The avian influenza A (H5N1) virus was first identified in southern China in 1996, leading to substantial outbreaks among poultry in Hong Kong in 1997 and resulting in 18 human infections. Even though the 1997 avian epidemic was controlled, the virus persisted in birds. It resurfaced in 2003, spreading extensively among birds throughout Asia and reaching Africa, Europe, and the Middle East, where it caused poultry outbreaks and sporadic human infections. Due to genomic reassortment, the currently circulating panzootic H5N1 viruses are genetically distinct from previous strains. In this study, the authors from the United States examined two dairy farms in Texas in April 2024, that were recovering from incursions of their cattle with highly pathogenic avian influenza A (HPAIV) subtype H5N1 viruses. To determine whether the farmworkers had been exposed to HPAIV subtype H5N1 viruses, neutralizing antibodies against recombinant H5N1 were measured.

Two decades after the first detection of “bird flu” in poultry in Southeast Asia, its descendants have resurged, triggering an H5N1 panzootic in wild birds, fueled by fast evolution through genomic reassortment. The disease spread rapidly, reaching South America and Antarctica for the first time. Millions of wild bird and poultry deaths across multiple continents were caused by HPAIV H5N1 viruses, which continued to diversify genetically. Other animal species including bears, bobcats, coyotes, foxes, goats, raccoons, sea lions, skunks, and cattle have also been infected with HPAIV H5N1 strains. The HPAIV subtype H5N1 clade 2.3.3.4b was particularly prevalent in these spillover events.

Since 2003, more than 22 countries have reported to WHO more than 900 sporadic human cases of influenza A (H5N1) infection. All avian influenza virus infections in humans have varied widely in severity, ranging from asymptomatic or mild to fatal diseases. Recent human infections with the H5N1 2.3.4.4b virus have significantly lower case fatality rates compared to prior H5N1 outbreaks in Asia, where half of people with reported infection died. The Center for Disease Control and Prevention (CDC) in the United States utilizes its flu surveillance systems to monitor for H5 bird flu activity in individuals exposed to infected birds, poultry, dairy cows, or other animals. The total number of confirmed human cases of H5 in the United States between February 2022 and now is 27 (13 in California, 10 in Colorado, 2 in Michigan, 1 in Missouri, and 1 in Texas).

About the Study

To isolate and characterize viruses from multiple farm specimens (cow nasal swab, milk specimens, fecal slurry, and a dead bird) the authors employed classical laboratory techniques for RNA extraction, cell culture, embryonated egg culture, and microneutralization (MN). The H5N1 HPAIV virus was detected with reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The specimens were also tested for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The researchers also analyzed the nasopharyngeal swabs and sera from the farmworkers who had recently shown symptoms. Following a pre-approved protocol, the authors were allowed to include up to 10 animal workers per farm in the study. Neutralizing antibodies against recombinant H5N1 were measured to determine whether the farmworkers had been exposed to the H5N1 HPAIV virus.

Results

HPAIV H5N1 viruses were detected in 64% (9/14) of milk specimens and 2.6% (1/39) of cattle nasal swab specimens, respectively. Surprisingly, SARS-CoV-2 was detected in a nasal swab from one sick cow.

Sanger sequencing results for the six milk samples and the dead bird showed the presence

of multiple basic amino acids at the HA cleavage site, indicating that strains were highly pathogenic avian influenza (HPAI) H5N1 viruses belonging to clade 2.3.4.4b. The isolates had multiple mutations associated with increased spillover potential. Mutation analysis revealed several mutations across the four viral genomes associated with viral virulence, host specificity shifts, drug binding sites, or drug resistance.

A total of 17 farmworkers, 10 on Farm A and 7 on Farm B, were enrolled, and 71% were men. In the last 30 days, 29% (5/17) of farmworkers reported experiencing recent respiratory illnesses and using different medications, like antibiotics, ibuprofen, multivitamins, and cough syrup. In Farm A, all allowed nasopharyngeal swabs and serum collection, whereas on Farm B, all allowed nasopharyngeal swab collections but only 4 permitted serum collection. The nasopharyngeal swab specimens from all farmworkers tested negative for influenza A viruses and SARS-CoV-2.

Microneutralization (MN) assays performed on the sera of 14 farmworkers revealed that 14.3% of them (2/14) had elevated titers of neutralizing antibodies against a recombinant H5N1 virus of clade 2.3.4.4b. The two MN-positive samples came from Farm A. The authors noted that workers on Farm A had more time (approximately 4 weeks) to develop antibodies against the H5N1 virus than those on Farm B (approximately 2 weeks), as Farm A experienced the H5N1 epizootic 14 days earlier than Farm B. The first dairy worker had a moderately elevated MN titer of 1:40. He reported no respiratory illnesses during the last 12 months but he did have a cough and took cough medication at the time of enrollment. The second worker had an MN titer of 1:80. She reported experiencing a fever, cough, or sore throat over the past 12 months and was around others at work with similar respiratory signs and symptoms. She had just recovered from a respiratory illness at the time of her enrollment.

Other authors have suggested that older people may have partial immunity to H5N1 due to their childhood exposure (“imprinting”) to seasonal H1N1 and H2N2 viruses, while younger people born after the 1968 H3N2 pandemic may be more susceptible to severe disease in a H5N1 pandemic. The researchers in the present study also said that they cannot rule out cross-reacting antibodies from previous influenza A virus infections or vaccines as a cause for the MN titer elevations. They emphasized, however, that neutralizing assays are often considered the best assay for virus-specific serological assessments.

Conclusion

This study showed that 14% of Texas farmworkers (2/14) had elevated titers of neutralizing antibodies against a recombinant H5N1 virus of clade 2.3.4.4b, indicating that farmworkers were exposed to HPAIV H5N1. There are currently no commercially available diagnostic tests that can detect H5N1 specifically. Experts are warning that human cases of the H5N1 avian flu could be undetected due to poor surveillance and a lack of diagnostic testing in at-risk groups. The authors concluded that continuous surveillance and reporting of farmworker results are critical in understanding outbreak trends. Genomic analyses of future H5N1 strains are also extremely important, not only to determine which viral strains are circulating but also to assess genetic markers associated with increased virulence and resistance to antiviral agrents.

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

Journal Reference

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