-21a
6	VRDL T87-0342	USA	1987	KJ364591	HAdV-21a
7	CDC V2148A	USA	1988	KJ364588	HAdV-21a
8	NHRC 5	USA	1996	KJ364578	HAdV-21a
9	VRDL T97-1745	USA	1997	KJ364589	HAdV-21a
10	VRDL T98-1269	USA	1998	KJ364592	HAdV-21a
11	NHRC 10030	USA	1998	KJ364586	HAdV-21a
12	NHRC 20007	USA	1998	KJ364580	HAdV-21a
13	NHRC 71139	USA	2004	KJ364583	HAdV-21a
14	NHRC 71252	USA	2005	KJ364585	HAdV-21a
15	NHRC 32493	USA	2005	KJ364574	HAdV-21a
16	NHRC 63218	USA	2006	KJ364575	HAdV-21a
17	HAdV-B21/USA/5L10/2009	USA	2009	OR753097	HAdV-21a
18	HAdV-B21/USA/4B5/2009	USA	2009	OQ518314	HAdV-21a

Continued

No.	Strain name	Country	Year	Accession No.	Type
19	Sibu-97	MAS	1997	KY307860	HAdV-21a
20	LRTI-2	GER	2008	KF802426	HAdV-21a
21	LRTI-3	GER	2010	KF577593	HAdV-21a
22	LRTI-4	GER	2012	KF938576	HAdV-21a
23	LRTI-5	GER	2012	KF577597	HAdV-21a
24	LRTI-6	GER	2013	KF577598	HAdV-21a
25	human/CHN/BB/201903/21	CHN	2019	MN686206	HAdV-21a
26	GZ06109.1	CHN	2019	MW091531	HAdV-21a
27	GZ09107.1	CHN	2019	MW151243	HAdV-21a
28	LRTI-7	CHE	2013	KY307857	HAdV-21a
29	LRTI-9	CHE	2016	KY307859	HAdV-21a
30	PEL0066	USA	2005	KJ364587	HAdV-21b
31	NHRC 71227	USA	2005	KJ364584	HAdV-21b
32	NHRC 32389	USA	2005	KJ364573	HAdV-21b
33	NHRC 52331	USA	2006	KJ364581	HAdV-21b
34	NHRC 44288	USA	2006	KJ364576	HAdV-21b
35	NHRC 91447	USA	2007	KJ364590	HAdV-21b
36	NHRC 64589	USA	2007	KJ364582	HAdV-21b
37	HAdV-B21/USA/2B2/2009	USA	2009	OQ518327	HAdV-21b
38	HAdV-B21/USA/2B10/2009	USA	2009	OQ518316	HAdV-21b
39	HAdV-B21/USA/4C7/2009	USA	2009	OQ518298	HAdV-21b
40	HAdV-B21/USA/6K2/2010	USA	2010	OQ518333	HAdV-21b
41	HAdV-B21/USA/9J5/2012	USA	2012	PP084098	HAdV-21b
42	HAdV-B21/USA/9I6/2012	USA	2012	OQ518343	HAdV-21b
43	HAdV-B21/USA/9M3/2012	USA	2012	OQ518308	HAdV-21b
44	HAdV-B21/USA/10E4/2012	USA	2012	OQ518304	HAdV-21b
45	HAdV-B21/USA/9M4/2012	USA	2012	OQ518286	HAdV-21b
46	HAdV-B21/USA/10E5/2012	USA	2012	OQ518284	HAdV-21b
47	HAdV-B21/USA/14A2/2017	USA	2017	OR777226	HAdV-21b
48	HAdVB/USA_NY/10208/2018/P21H21F21	USA	2018	MH923228	HAdV-21b
49	LRTI-1	GER	2005	KF577595	HAdV-21b
50	LRTI-8	GER	2016	KY307858	HAdV-21b
51	GB	USA	1953	AY599834	HAdV-B3
52	Gomen	USA	1954	AY594255	HAdV-B7
53	Ad11p Slobitski	USA	1957	AY163756	HAdV-B11
54	de Wit; ATCC VR-1091	NLD	1955	AY803294	HAdV-B14
55	ch. 79	USA	1955	AY601636	HAdV-B16
56	Compton; ATCC VR-716	USA	1972	AY737797	HAdV-B34
57	ATCC VR-718; Holden	USA	1973	AY128640	HAdV-B35
58	Wan; ATCC VR-1502	USA	1988	AY737798	HAdV-B50
59	QS-DLL	CHN	2006	FJ643676	HAdV-B55
60	87-922	ARG	1987	JN860676	HAdV-B66
61	Arg 827/04	ARG	2004	JN860678	HAdV-B68

Continued

No.	Strain name	Country	Year	Accession No.	Type
62	human/DEU/HEIM_00086/X/X[PXHXFX]	DEU	2013	KF633445	HAdV-B76
63	human/DEU/HEIM_00092/1985/NEW[P35H34F7]	DEU	1985	KF268328	HAdV-B77
64	HAdV-B/USA/CHOP2146-10810/2013/[P11H11F7]	USA	2013	KT970441	HAdV-B78
65	HAdV-B/JPN/T150125/2015/79[P11H34F11]	JPN	2015	LC177352	HAdV-B79
66	HAdV-B106[P11H11F35]	DEU	2020	ON393912	HAdV-B106
67	Human mastadenovirus B114	DEU	2023	OR853835	HAdV-B114

SUPPLEMENTARY TABLE S2-1. Genomic annotations of strains Jilin2023-296, Jilin2023-595, Ningxia2024-533, and Ningxia2024-595.

