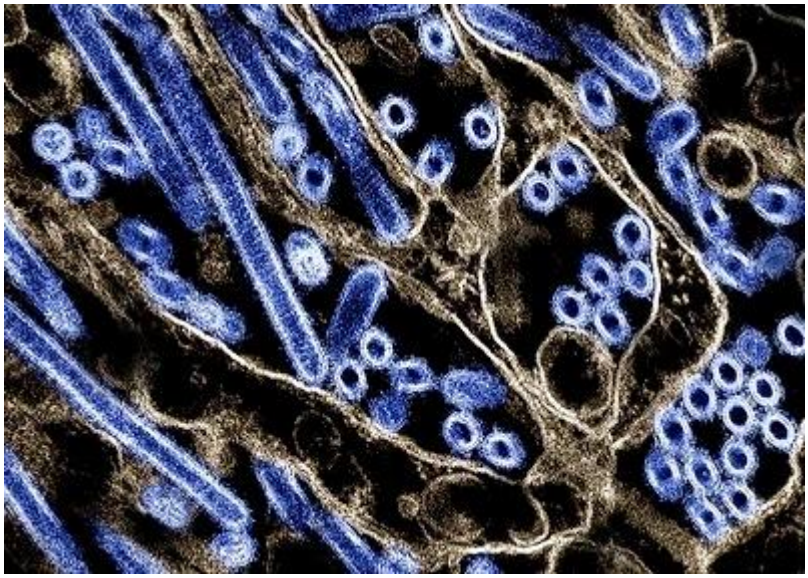
Since 1996, when a highly pathogenic avian influenza (HPAI) virus H5N1 of the lineage A/Goose/Guangdong/1/96 (GsGd) emerged in domestic geese, this lineage has spread worldwide. Since 2022, the HPAI H5N1 virus of clade 2.3.4.4b has been circulating in avian species in North America, and recent spillovers of this virus into dairy cows in the United States resulted in infection of dairy farmworkers. In this study, the Dutch authors compared the attachment pattern in the human upper or lower respiratory tract of the H5N1 virus of clade 2.3.4.4b with the attachment pattern of the H5N1 virus of clade 2.1.3.2 and a seasonal H3N2 virus. They also investigated replication and infection of human nasal and tracheal/bronchial respiratory epithelium with these viruses and associated innate immune responses.

The broadened host range of clade 2.3.4.4b H5N1 viruses and unprecedented transmission levels between mammals raised concerns about potential spillover into humans, especially when these viruses were detected in tissue samples and milk from infected dairy cows and humans. https://discovermednews.com/transmission-of-influenza-a-h5n1-virus-through-raw-milk/



About the study

The authors investigated the attachment pattern of the viruses H5N12022, H5N12005, and H3N22003 to the upper respiratory tract (nasal respiratory mucosa and olfactory mucosa) and the lower respiratory tract (trachea, bronchus, bronchioles, and alveoli) of humans using virus histochemistry. They also investigated the replication efficiency of the HPAI H5N12022 virus isolate in human nasal respiratory epithelium and tracheal/bronchiolar epithelium differentiated from airway organoids at the air-liquid interface. To characterize cytokine responses in cultures of nasal and tracheal/bronchiolar respiratory epithelium following infection with H5N12022, H5N12005, and H3N22003 viruses, the researchers used a multibead cytokine assay.

Results

The attachment to ciliated epithelial cells of the upper respiratory tract varied widely among viruses. The attachment of the H5N12005 virus was rare, and that of the H5N12022 virus was moderate. However, the seasonal H3N22003 virus attached more abundantly than both H5 viruses.

In the lower respiratory tract, the H5N12022 virus was found to attach more abundantly to the apical side of ciliated and non-ciliated epithelial cells of the trachea, bronchus, and bronchiole, and alveolar type-I and type-II pneumocytes than H5N12005 virus, which only attached to alveolar type-II pneumocytes and less abundantly to the other aforementioned cell types. The H3N22003 virus attached most abundantly to ciliated and non-ciliated epithelial cells in the airways and alveolar type-I pneumocytes, but not to type-II pneumocytes.

