

Letter to Editor

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Molecular surveillance of human adenovirus in fecal specimens from Bangkok, Thailand, 2022–2023: Predominance of HAdV–F41

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Human adenoviruses (HAdVs) consist of several species and genotypes associated with respiratory, ophthalmic, hepatic, and gastrointestinal diseases[1,2]. Enteric HAdV-F40 and HAdV-F41 are recognized etiological agents of acute gastroenteritis in children; however, non-enteric HAdV genotypes may also be identified in fecal samples due to gastrointestinal involvement, extended shedding, or simultaneous systemic or respiratory infections[3,4]. Current sequence-derived data regarding HAdV genotypes identified in fecal samples from Thailand are limited. We report an updated dual-marker molecular surveillance investigation of HAdV in leftover fecal samples from individuals with acute diarrhea at Siriraj Hospital, Bangkok, Thailand, throughout 2022–2023.

This study analyzed 672 residual fecal samples from routine rotavirus testing of patients with acute diarrhea at the Department of Microbiology, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand. The study was approved by the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (Approval certificate No. Si319/2022; approval date: April 19, 2022). All study procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki. The sample comprised 376 males and 296 females, categorized into age groups: <1 month ($n=100$), 1–11 months ($n=162$), 1–5 years ($n=260$), 6–10 years ($n=67$), 11–15 years ($n=48$), and >15 years ($n=35$) (Table 1).

Viral nucleic acids were extracted from fecal suspensions, and HAdV detection targeted a conserved partial E2B DNA polymerase region using previously described primers[5]. Each PCR run included no-template negative controls, and all controls remained negative. E2B-positive samples were then characterized by partial hexon nested PCR using universal primer sets[6]; every E2B-positive specimen also exhibited positivity in hexon amplification. PCR products from both loci were sequenced bidirectionally by Sanger sequencing.

Table 1. Demographic characteristics and HAdV genotype distribution among 672 included fecal samples.

Characteristic	<i>n</i> (%) [*]
Total samples	672 (100.0)
Sex	
Male	376 (56.0)
Female	296 (44.0)
Age group	
<1 month	100 (14.9)
1 to 11 months	162 (24.1)
1 to 5 years old	260 (38.7)
6 to 10 years old	67 (10.0)
11 to 15 years old	48 (7.1)
>15 years old	35 (5.2)
Human adenovirus (HAdV) ^{**}	
Positive	149 (22.2)
HAdV-B3	1 (0.1)
HAdV-C1	5 (0.7)
HAdV-C2	13 (1.9)
HAdV-C5	7 (1.0)
HAdV-C6	2 (0.3)
HAdV-D17	1 (0.1)
HAdV-D8	1 (0.1)
HAdV-F41	119 (17.7)
Negative	523 (77.8)

^{*}Percentages were calculated using the total number of included samples ($n=672$); ^{**}HAdV genotype rows represent corrected hexon-supported assignments.

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Consensus sequences were determined after an exhaustive analysis of the chromatograms. Genotype categorization has been corroborated through local BLAST analysis utilizing curated reference sequences that represent the identified genotypes[7]. Final genotype classifications were primarily determined using hexon sequences, as hexon offers superior genotype-level differentiation compared to E2B DNA polymerase. When the initial genotype annotation opposed the local BLAST findings, the genotype was amended according to the highest-identity hexon BLAST alignment. E2B DNA polymerase was retained as a conserved detection marker and complementary phylogenetic locus, not as a standalone marker for genotype reassignment. All sequences were submitted to

GenBank with accession numbers PQ148470-PQ148767.

HAdV was identified in 149 out of 672 samples (22.2%). According to hexon-supported genotype classification, HAdV-F41 was the most prevalent, representing 119 HAdV-positive samples and 17.7% of the total cohort. The rarer genotypes were HAdV-C2 ($n=13$), HAdV-C5 ($n=7$), HAdV-C1 ($n=5$), HAdV-C6 ($n=2$), HAdV-B3 ($n=1$), HAdV-D8 ($n=1$), and HAdV-D17 ($n=1$). The remaining 523 specimens were HAdV-negative (Table 1). These findings are consistent with previous Thai studies reporting enteric and non-enteric HAdV genotypes in children with acute gastroenteritis[3,4], while extending available surveillance data to a Bangkok cohort sampled during 2022-2023.