Gene	Encoded product	Jilin2023-296	Jilin2023-595	Ningxia2024-533	Ningxia2024-595
E1A	28.4 kDa protein	575–1,156; 1250–1,453	575–1,156; 1250–1,453	567–1,148; 1242–1,445	575–1,156; 1250–1,453
	24.6 kDa protein	575–1,063; 1250–1,453	575–1,063; 1250–1,453	567–1,055; 1242–1,445	575–1,063; 1250–1,453
	6.8 kDa protein	575–646; 1,248–1,349	575–646; 1,248–1,349	567–638; 1,240–1,341	575–646; 1,248–1,349
	small T antigen	1,601–2,137	1,601–2,137	1,593–2,129	1,601–2,137
E1B	large T antigen (55K)	1,906–3,384	1,906–3,384	1,898–3,376	1,906–3,384
IX	hexon-associated protein IX	3,478–3,894	3,478–3,894	3,470–3,886	3,478–3,894
IVa2	maturation protein IVa2	3,947–5,280; 5,559–5,571	3,947–5,280; 5,559–5,571	3,939–5,272; 5,551–5,563	3,947–5,280; 5,559–5,571
E2B	DNA polymerase	5,050–8,622; 13,841–13,849	5,050–8,622; 13,841–13,849	5,042–8,614; 13,834–13,842	5,050–8,622; 13,840–13,848
	hypothetical 11.5 kDa protein	6,143–6,463	6,143–6,463	6,135–6,455	6,143–6,463
	hypothetical 19 kDa protein	6,867–7,388	6,867–7,388	6,859–7,380	6,867–7,388
	hypothetical 10.4 kDa protein	7,132–7,419	7,132–7,419	7,124–7,411	7,132–7,419
	DNA-binding protein	7,828–8,229; 9,479–9,496	7,828–8,229; 9,479–9,496	7,820–8,221; 9,471–9,488	7,828–8,622; 9,479–9,496
	hypothetical 12.6 kDa protein	8,228–8,572	8,228–8,572	8,220–8,564	8,228–8,572
	terminal protein precursor	8,421–10,388; 13,841–13,849	8,421–10,388; 13,841–13,849	8,413–10,380; 13,834–13,842	8,421–10,388; 13,840–13,848
	hypothetical 14.5 kDa protein	8,547–8,948	8,547–8,948	8,539–8,940	8,547–8,948
	hypothetical 11.5 kDa protein	9,542–9,856	9,542–9,856	9,534–9,848	9,542–9,856
	hypothetical 9.7 kDa protein	9,756–10,031	9,756–10,031	9,748–10,023	9,756–10,031
	52 kDa protein	10,858–12,027	10,858–12,027	10,851–12,020	10,857–12,026
	protein IIIa precursor	12,055–13,818	12,055–13,818	12,048–13,811	12,054–13,817
L2	penton protein	13,891–15,540	13,891–15,540	13,884–15,533	13,890–15,539
	protein VII precursor	15,549–16,127	15,549–16,127	15,542–16,120	15,548–16,126
	protein V precursor	16,167–17,228	16,167–17,228	16,160–17,221	16,166–17,227
	protein X	17,257–17,487	17,257–17,487	17,250–17,480	17,256–17,486
L3	protein VI precursor	17,560–18,309	17,560–18,309	17,553–18,302	17,559–18,308
	hexon protein	18,423–21,272	18,423–21,272	18,416–21,265	18,422–21,271
	23 kDa proteinase	21,309–21,938	21,309–21,938	21,302–21,931	21,308–21,937
E2A	DNA-binding protein	22,027–23,580	22,027–23,580	22,020–23,573	22,026–23,579
L4	100 kDa hexon-assembly associated protein	23,611–26,094	23,611–26,094	23,604–26,087	23,610–26,093
	33 kDa protein	25,796–26,669	25,796–26,669	25,789–26,662	25,795–26,668
	22 kDa protein	25,796–26,395	25,796–26,395	25,789–26,388	25,795–26,394
	protein VIII	26,740–27,423	26,740–27,423	26,733–27,416	26,739–27,422