According to these data, the H5N12022 virus was attached to the upper and lower respiratory tract of humans more abundantly than the H5N12005 virus, suggesting an expanded receptor binding repertoire for the H5N12022 virus.

These findings are consistent with the results of a study conducted by van Riel D et al, who also used virus histochemistry to compare the viral attachment pattern between the H5N1 virus and two human influenza viruses (H1N1 and H3N2) in respiratory tract tissues from humans, mice, ferrets, cats, and pigs. In contrast to human influenza A viruses, the attachment of avian influenza viruses was rare in the trachea and gradually increased towards the bronchioles. The avian influenza viruses preferentially attached to different cell

types compared to the human influenza A viruses: to acinar cells in the tracheal and bronchial submucosal glands, nonciliated cuboidal cells in the bronchioles, alveolar type II pneumocytes, and alveolar macrophages. (van Riel D, et al, Human and Avian Influenza Viruses Target Different Cells in the Lower Respiratory Tract of Humans and Other Mammals. The American Journal of Pathology, Vol. 171, No. 4, October 2007) https://pmc.ncbi.nlm.nih.gov/articles/PMC1988871/

In the nasal (upper) respiratory tract epithelium cultures, both H5N1 viruses showed limited replication compared to the H3N22003 virus although the H5N12022 virus replicated to higher titers than the H5N12005 virus. In the tracheal/bronchiolar (lower) respiratory tract epithelium cultures, the H5N12022 virus replicated to higher titers than the H5N12005 virus and to similar titers as the H3N22003 virus.

24 hours after inoculation, immunohistochemical staining of nasal respiratory epithelium and tracheal/bronchiolar epithelium with anti-nucleoprotein as the primary antibody revealed that the H3N22003 virus infected many cells. Both H5 viruses infected a limited number of ciliated epithelial cells in the nasal respiratory epithelium, whereas in the tracheal/bronchiolar epithelium, the $H5N1^{2022}$ virus infected more cells than the H5N12005 virus.

Importantly, 72 hours after inoculation, the epithelial cell layer infected with H5N12022 or H3N22003 viruses displayed epithelial necrosis and degeneration, occasional giant epithelial cells, and loss of cilia.

The analysis of the cytokine response in the cultures of nasal respiratory epithelium and tracheal/bronchiolar epithelium following the infection with the H5N12022, $H5N1^{2005}$, or $H3N2^{2003}$ viruses revealed that infection with the virus H5N12022 induced a robust innate immune response and a cytokine profile that was similar to that of H3N22003 virus, but different from that of the H5N12005 virus.

The H3N22003 virus induced a robust type-I-interferon (IFN) and type-III-IFN response consisting of IFN-α2 and IFN-β, IFN-λ1 and IFN-λ2/3. The H5N12022 virus also induced type-I-IFN and type-III-IFN responses in both respiratory cultures, whereas the H5N12005 virus only induced the release of IFN-α1 in the nasal respiratory epithelium. In both respiratory cultures, inoculation of both H5 viruses resulted in the production of interferon-γ-induced protein 10 (IP-10). Both H5 viruses induced the interleukin-6 release in the tracheal/bronchiolar (lower) respiratory tract epithelium cultures, and not in the nasal

(upper) respiratory tract epithelium cultures.

Conclusion

This study demonstrated that currently circulating HPAI H5N1 virus of clade 2.3.4.4b attached better to the respiratory tract of humans than the H5N1 virus of clade 2.1.3.2.

More abundant attachment to the human respiratory tract of the $H5N1^{2022}$ virus was associated with its more effective replication. This difference likely contributed to a more robust innate immune response in respiratory epithelial cells of humans following the infection with the $H5N1^{2022}$ virus compared to that of the $H5N1^{2005}$ virus.

This study has been published on a preprint server and is currently being peer-reviewed.

Journal Reference

Bauer L, Leijten L, Iervolino M etal. A 2022 avian H5N1 influenza A virus from clade 2.3.4.4b attaches to and replicates better in human respiratory epithelium than a 2005 H5N1 virus from clade 2.3.2.1. bioRxiv preprint. https://doi.org/10.1101/2024.11.27.625596