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RESEARCH LETTER

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Characterization of the first detected Avian Influenza A(H9N2) human case in Ghana

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ABSTRACT

Avian influenza A(H9N2) has been circulating in poultry across Asia, the Middle East, and Africa, posing human health risks. In Ghana, it has co-circulated among poultry with influenza A (H5N1). This report describes Ghana's first confirmed human case of avian influenza A(H9N2) virus infection in a two-year-old boy from Upper East Region, identified through active respiratory surveillance. Molecular and genomic analyses confirmed the virus was of the G1 lineage, closely related to other West African strains, with mammalian adaptive mutations known to increase human infection potential. The child experienced mild symptoms, received outpatient care, and recovered. Health authorities conducted epidemiological investigations. No source was identified for the child's infection; no additional human infections were detected. This case highlights the importance of robust avian influenza surveillance in animals and humans, particularly in regions with human-animal interactions. It underscores the importance of national and global collaboration using a One Health approach to detect and prevent zoonotic spillovers and potential pandemics.

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KEYWORDS Avian influenza; surveillance; cross over event; human; One-Health; Ghana

Introduction

Avian influenza viruses (AIVs) of the subtype A(H9N2) were first detected among poultry in Asia in 1975. They have subsequently become enzootic in poultry in large parts of Asia, the Middle East, and some parts of Africa [1, 2]. Although A(H9N2) viruses are considered low-pathogenic among domestic poultry, they can cause decrease in egg production, resulting in significant economic losses to farmers [3]. Since 1998, AIV of the A(H9N2) subtype have infected humans in zoonotic spillover events in Hong Kong, China Mainland, Egypt, Bangladesh, Pakistan, and Oman, and have been classified as viruses of pandemic concern [1, 2]. A(H9N2) human virus infections are usually mild with only one known mortality case to date, likely due to an underlying medical condition [1]. AIV of the H9N2 subtype, were first detected in Africa in Egypt and Tunisia in 2011. They were further detected in Morocco in 2016 and has since spread to other regions in Africa including West Africa. AIV A(H9N2) were first detected in West Africa in Burkina Faso in 2017, then subsequently in Senegal, Ghana, Nigeria, Benin and Togo. Viruses circulating in West Africa have primarily belonged to the G1 lineage, highly related to viruses circulating in Northern Africa and the Middle East [1, 2].

AIVs were first documented among poultry in Ghana in 2007, when infections with highly pathogenic avian influenza viruses (HPAIV) of the subtype A(H5N1) led to large die-offs and culling of poultry, greatly impacting the livelihoods of farmers [4]. HPAIV A(H5N1) of clade 2.3.2.1c harbouring mammalian adaptive mutations re-emerged among poultry in 2015 [5]. In recent years, A(H5N1) clade 2.3.4.4b has circulated globally and been detected in Ghana. A(H9N2) AIVs have been detected among poultry in

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Ghana since 2017 [3, 6]. Between November 2017 and February 2018, there were reports from farmers of a dramatic increase in poultry mortalities and a significant drop in egg production on several farms. The Veterinary Services Directorate in Ghana later confirmed the presence of A (H9N2) among poultry during this period. Whole genome sequencing confirmed these viruses were of the G1 lineage [3]. Genetic analysis showed that these viruses, although less pathogenic, possessed mammalian adaptive motifs [6]. Recent surveillance activities have observed co-circulation of A(H9N2) HPAIV viruses with A(H5N1) clade 2.3.4.4b viruses among poultry, increasing the potential for genetic reassortment that could lead to the emergence of novel strains with enhanced transmissibility and/or pathogenicity [3, 6].

In Ghana, backyard farms and open live bird markets in most major markets are common and provide an opportunity for close proximity of humans and domestic or agricultural animals, a suitable condition for possible spillover of AIVs to humans. Early identification of AIV outbreaks can help human and animal health authorities interrupt transmission to prevent further spread across the country and globally. In response to the 2007 HPAIV A(H5N1) outbreaks, Ghana established sentinel surveillance for influenza, which currently includes 36 sentinel sites in hospitals, polyclinics, and military medical reception stations in all the 16 regions of the country [7]. This respiratory pathogen surveillance system detected the first case of influenza A(H1N1)pdm09 in 2010 and the first case of SARS-CoV-2 in 2020 in Ghana [8].

We present a probable spillover event of A(H9N2) AIV from animals to a human in Ghana detected through the respiratory pathogen surveillance system and the ensuing public health response to detect and prevent additional human infections. This report describes the first confirmed human case of AIV A(H9N2) in Ghana which was detected in a young child from the Upper East Region, which shares borders with Burkina Faso.