Continued

Gene	Encoded product	Jilin2023-296	Jilin2023-595	Ningxia2024-533	Ningxia2024-595
E3	12.1 kDa protein	27,423–27,743	27,423–27,743	27,416–27,736	27,422–27,742
	16 kDa protein	27,697–28,137	27,697–28,137	27,690–28,130	27,696–28,136
	19.2 kDa protein	28,122–28,640	28,122–28,640	28,115–28,633	28,121–28,639
	20 kDa protein	28,670–29,209	28,670–29,209	28,663–29,202	28,669–29,208
	21.2 kDa protein	29,222–29,834	29,222–29,834	29,215–29,827	29,221–29,833
	8.8 kDa protein	29,864–30,090	29,864–30,090	29,857–30,083	29,863–30,089
	10.3 kDa protein	30,130–30,405	30,130–30,405	30,123–30,398	30,129–30,404
	16.6 kDa protein	30,377–30,814	30,377–30,814	30,370–30,807	30,376–30,813
	15.3 kDa protein	30,807–31,214	30,807–31,214	30,800–31,207	30,806–31,213
L5	fiber protein	31,412–32,383	31,412–32,383	31,405–32,376	31,411–32,382
E4	16 kDa protein	32,415–32,666; 33,389–33,562	32,415–32,666; 33,389–33,562	32,408–32,659; 33,382–33,555	32,414–32,665; 33,388–33,561
	34.7 kDa protein	32,663–33,562	32,663–33,562	32,656–33,555	32,662–33,561
	14.3 kDa protein	33,465–33,833	33,465–33,833	33,458–33,826	33,464–33,832
L5	agnoprotein	33,688–34,197	33,688–34,197	33,681–34,190	33,687–34,196
E4	13.6 kDa protein	33,842–34,195	33,842–34,195	33,835–34,188	33,841–34,194
	14.4 kDa protein	34,192–34,581	34,192–34,581	34,185–34,574	34,191–34,580
	14.2 kDa protein	34,623–35,000	34,623–35,000	34,616–34,993	34,622–34,999

SUPPLEMENTARY TABLE S2-2. Genomic annotations of strains Ningxia2024-608, Ningxia2024-618, Ningxia2024-669, and Ningxia2024-671.

Gene	Encoded product	Ningxia2024-608	Ningxia2024-618	Ningxia2024-669	Ningxia2024-671
E1A	28.4 kDa protein	575–11,56; 1,250–1,453	560–1,141; 1,235–1,438	575–1,156; 1,250–1,453	575–1,156; 1,250–1,453
	24.6 kDa protein	575–1,063; 1,250–1,453	560–1,048; 1,235–1,438	575–1,063; 1,250–1,453	575–1,063; 1,250–1,453
	6.8 kDa protein	575–646; 1,248–1,349	560–631; 1,233–1,334	575–646; 1,248–1,349	575–646; 1,248–1,349
	small T antigen	1,601–2,137	1,586–2,122	1,601–2,137	1,601–2,137
E1B	large T antigen (55K)	1,906–3,384	1,891–3,369	1,906–3,384	1,906–3,384
IX	hexon-associated protein IX	3,478–3,894	3,463–3,879	3,478–3,894	3,478–3,894
IVa2	maturation protein IVa2	3,947–5,280; 5,559–5,571	3,932–5,265; 5,544–5,556	3,947–5,280; 5,559–5,571	3,947–5,280; 5,559–5,571
E2B	DNA polymerase	5,050–8,622; 13,840–13,848	5,035–8,607; 13,826–13,834	5,050–8,622; 13,841–13,849	5,050–8,622; 13,841–13,849
	hypothetical 11.5 kDa protein	6,143–6,463	6,128–6,448	6,143–6,463	6,143–6,463
	hypothetical 19 kDa protein	6,867–7,388	6,852–7,373	6,867–7,388	6,867–7,388
	hypothetical 10.4 kDa protein	7,132–7,419	7,117–7,404	7,132–7,419	7,132–7,419
	DNA-binding protein	7,828–8,229; 9,479–9,496	7,813–8,214; 9,464–9,481	7,828–8,229; 9,479–9,496	7,828–8,229; 9,479–9,496
	hypothetical 12.6 kDa protein	8,228–8,572	8,213–8,557	8,228–8,572	8,228–8,572
	terminal protein precursor	8,421–10,388; 13,840–13,848	8,406–10,373; 13,826–13,834	8,421–10,388; 13,841–13,849	8,421–10,388; 13,841–13,849
	hypothetical 14.5 kDa protein	8,547–8,948	8,532–8,933	8,547–8,948	8,547–8,948
	hypothetical 11.5 kDa protein	9,542–9,856	9,527–9,841	9,542–9,856	9,542–9,856
	hypothetical 9.7 kDa protein	9,756–10,031	9,741–10,016	9,756–10,031	9,756–10,031
	52 kDa protein	10,857–12,026	10,843–12,012	10,858–12,027	10,858–12,027
	protein IIIa precursor	1,2054–1,3817	12,040–13,803	12,055–13,818	12,055–13,818

