# Genomic Surveillance for Human Adenovirus Infections among Department of Defense Beneficiaries in the 2018-2019 Season

Adam R. Pollio[1], Anthony C. Fries[2], Yu Yang[1], Jerry J. Hughes[1], Christian K. Fung[1], Matthew A. Conte[1], Robert A. Kuschner[1], Natalie D. Collins[1], Elizabeth Macias[2], and Jun Hang[1]

[1] Walter Reed Army Institute of Research, Silver Spring, MD; [2] US Air Force School of Aerospace Medicine, Wright-Patterson AFB, OH

## Background

Human adenoviruses (family *Adenoviridae*, genus *Mastadenovirus*) (HAdV) species B, C and E cause acute respiratory illnesses (ARI). In particular, respiratory HAdV types 3, 4, 7, 11, 14, 21 and 55 were associated with ARI outbreaks in the US and in other countries. The risk of outbreaks can be effectively controlled by implementation of the live HAdV types 4 and 7 vaccines or mitigated by screening and preventive measures. Active surveillance on respiratory HAdV infection with detailed characterization is desired to inform mitigation measures to protect high risk populations. The US military has successfully used licensed HAdV vaccines to prevent ARI outbreak during recruit basic training, and experienced persistent ARI outbreaks when HAdV vaccines were not used. The US Department of Defense Global Emerging Infection Surveillance (DoD GEIS) program actively monitors infectious pathogens including respiratory viruses to better protect the health of the DoD beneficiaries. In this study we conducted HAdV genomic surveillance and identified HAdV types 4, 7 and 14 that caused clustered cases in active duty members and their dependents during the season 2018 – 2019. Whole genome comparative analysis revealed novel genetic mutation, but overall conservation of adenovirus genomes.

## Methods

**Sample collection, extraction and sequencing:** The HAdV isolates were obtained from the DoD Global Respiratory Pathogen Surveillance Program (DoDGRPSP) at the US Air Force School of Aerospace Medicine (USAFSAM), Wright-Patterson AFB, Ohio. The respiratory specimens positive for HAdV in molecular assay were subjected to viral culture using cell lines A549, RM2, etc. The supernatant from cultures with positive cytopathic effect (CPE) were extracted to purify HAdV genomic DNA for whole genome sequencing using QIAseq FX DNA Library Kit (QIAGEN, https://www.qiagen.com) and MiSeq next-generation sequencing (NGS) system and Reagent Kit v3 (600-cycle) (Illumina, https://www.illumina.com) at the Walter Reed Army Institute of Research (WRAIR).

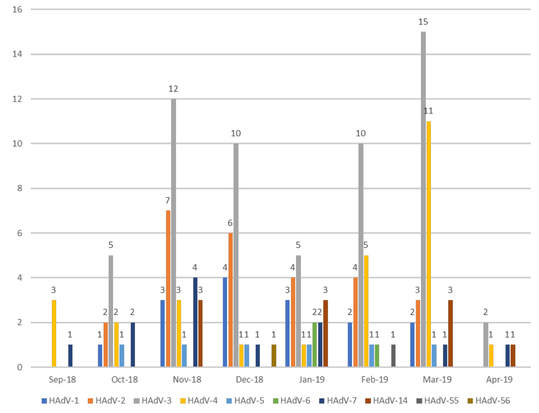
**Data Analysis:** The bioinformatics tools, NGS_Mapper, Geneious R10 (Biomatters Ltd., https://www.geneious.com), Integrative Genomics Viewer (IGV) (Broad Institute, https://igv.org), were used for whole genome sequence assembly and visualization. Multiple sequence alignments were generated using MUSCLE and phylogenetic trees built using Neighbor-Joining method and Tamura-Nei genetic distance model, with 500 bootstrap replicates. Representative complete genome sequences for HAdV types 4, 7 and 14 strains from different countries and collection years were retrieved from GenBank and included in whole genome phylogenetic analyses. Sequences containing large segment recombinant, such as HAdV7 strains HAdV 7/Haiti-0707/2014 (MN531562) and HAdV-B/USA/8010/1998 (MH910665), were excluded.

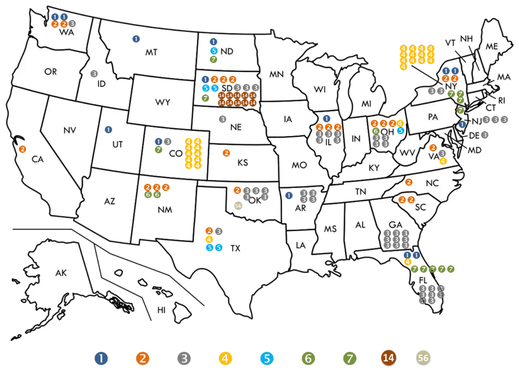
### Disclaimer

Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense.