Case report

On 7 May 2024, a two-year-old boy presented to an outpatient sentinel surveillance site in the Upper East Region reporting fever, sore throat, and cough with symptom onset occurring on 5 May 2024. His body temperature at the time of consultation was 36.6°C. A diagnosis of mild influenza-like illness was made, and the patient was treated with antipyretics, antihistamines, and antibiotics. Respiratory specimens (combined oropharyngeal and nasopharyngeal swabs) were collected as part of routine surveillance during the visit. The patient returned to the same hospital on July 17 as his condition showed no visible improvement with persistence of symptoms. He was re-treated as an outpatient case with antipyretics, antihistamines, and antibiotics after which the symptoms subsided and he fully recovered without influenza antiviral treatment.

Laboratory investigation and confirmation

The respiratory specimens collected from the patient on May 7 were tested for influenza and SARS-CoV-2 by real-time reverse transcription polymerase chain reaction (RT–PCR) on May 15th at the Noguchi Memorial Institute for Medical Research (NMIMR) in Accra for laboratory testing. Briefly, RNA was isolated from combined oro/naso pharyngeal swab transported cold to the laboratory using the QIAamp Viral RNA extraction kit (Qiagen, Germany) following the manufacturer's protocol. RNA was subjected to RT–PCR using primers/probes form the US CDC initially targeting influenza A Matrix gene using the AgPath-ID One Step RT–PCR (Thermo Fischer Scientific) kit. The specimen tested positive for influenza A. The influenza A positive specimen was further subtyped using specific primers for influenza A H3N2 and pdmInfA, which confirmed it as H3N2. On July 9th, routine genomic sequencing was performed on the specimen using a nanopore-based method [9] at the NMIMR. The QIAamp Viral RNA Mini kit (Qiagen, Germany) was used with a modified manufacturer's protocol, utilizing 280 µL of starting material to isolate 35 µL RNA. All gene fragments of the viruses detected were amplified using a universal primer in combination with gene-specific primers. PCR amplification was performed using the SuperScript™ III One-Step RT–PCR System with Platinum™ Taq DNA Polymerase in a 25 µL reaction volume. Thermal cycling conditions were as follows: reverse transcription at 42 °C for 50 min, followed by incubation at 50 °C for 10 min, and initial denaturation at 94 °C for 2 mins. This was followed by 4 cycles of denaturation at 94 °C for 30 s, annealing at 43 °C for 30 s, and extension at 68 °C for 3 min 50 s. Subsequently, 30 cycles were run with denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, and extension at 68 °C for 3 min 30 s. A final extension was performed at 68 °C for 10 min. PCR amplicons were confirmed on 1% agarose gel. Amplicons were purified with Agencourt AMPure XP (Beckman Coulter Inc., TX USA) beads. Full-genome sequences on a GridION platform (Oxford Nanopore Technologies, ONT, Oxford, UK) were generated and results analysed using the MIRA Assembly and Curation platform [10]. The results indicated that the virus was AIV A(H9N2) rather than A(H3N2).

For confirmation, the specimen was shared with The Francis Crick Institute in the U.K. and the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta for further testing. CDC confirmed the specimen as AIV A(H9N2) by RT–PCR after testing for a panel of influenza A subtypes including: H7, H5, H3 and