Continued

Gene	Encoded product	Ningxia2024-608	Ningxia2024-618	Ningxia2024-669	Ningxia2024-671
L2	penton protein	13,890–15,539	13,876–15,525	13,891–15,540	13,891–15,537
	protein VII precursor	15,548–16,126	15,534–16,112	15,549–16,127	15,551–16,129
	protein V precursor	16,166–17,227	16,152–17,213	16,167–17,228	16,169–17,230
	protein X	17,256–17,486	17,242–17,472	17,257–17,487	17,259–17,489
L3	protein VI precursor	17,559–18,308	17,545–18,294	17,560–18,309	17,562–18,311
	hexon protein	18,422–21,271	18,408–21,257	18,423–21,272	18,425–21,274
	23 kDa proteinase	21,308–21,937	21,294–21,923	21,309–21,938	21,311–21,940
E2A	DNA-binding protein	22,026–23,579	22,012–23,565	22,027–23,580	22,029–23,582
L4	100 kDa hexon-assembly associated protein	23,610–26,093	23,596–26,079	23,611–26,094	23,613–26,096
	33 kDa protein	25,795–26,668	25,781–26,654	25,796–26,669	25,798–26,671
	22 kDa protein	25,795–26,394	25,781–26,380	25,796–26,395	25,798–26,397
	protein VIII	26,739–27,422	26,725–27,408	26,740–27,423	26,742–27,425
E3	12.1 kDa protein	27,422–27,742	27,408–27,728	27,423–27,743	27,425–27,745
	16 kDa protein	27,696–28,136	27,682–28,122	27,697–28,137	27,699–28,139
	19.2 kDa protein	28,121–28,639	28,107–28,625	28,122–28,640	28,124–28,642
	20 kDa protein	28,669–29,208	28,655–29,194	28,670–29,209	28,672–29,211
	21.2 kDa protein	29,221–29,833	29,207–29,819	29,222–29,834	29,224–29,836
	8.8 kDa protein	29,863–30,089	29,849–30,075	29,864–30,090	29,866–30,092
	10.3 kDa protein	30,129–30,404	30,115–30,390	30,130–30,405	30,132–30,407
	16.6 kDa protein	30,376–30,813	30,362–30,799	30,377–30,814	30,379–30,816
	15.3 kDa protein	30,806–31,213	30,792–31,199	30,807–31,214	30,809–31,216
L5	fiber protein	31,411–32,382	31,397–32,368	31,412–32,383	31,414–32,385
E4	16 kDa protein	32,414–32,665; 33,388–33,561	32,400–32,651; 33,374–33,547	32,415–32,666; 33,389–33,562	32,417–32,668; 33,391–33,564
	34.7 kDa protein	32,662–3,3561	32,648–33,547	32,663–33,562	32,665–33,564
	14.3 kDa protein	33,464–33,832	33,450–33,818	33,465–33,833	33,467–33,835
L5	agnoprotein	33,687–34,196	33,673–34,182	33,688–34,197	33,690–34,199
E4	13.6 kDa protein	33,841–34,194	33,827–34,180	33,842–34,195	33,844–34,197
	14.4 kDa protein	34,191–34,580	34,177–34,566	34,192–34,581	34,194–34,583
	14.2 kDa protein	34,622–34,999	34,608–34,985	34,623–35,000	34,625–35,002

SUPPLEMENTARY TABLE S2-3. Genomic annotations of the strains Ningxia2024-674, Ningxia2024-725, Ningxia2024-747, and Ningxia2024-754.

Gene	Encoded product	Ningxia2024-674	Ningxia2024-725	Ningxia2024-747	Ningxia2024-754
E1A	28.4 kDa protein	574–1,155; 1,249–1,452	575–1,156; 1,250–1,453	575–1,156; 1,250–1,453	575–1,156; 1,250–1,453
	24.6 kDa protein	574–1,062; 1,249–1,452	575–1,063; 1,250–1,453	575–1,063; 1,250–1,453	575–1,063; 1,250–1,453
	6.8 kDa protein	574–645; 1,247–1,348	575–646; 1,248–1,349	575–646; 1,248–1,349	575–646; 1,248–1,349
	small T antigen	1,600–2,136	1,601–2,137	1,601–2,137	1,601–2,137
E1B	large T antigen (55K)	1,905–3,383	1,906–3,384	1,906–3,384	1,906–3,384
IX	hexon-associated protein IX	3,477–3,893	3,478–3,894	3,478–3,894	3,478–3,894
IVa2	maturation protein IVa2	3,946–5,279; 5,558–5,570	3,947–5,280; 5,559–5,571	3,947–5,280; 5,559–5,571	3,947–5,280; 5,559–5,571