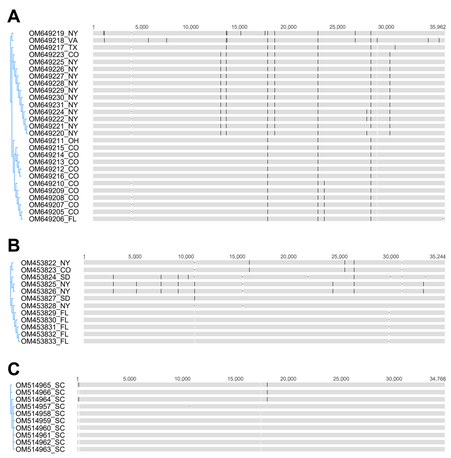
## Results

| | n | N[a] | % |
|---|---|---|---|
| **Sex** | | | |
| Male | 116 | 166 | 70.0 |
| Female | 50 | 166 | 30.1 |
| **Age bracket (years)** | | | |
| 0-5 | 69 | 166 | **41.6** |
| 6-10 | 26 | 166 | 15.7 |
| 11-15 | 10 | 166 | 6.0 |
| 16-20 | 28 | 166 | 16.9 |
| 21-25 | 19 | 166 | 11.5 |
| 26-30 | 7 | 166 | 4.2 |
| 31-35 | 5 | 166 | 4.1 |
| 36-40 | 1 | 166 | 0.6 |
| 41-45 | 1 | 166 | 0.6 |
| **Service category** | | | |
| Active duty | 54 | 162 | 33.3 |
| Dependents | 108 | 162 | **66.7** |
| **Clinical outcomes** | | | |
| Hospitalized | 2 | 98 | 2.0 |
| Pneumonia | 0 | 119 | 0.0 |



During the influenza season 2018 – 2019, the DoD Global Respiratory Pathogen Surveillance Program (DoDGRS) received 24,300 specimens from its surveillance network. In total, 322 (1.3%) respiratory specimens were positive for HAdV in molecular assays. Out of 166 viral culture samples available for NGS, whole genome sequences were obtained for 161 isolates. Most patients identified were military dependents (67%) between the ages of 0-5 (42%). A variety types of HAdV were identified, including HAdV-1 (N = 15), HAdV-2 (N = 26), HAdV-3 (N = 60), HAdV-4 (N = 13), HAdV-5 (N = 6), HAdV-6 (N = 3), HAdV-7 (N = 12), HAdV-14 (N = 10), HAdV-55 (N = 1), and HAdV-56 (N = 1). HAdV 3 is the most prevalent type identified, however, it was identified in several locations and was not considered significant at this time. There were clusters of HAdV 4, 7 and 14 cases, suggesting possible outbreaks.




Multiple sequence alignment of complete genome sequences of human adenoviruses (HAdV) of season 2018 – 2019 in this study. (A) HAdV 4; (B) HAdV 7; (C) HAdV 14. Genomes are arranged based on phylogenetic relatedness.


Whole genome phylogeny of human adenoviruses (HAdV) in this study and representative HAdV sequences in GenBank. (A) HAdV 4; (B) HAdV 7; (C) HAdV 14. Phylogentic analyses revealed clustered cases of HAdV 4, 7 and 14 infections in several states in the season 2018 – 2019. Neighbor-Joining phylogenetic tree with bootstrap support values from 500 replicates shown at the branches. The scale bar represents estimated nucleotide substitutions per site. The dots indicate the HAdVs from season 2018-2019 in this study.

## Conclusion

A variety types of HAdV were identified in this genomic surveillance study of HAdV isolates from DoDGRS for the influenza season 2018 - 2019. HAdV types 4, 7 and 14, which were reported causing ARI outbreaks in the US military and other congregate settings, were found in clustered cases in several US states. Routine clinical respiratory pathogen diagnostics or molecular surveillance of respiratory diseases detect adenovirus without determination of HAdV type. Human adenoviruses are genetically diverse and can infect a number of tissues with severities varied from mild to fatal. Enhanced genomic surveillance of HAdV in the US and worldwide will shed light on prevalence, genetic divergence, and viral evolution of human adenoviruses and inform timely risk assessment and countermeasures.