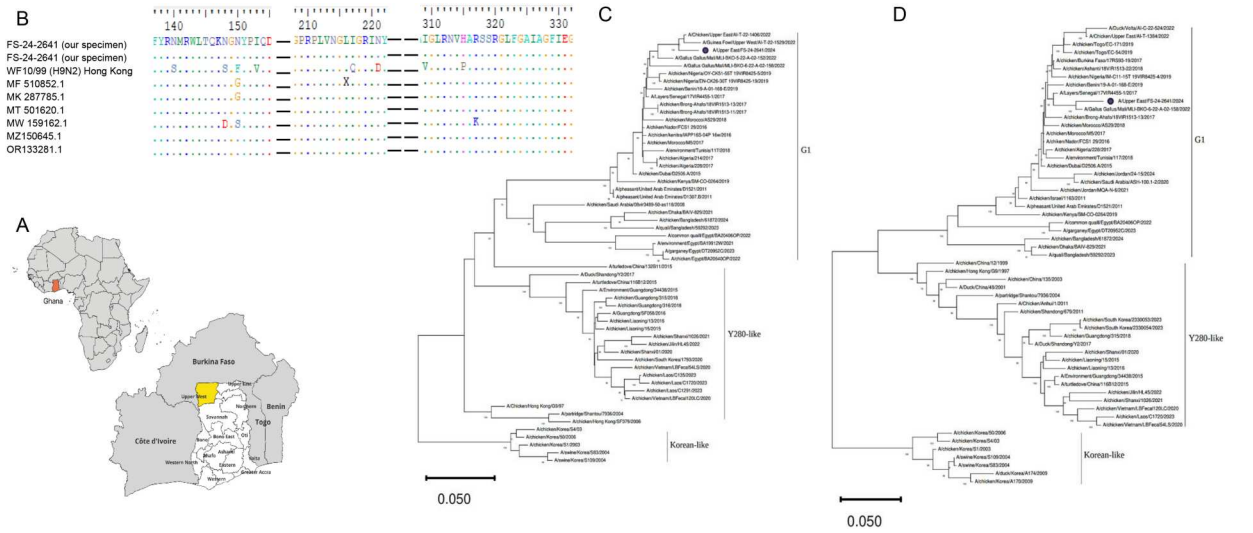


Figure 1. Characterization of the first human case of avian influenza virus infection in human detected in Ghana. A. Map of Africa showing Ghana, in West Africa. Yellow highlight shows the region where the case was detected. Maps were created using QGIS version 3.26.1-Buenos Aires with the Ghana boundary coordinates obtained from Ghana Statistical Service (GSS). B. Genetic analyses of HA showing RSSR/GLF at cleavage site, an indication of low pathogenicity and 145 T and 216L mammalian adaptive motifs (mature HA numbering) C. Phylogenetic analysis of the Neuraminidase gene from AIV A(H9N2) isolated from a child in Ghana D. Phylogenetic analysis of the Haemagglutinin gene from AIV A(H9N2) isolated from a child in Ghana. Sequence from this case is shown with a purple dot. Sequences belong to the G1 A(H9N2) lineage and closely clusters with other A(H9N2) sequences circulating among poultry in West Africa and North Africa. Evolutionary analyses were done using MEG11 software (http://www.megasoftware.net). The scale bar signifies the number of nucleotide substitutions per site 12. https://www.who.int/publications/i/item/9789241580496.

pdmInfA and sequencing using a Nanopore-based method [9]. Subsequently, amplification was attempted with influenza A specific H3 primers in Ghana, but this did not yield any results, an indication that the A(H3N2) was not present in the specimen.

The AIV A(H9N2) sequences from Ghana and CDC perfectly matched. Both laboratories were able to sequence the haemagglutinin (HA), matrix (MP), neuraminidase (NA), nucleoprotein (NP), and non-structural protein (NS) segments. Sequencing attempts for Basic polymerase 1 and 2 (PB1,PB2) as well as acidic polymerase (PA) gene segments, important determinants of mammalian adaptation were not successful. Basic Local Alignment Search Tool (BLAST) confirmed that the successfully sequenced segments belong to the A(H9N2) AIV subtype with the HA showing 97.22% similarity to Influenza A virus (A/Gallus Gallus/Mali/MLI-BKO-6-22-A-02-158/2022 (H9N2)) segment 4 hemagglutinin (HA) gene from Mali. The HA cleavage site displayed a 317-RSSR/GLF-323 (mature H9 numbering) motif, characteristic of low pathogenicity. Amino acid changes 145 T and 216L (mature H9 numbering) in HA, known to switch receptor binding preference to human-like receptors were also observed [11]. The NA segment did not express the 274Y mutation (mature H9 numbering) associated with resistance to Oseltamivir. A 116S and V154I detected in MP, have been linked to enhanced human to human transmission. No mammalian adaptive motifs were detected for NP gene and a BLAST revealed 100% identity of NP from this study to (NP of A/Gallus Gallus/Mali/MLI-BKO-6-22-A-02-158/2022 (H9N2)) from Mali. Sequences were deposited in GISAID (accession number EPI_ISL_19266347). A maximum likelihood phylogenetic analysis of the complete HA and NA gene segments showed that the strain belonged to the G1 lineage and clustered closely with AIV A(H9N2) viruses circulating among poultry in West and North Africa (Figure 1).

Specimens collected on July 17 from the patient tested negative for influenza A virus, influenza B virus, and SARS-CoV-2 by RT–PCR at NMIMR.