Continued

Gene	Encoded product	Ningxia2024-674	Ningxia2024-725	Ningxia2024-747	Ningxia2024-754
E2B	DNA polymerase	5,049–8,621; 13,839–13,847	5,050–8,622; 13,840–13,848	5,050–8,622; 13,841–13,849	5,050–8,622; 13,840–13,848
	hypothetical 11.5 kDa protein	6,142–6,462	6,143–6,463	6,143–6,463	6,143–6,463
	hypothetical 19 kDa protein	6,866–7,387	6,867–7,388	6,867–7,388	6,867–7,388
	hypothetical 10.4 kDa protein	7,131–7,418	7,132–7,419	7,132–7,419	7,132–7,419
	DNA-binding protein	7,827–8,228; 9,478–9,495	7,828–8,229; 9,479–9,496	7,828–8,229; 9,479–9,496	7,828–8,229; 9,479–9,496
	hypothetical 12.6 kDa protein	8,227–8,571	8,228–8,572	8,228–8,572	8,228–8,572
	terminal protein precursor	8,420–10,387; 13,839–13,847	8,421–10,388; 13,840–13,848	8,421–10,388; 13,841–13,849	8,421–10,388; 13,840–13,848
	hypothetical 14.5 kDa protein	8,546–8,947	8,547–8,948	8,547–8,948	8,547–8,948
	hypothetical 11.5 kDa protein	9,541–9,855	9,542–9,856	9,542–9,856	9,542–9,856
	hypothetical 9.7 kDa protein	9,755–10,030	9,756–10,031	9,756–10,031	9,756–10,031
L1	52 kDa protein	10,856–12,025	10,857–12,026	10,858–12,027	10,857–12,026
	protein IIIa precursor	12,053–13,816	12,054–13,817	12,055–13,818	12,054–13,817
L2	penton protein	13,889–15,535	13,890–15,536	13,891–15,537	13,890–15,536
	protein VII precursor	15,549–16,127	15,548–16,126	15,551–16,129	15,548–16,126
	protein V precursor	16,167–17,228	16,166–17,227	16,169–17,230	16,166–17,227
	protein X	17,257–17,487	17,256–17,486	17,259–17,489	17,256–17,486
L3	protein VI precursor	17,560–18,309	17,559–18,308	17,562–18,311	17,559–18,308
	hexon protein	18,426–21,275	18,422–21,271	18,425–21,274	18,422–21,271
	23 kDa proteinase	21,312–21,941	21,308–21,937	21,311–21,940	21,308–21,937
E2A	DNA-binding protein	22,030–23,583	22,026–23,579	22,029–23,582	22,026–23,579
L4	100 kDa hexon-assembly associated protein	23,614–26,097	23,610–26,093	23,613–26,096	23,610–26,093
	33 kDa protein	25,799–26,672	25,795–26,668	25,798–26,671	25,795–26,668
	22 kDa protein	25,799–26,398	25,795–26,394	25,798–26,397	25,795–26,394
	protein VIII	26,743–27,426	26,739–27,422	26,742–27,425	26,739–27,422
E3	12.1 kDa protein	27,426–27,746	27,422–27,742	27,425–27,745	27,422–27,742
	16 kDa protein	27,700–28,140	27,696–28,136	27,699–28,139	27,696–28,136
	19.2 kDa protein	28,125–28,643	28,121–28,639	28,124–28,642	28,121–28,639
	20 kDa protein	28,673–29,212	28,669–29,208	28,672–29,211	28,669–29,208
	21.2 kDa protein	29,225–29,837	29,221–29,833	29,224–29,836	29,221–29,833
	8.8 kDa protein	29,867–30,093	29,863–30,089	29,866–30,092	29,863–30,089
	10.3 kDa protein	30,133–30,408	30,129–30,404	30,132–30,407	30,129–30,404
	16.6 kDa protein	30,380–30,817	30,376–30,813	30,379–30,816	30,376–30,813
	15.3 kDa protein	30,810–31,217	30,806–31,213	30,809–31,216	30,806–31,213
	fiber protein	31,415–32,386	31,411–32,382	31,414–32,385	31,411–32,382
E4	16 kDa protein	32,418–32,669; 33,392–33,565	32,414–32,665; 33,388–33,561	32,417–32,668; 33,391–33,564	32,414–32,665; 33,388–33,561
	34.7 kDa protein	32,666–33,565	32,662–33,561	32,665–33,564	32,662–33,561
	14.3 kDa protein	33,468–33,836	33,464–33,832	33,467–33,835	33,464–33,832
L5	agnoprotein	33,691–34,200	33,687–34,196	33,690–34,199	33,687–34,196
E4	13.6 kDa protein	33,845–34,198	33,841–34,194	33,844–34,197	33,841–34,194
	14.4 kDa protein	34,195–34,584	34,191–34,580	34,194–34,583	34,191–34,580
	14.2 kDa protein	34,626–35,003	34,622–34,999	34,625–35,002	34,622–34,999

SUPPLEMENTARY TABLE S2-4. Genomic annotation of strains Ningxia2024-868, Ningxia2024-907, Ningxia2024-944, and Beijing2024-330.