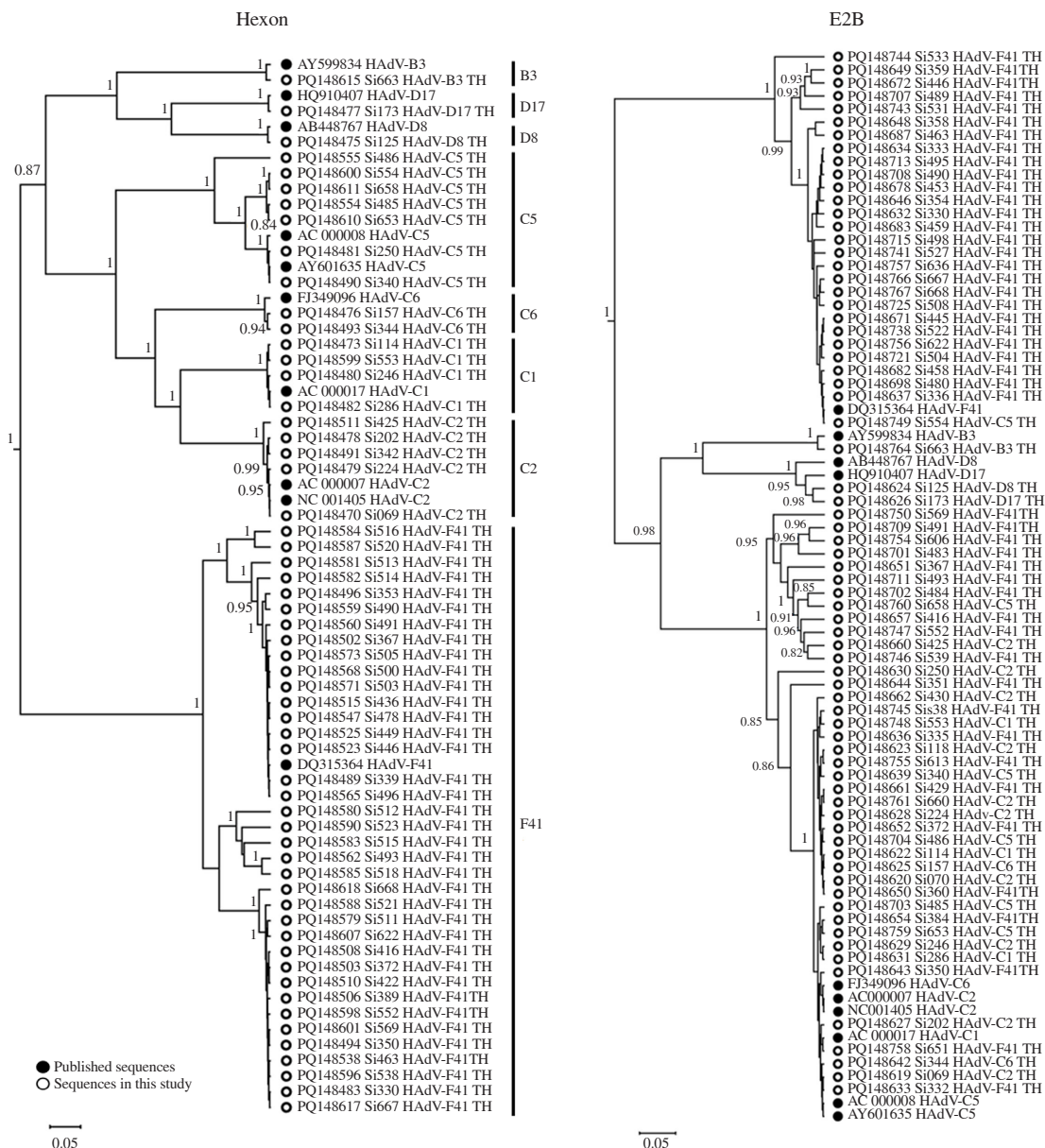


Figure 1. Bayesian phylogenetic analysis of partial hexon and E2B DNA polymerase sequences of human adenovirus (HAdV) detected in Thailand. Maximum clade credibility trees were inferred separately for the partial hexon gene and the partial E2B DNA polymerase gene. Study-generated sequences are shown as open circles, and curated genotype reference sequences as filled circles. Node labels indicate posterior probabilities for major supported clades (only values ≥ 0.85 are shown). The hexon tree was used as the primary genotype-resolving analysis, whereas the E2B DNA polymerase tree was interpreted as complementary phylogenetic evidence because the analyzed fragment was shorter and more conserved. Scale bars indicate substitutions per site.

Bayesian phylogenetic analyses were conducted independently for the partial hexon and E2B DNA polymerase datasets utilizing BEAST X v10.5.0[8]. The hexon tree provided the clearest genotype-resolving signal, with study sequences clustering with corresponding curated reference genotypes (Figure 1). The HAdV-F41 sequences formed the largest clade and showed short branch lengths across the analyzed partial hexon region, consistent with a dominant group of closely related HAdV-F41-like strains during the study period. In contrast, the E2B DNA polymerase tree showed lower genotype-discriminating resolution, consistent with the conserved nature of this region and the shorter fragment analyzed. Numerous E2B sequences did not group exclusively based on their hexon-supported genotypes (Figure 1). This inconsistency might indicate a confined phylogenetic signal, a marker-specific evolutionary trajectory, or potential recombination; yet, incomplete E2B and hexon sequences are inadequate to validate recombination occurrences. Whole-genome sequencing or broader multi-locus analysis would be required for that purpose.

This study should be interpreted as hospital-based molecular surveillance rather than a nationwide estimate of HAdV circulation in Thailand. The clinical severity, treatment results, and co-infections were not evaluated. The data, however, indicate the ongoing prevalence of HAdV-F41, reveal co-detection of the rarer HAdV-B, HAdV-C, and HAdV-D genotypes in fecal samples, and provide paired E2B/hexon sequences for subsequent comparative monitoring.

Conflict of interest statement

The authors declare no conflict of interest.

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Data availability

GenBank accessions PQ148470-PQ148767; additional analysis files are available upon reasonable request. AI-assisted tools were used only for language polishing and editorial refinement; the authors take full responsibility for the manuscript.

Authors' contributions

TC and KS designed the study; KS, BK, and NS curated specimens; TC performed experiments, analyzed data, and drafted the manuscript; all authors reviewed and approved the final version.

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