Public health actions

Following the confirmation of AIV A/H9, the Ghana Health Service (GHS) reported the event to the World Health Organization (WHO) as well as other relevant partners, following International Health Regulations [12]. In addition, GHS initiated several public health actions, including contact tracing, enhanced surveillance and influenza case finding, strengthening infection prevention and control protocols, and providing public health guidance to healthcare providers and the public. Subsequent epidemiologic investigation found that the patient had no known history of exposure to poultry, contact with a sick person with similar symptoms, or history of travel in the past 6 months. There were reports of poultry die-offs in the patient's community around the time of infection; however, our investigation was

unable to verify these reports. Respiratory specimens from close contacts tested negative for influenza A virus, influenza B virus, and SARS-CoV-2. No additional cases of human infection with AIV A(H9N2) viruses were identified in the community.

Discussion

AIV A(H9N2) has been detected in poultry populations in West Africa, including Ghana, where it continues to be enzootic in the poultry population [13]. We present the first confirmed human case of infection with a zoonotic influenza virus, identified as influenza A(H9N2) in Ghana [14].

The patient in this case report was two years old at the time of infection, which is consistent with most human AIV A(H9) infections. A 2019 report demonstrated that globally, the majority of A(H9N2) infections have been detected among children 8 years old or younger [66% (39/59)], with a median age of 4 years [1]. Understanding risk factors that might predispose children to infection with influenza A(H9N2) viruses following contact with infected poultry or their environment is crucial for informing public health efforts to contain the spread of AIVs to humans. It is unclear if young children are at a greater risk for infection with influenza A(H9N2) virus and what might lead to a greater frequency of detection, such as types or frequency of exposure to infected poultry, environmental exposures, or other factors.

In 2020, a laboratory-confirmed human case of AIV A(H9N2) was reported in Senegal. This case was particularly significant as it was the first known reported human A(H9N2) virus infection in West Africa [15]. At the time, Senegal had not experienced documented AIV A/H9 infections among poultry, even though unusually high poultry mortalities associated with unknown disease were occasionally reported [15]. Similarly, the patient of the current report did not have any known contact with infected poultry or other animals, although our investigation revealed there were unconfirmed reports of guinea fowl die-offs in his rural vicinity around the time of the infection. Guinea fowls are a local delicacy for the area. Previous cross over events have been linked with preparation of birds. For most low – and middle-income countries, birds are culled when illness/sickness is noticed. However, this case reports no known contact with birds. Further public health investigations did not find any symptomatic close contacts of the case, which suggests that this might have been an isolated event.

Although this infection was confirmed as the A(H9N2) subtype and having mammalian adaptive motifs in HA, the existing epidemiological and virological evidence suggests that the virus detected from this patient had not acquired the capacity for efficient human-to-human transmission [8]. However, given that domestic poultry is present throughout living areas and communal areas, rather than solely confined to farms in Ghana and many other African countries, surveillance for spillover events is critical for early detection to prevent epidemics among people.

Epidemiological investigations for this case took place two months after the patient first presented for outpatient care because of the initial seasonal influenza A(H3N2) result. The late diagnosis made it difficult to trace the true source of the infection and therefore does not rule out the possibility that close contacts could have had symptomatic infection before or around the same time. Also, we cannot rule out asymptomatic infections among close contacts because serology testing was not conducted due to logistical challenges. Further, our sequencing attempts failed for the polymerase genes. Nevertheless, sequencing was successful for 5 gene segments including HA and NA which are important for mammalian adaptation.

This case highlights the importance of surveillance for influenza among animal populations and early detection to mitigate the risks of widespread transmission. It further highlights the importance of strengthening collaborations at the national, regional, and global levels to closely monitor the avian influenza situation using a One Health approach and improve preparedness efforts.

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Author contributions

Conceptualization: IAA, WKA; Laboratory analyses: LB, LK, JAN, GMS, VM, JW, SA, EAAA, JW, RT, ANSY, CT; Data analyses and curation: OBO, JAQ, SON, ID; Initial Draft: NAAN, AAA, MAP; Revisions: PD, NTN, DO, SNP, FAB, DOL, MC; Final reviews: All.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Ethical considerations

Surveillance for respiratory pathogens falls under public health surveillance and therefore, ethical review is not required. The US Naval Medical Research Command Institutional Review Board – Office of Research Administration (NAMRU3-PJT-21-01) also determined this activity as public health surveillance. The sites for routine national influenza virus sentinel surveillance in Ghana were set up in collaboration with the Ghana Health Service and the Ghana Armed Forces as part of the Integrated Disease Surveillance and Response (IDSR) system. All procedures were performed according to relevant guidelines and regulation. No administrative permissions were required to access these data. Data were anonymized before use, and only laboratory identities were used during analysis. "Surveillance for domestic and wild bird populations in Ghana" is approved by the Scientific and Technical Committee of the Noguchi Memorial Institute for Medical Research, University of Ghana (STC paper 4(3) 2023–2024).

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