Gene	Encoded product	Ningxia2024-868	Ningxia2024-907	Ningxia2024-944	Beijing2024-330
E1A	28.4 kDa protein	575–1,156; 1,250–1,453	575–1,156; 1,250–1,453	576–1,148; 1,242–1,445	567–1,148; 1,242–1,445
	24.6 kDa protein	575–1,063; 1,250–1,453	575–1,063; 1,250–1,453	576–1,064; 1,242–1,445	567–1,055; 1,242–1,445
	6.8 kDa protein	575–646; 1,248–1,349	575–646; 1,248–1,349	567–638; 1,240–1,341	567–638; 1,240–1,341
E1B	small T antigen	1,601–2,137	1,601–2,137	1,593–2,129	1,593–2,129
	large T antigen (55K)	1,906–3,384	1,906–3,384	1,898–3,376	1,898–3,376
IX	hexon-associated protein IX	3,478–3,894	3,478–3,894	3,470–3,886	3,470–3,886
IVa2	maturation protein IVa2	3,947–5,280; 5,559–5,571	3,947–5,280; 5,559–5,571	3,939–5,272; 5,551–5,563	3,939–5,272; 5,551–5,563
E2B	DNA polymerase	5,050–8,622; 13,840–13,848	5,050–8,622; 13,841–13,849	5,042–8,614; 13,831–13,839	5,042–8,614; 13,832–13,840
	hypothetical 11.5 kDa protein	6,143–6,463	6,143–6,463	6,135–6,455	6,135–6,455
	hypothetical 19 kDa protein	6,867–7,388	6,867–7,388	6,859–7,380	6,859–7,380
	hypothetical 10.4 kDa protein	7,132–7,419	7,132–7,419	7,124–7,411	7,124–7,411
	DNA-binding protein	7,828–8,229; 9,479–9,496	7,828–8,229; 9,479–9,496	7,820–8,221; 9,471–9,488	7,820–8,221; 9,471–9,488
	hypothetical 12.6 kDa protein	8,228–8,572	8,228–8,572	8,220–8,564	8,220–8,564
	terminal protein precursor	8,421–10,388; 13,840–13,848	8,421–10,388; 13,841–13,849	8,413–10,380; 13,831–13,839	8,413–10,380; 13,832–13,840
	hypothetical 14.5 kDa protein	8,547–8,948	8,547–8,948	8,539–8,940	8,539–8,940
	hypothetical 11.5 kDa protein	9,542–9,856	9,542–9,856	9,534–9,848	9,534–9,848
	hypothetical 9.7 kDa protein	9,756–10,031	9,756–10,031	9,748–10,023	9,748–10,023
	52 kDa protein	10,857–12,026	10,858–12,027	10,848–12,017	10,849–12,018
L1	protein IIIa precursor	12,054–13,817	12,055–13,818	12,045–13,808	12,046–13,809
L2	penton protein	13,890–15,536	13,891–15,537	13,881–15,527	13,882–15,531
	protein VII precursor	15,548–16,126	15,549–16,127	15,539–16,117	15,540–16,118
	protein V precursor	16,166–17,227	16,167–17,228	16,157–17,218	16,158–17,219
	protein X	17,256–17,486	17,257–17,487	17,247–17,477	17,248–17,478
L3	protein VI precursor	17,559–18,308	17,560–18,309	17,550–18,299	17,551–18,300
	hexon protein	18,422–21,271	18,423–21,272	18,413–21,262	18,414–21,263
	23 kDa proteinase	21,308–21,937	21,309–21,938	21,299–21,928	21,300–21,929
E2A	DNA-binding protein	22,026–23,579	22,027–23,580	22,017–23,570	22,018–23,571
L4	100 kDa hexon-assembly associated protein	23,610–26,093	23,611–26,094	23,601–26,084	23,602–26,085
	33 kDa protein	25,795–26,668	25,796–26,669	25,786–26,659	25,787–26,660
	22 kDa protein	25,795–26,394	25,796–26,395	25,786–26,385	25,787–26,386
	protein VIII	26,739–27,422	26,740–27,423	26,730–27,413	26,731–27,414
E3	12.1 kDa protein	27,422–27,742	27,423–27,743	27,413–27,733	27,414–27,734
	16 kDa protein	27,696–28,136	27,697–28,137	27,687–28,127	27,688–28,128
	19.2 kDa protein	28,121–28,639	28,122–28,640	28,112–28,630	28,113–28,631
	20 kDa protein	28,669–29,208	28,670–29,209	28,660–29,199	28,661–29,200
	21.2 kDa protein	29,221–29,833	29,222–29,834	29,212–29,824	29,213–29,825
	8.8 kDa protein	29,863–30,089	29,864–30,090	29,854–30,080	29,855–30,081
	10.3 kDa protein	30,129–30,404	30,130–30,405	30,120–30,395	30,121–30,396
	16.6 kDa protein	30,376–30,813	30,377–30,814	30,367–30,804	30,368–30,805
	15.3 kDa protein	30,806–31,213	30,807–31,214	30,797–31,204	30,798–31,205

Continued

Gene	Encoded product	Ningxia2024-868	Ningxia2024-907	Ningxia2024-944	Beijing2024-330
L5	fiber protein	31,411–32,382	31,412–32,383	31,402–32,373	31,403–32,374
E4	16 kDa protein	32,414–32,665; 33,388–33,561	32,415–32,666; 33,389–33,562	32,405–32,656; 33,379–33,552	32,406–32,657; 33,380–33,553
	34.7 kDa protein	32,662–33,561	32,663–33,562	32,653–33,552	32,654–33,553
	14.3 kDa protein	33,464–33,832	33,465–33,833	33,455–33,823	33,456–33,824
L5	agnoprotein	33,687–34,196	33,688–34,197	33,678–34,187	33,679–34,188
E4	13.6 kDa protein	33,841–34,194	33,842–34,195	33,832–34,185	33,833–34,186
	14.4 kDa protein	3,4191–34,580	34,192–34,581	34,182–34,571	34,183–34,572
	14.2 kDa protein	34,622–34,999	34,623–35,000	34,613–34,990	34,614–34,991

SUPPLEMENTARY TABLE S2-5. Genomic annotation of strains Beijing2024-364, Beijing2024-378, Shenzhen2024-077, and Shenzhen2024-201.

Gene	Encoded product	Beijing2024-364	Beijing2024-378	Shenzhen2024-077	Shenzhen2024-201
E1A	28.4 kDa protein	575–1,156; 1,250–1,453	575–1,156; 1,250–1,453	575–1,156; 1,250–1,453	566–1,147; 1,241–1,444
	24.6 kDa protein	575–1,063; 1,250–1,453	575–1,063; 1,250–1,453	575–1,063; 1,250–1,453	566–1,054; 1,241–1,444
	6.8 kDa protein	575–646; 1,248–1,349	575–646; 1,248–1,349	575–646; 1,248–1,349	566–637; 1,239–1,340
	small T antigen	1,601–2,137	1,601–2,137	1,601–2,137	1,592–2,128
E1B	large T antigen (55K)	1,906–3,384	1,906–3,384	1,906–3,384	1,897–3,375
IX	hexon-associated protein IX	3,478–3,894	3,478–3,894	3,478–3,894	3,469–3,885
Iva2	maturation protein Iva2	3,947–5,280; 5,559–5,571	3,947–5,280; 5,559–5,571	3,947–5,280; 5,559–5,571	3,938–5,271; 5,550–5,562
E2B	DNA polymerase	5,050–8,622; 1,3841–1,3849	5,050–8,622; 13,841–13,849	5,050–8,622; 13,841–13,849	5,041–8,613; 13,833–13,841
	hypothetical 11.5 kDa protein	6,143–6,463	6,143–6,463	6,143–6,463	6,134–6,454
	hypothetical 19 kDa protein	6,867–7,388	6,867–7,388	6,867–7,388	6,858–7,379
	hypothetical 10.4 kDa protein	7,132–7,419	7,132–7,419	7,132–7,419	7,123–7,410
	DNA-binding protein	7,828–8,229; 9,479–9,496	7,828–8,229; 9,479–9,496	7,828–8,229; 9,479–9,496	7,819–8,220; 9,470–9,487
	hypothetical 12.6 kDa protein	8,228–8,572	8,228–8,572	8,228–8,572	8,219–8,563
	terminal protein precursor	8,421–10,388; 13,841–13,849	8,421–10,388; 13,841–13,849	8,421–10,388; 13,841–13,849	8,412–10,379; 13,833–13,841
	hypothetical 14.5 kDa protein	8,547–8,948	8,547–8,948	8,547–8,948	8,538–8,939
	hypothetical 11.5 kDa protein	9,542–9,856	9,542–9,856	9,542–9,856	9,533–9,847
	hypothetical 9.7 kDa protein	9,756–10,031	9,756–10,031	9,756–10,031	9,747–10,022
	52 kDa protein	10,858–12,027	10,858–12,027	10,858–12,027	10,850–12,019
	protein IIIa precursor	12,055–13,818	12,055–13,818	12,055–13,818	12,047–13,810
L2	penton protein	13,891–15,540	13,891–15,540	13,891–15,537	13,883–15,529
	protein VII precursor	15,549–16,127	15,549–16,127	15,549–16,127	15,540–16,118
	protein V precursor	16,167–17,228	16,167–17,228	16,167–17,228	16,158–17,219
L3	protein X	17,257–17,487	17,257–17,487	17,257–17,487	17,248–17,478
	protein VI precursor	17,560–18,309	17,560–18,309	17,560–18,309	17,551–18,300
	hexon protein	18,423–21,272	18,423–21,272	18,423–21,272	18,417–21,266
E2A	23 kDa proteinase	21,309–21,938	21,309–21,938	21,309–21,938	21,303–21,932
	DNA-binding protein	22,027–23,580	22,027–23,580	22,027–23,580	22,021–23,574

Continued

Gene	Encoded product	Beijing2024-364	Beijing2024-378	Shenzhen2024-077	Shenzhen2024-201
L4	100 kDa hexon-assembly associated protein	23,611–26,094	23,611–26,094	23,611–26,094	23,605–26,088
	33 kDa protein	25,796–26,669	25,796–26,669	25,796–26,669	25,790–26,663
	22 kDa protein	25,796–26,395	25,796–26,395	25,796–26,395	25,790–26,389
	protein VIII	26,740–27,423	26,740–27,423	26,740–27,423	26,734–27,417
E3	12.1 kDa protein	27,423–27,743	27,423–27,743	27,423–27,743	27,417–27,737
	16 kDa protein	27,697–28,137	27,697–28,137	27,697–28,137	27,691–28,131
	19.2 kDa protein	28,122–28,640	28,122–28,640	28,122–28,640	28,116–28,634
	20 kDa protein	28,670–29,209	28,670–29,209	28,670–29,209	28,664–29,203
	21.2 kDa protein	29,222–29,834	29,222–29,834	29,222–29,834	29,216–29,828
	8.8 kDa protein	29,864–30,090	29,864–30,090	29,864–30,090	29,858–30,084
	10.3 kDa protein	30,130–30,405	30,131–30,406	30,130–30,405	30,124–30,399
	16.6 kDa protein	30,377–30,814	30,378–30,815	30,377–30,814	30,371–30,808
	15.3 kDa protein	30,807–31,214	30,808–31,215	30,807–31,214	30,801–31,208
L5	fiber protein	31,412–32,383	31,413–32,384	31,412–32,383	31,406–32,377
E4	16 kDa protein	32,415–32,666; 33,389–33,562	32,416–32,667; 33,390–33,563	32,415–32,666; 33,389–33,562	32,409–32,660; 33,383–33,556
	34.7 kDa protein	32,663–33,562	32,664–33,563	32,663–33,562	32,657–33,556
	14.3 kDa protein	33,465–33,833	33,466–33,834	33,465–33,833	33,459–33,827
L5	agnoprotein	33,688–34,197	33,689–34,198	33,688–34,197	33,682–34,191
E4	13.6 kDa protein	33,842–34,195	33,843–34,196	33,842–34,195	33,836–34,189
	14.4 kDa protein	34,192–34,581	34,193–34,582	34,192–34,581	34,186–34,575
	14.2 kDa protein	34,623–35,000	34,624–35,001	34,623–35,000	34,617–34,994

SUPPLEMENTARY TABLE S2-6. Genomic annotations of strains Chongqing2024-069, Shenyang2024-163, and Gansu2024-215.

Gene	Encoded product	Chongqing2024-069	Shenyang2024-163	Gansu2024-215
E1A	28.4 kDa protein	575–1,156; 1,250–1,453	575–1,156; 1,250–1,453	575–1,156; 1,250–1,453
	24.6 kDa protein	575–1,063; 1,250–1,453	575–1,063; 1,250–1,453	575–1,063; 1,250–1,453
	6.8 kDa protein	575–646; 1,248–1,349	575–646; 1,248–1,349	575–646; 1,248–1,349
	small T antigen	1,601–2,137	1,601–2,137	1,601–2,137
E1B	large T antigen (55K)	1,906–3,384	1,906–3,384	1,906–3,384
IX	hexon-associated protein IX	3,478–3,894	3,478–3,894	3,478–3,894
IVa2	maturation protein IVa2	3,947–5,280; 5,559–5,571	3,947–5,280; 5,559–5,571	3,947–5,280; 5,559–5,571
E2B	DNA polymerase	5,050–8,622; 13,841–13,849	5,050–8,622; 13,841–13,849	5,050–8,622; 13,841–13,849
	hypothetical 11.5 kDa protein	6,143–6,463	6,143–6,463	6,143–6,463
	hypothetical 19 kDa protein	6,867–7,388	6,867–7,388	6,867–7,388
	hypothetical 10.4 kDa protein	7,132–7,419	7,132–7,419	7,132–7,419
	DNA-binding protein	7,828–8,229; 9,479–9,496	7,828–8,229; 9,479–9,496	7,828–8,229; 9,479–9,496
	hypothetical 12.6 kDa protein	8,228–8,572	8,228–8,572	8,228–8,572
	terminal protein precursor	8,421–10,388; 13,841–13,849	8,421–10,388; 13,841–13,849	8,421–10,388; 13,841–13,849
	hypothetical 14.5 kDa protein	8,547–8,948	8,547–8,948	8,547–8,948
	hypothetical 11.5 kDa protein	9,542–9,856	9,542–9,856	9,542–9,856
	hypothetical 9.7 kDa protein	9,756–10,031	9,756–10,031	9,756–10,031

Continued

Gene	Encoded product	Chongqing2024-069	Shenyang2024-163	Gansu2024-215
L1	52 kDa protein	10,858–12,027	10,858–12,027	10,858–12,027
	protein IIIa precursor	12,055–13,818	12,055–13,818	12,055–13,818
L2	penton protein	13,891–15,540	13,891–15,537	13,891–15,537
	protein VII precursor	15,549–16,127	15,549–16,127	15,549–16,127
	protein V precursor	16,167–17,228	16,167–17,228	16,167–17,228
	protein X	17,257–17,487	17,257–17,487	17,257–17,487
L3	protein VI precursor	17,560–18,309	17,560–18,309	17,560–18,309
	hexon protein	18,423–21,272	18,423–21,272	18,423–21,272
	23 kDa proteinase	21,309–21,938	21,309–21,938	21,309–21,938
E2A	DNA-binding protein	22,027–23,580	22,027–23,580	22,027–23,580
L4	100 kDa hexon-assembly associated protein	23,611–26,094	23,611–26,094	23,611–26,094
	33 kDa protein	25,796–26,669	25,796–26,669	25,796–26,669
	22 kDa protein	25,796–26,395	25,796–26,395	25,796–26,395
	protein VIII	26,740–27,423	26,740–27,423	26,740–27,423
E3	12.1 kDa protein	27,423–27,743	27,423–27,743	27,423–27,743
	16 kDa protein	27,697–28,137	27,697–28,137	27,697–28,137
	19.2 kDa protein	28,122–28,640	28,122–28,640	28,122–28,640
	20 kDa protein	28,670–29,209	28,670–29,209	28,670–29,209
	21.2 kDa protein	29,222–29,834	29,222–29,834	29,222–29,834
	8.8 kDa protein	29,864–30,090	29,864–30,090	29,864–30,090
	10.3 kDa protein	30,130–30,405	30,130–30,405	30,130–30,405
	16.6 kDa protein	30,377–30,814	30,377–30,814	30,377–30,814
	15.3 kDa protein	30,807–31,214	30,807–31,214	30,807–31,214
L5	fiber protein	31,412–32,383	31,412–32,383	31,412–32,383
E4	16 kDa protein	32,415–32,666; 33,389–33,562	32,415–32,666; 33,389–33,562	32,415–32,666; 33,389–33,562
	34.7 kDa protein	32,663–33,562	32,663–33,562	32,663–33,562
	14.3 kDa protein	33,465–33,833	33,465–33,833	33,465–33,833
L5	agnoprotein	33,688–34,197	33,688–34,197	33,688–34,197
E4	13.6 kDa protein	33,842–34,195	33,842–34,195	33,842–34,195
	14.4 kDa protein	34,192–34,581	34,192–34,581	34,192–34,581
	14.2 kDa protein	34,623–35,000	34,623–35,000	34,623–35,000