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Community Based Surveillance of Influenza A Virus among Life Bird Handlers from Selected Bird Markets and Poultry Farms in Ibadan, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Study designed was done by authors JOO, ASB and GNO. Sample collection, tissue culture and egg inoculation were carried out by author JOO. Authors GNO and JOO supplied the laboratory materials. Authors JOO and ASB carried out molecular analysis of the isolates. Author JOO wrote the manuscript and the authors read and approved it.

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ABSTRACT

Background: Influenza is an acute respiratory disease that has caused pandemic in birds and humans. Therefore, this study was designed to isolate and identify influenza A virus strains from live bird handlers in life bird markets (LBM) and poultry farms in Ibadan metropolis.
Methods: A total of 43 oropharyneal swabs were collected over a period of four months and tested for influenza A virus. Isolation was done by virus culture in MDCK cells and ten to twelve day old embryonated chicken eggs. Detection of RNA of the virus was carried out using real time PCR. Statistical tools employed were percentages (Multiple Bar Chart) chi square (P=.05 and 1 degree of freedom).

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Results: Out of 43 samples collected and tested, 5 (11.6%) were positive for influenza virus in MDCK, 2 (4.7%) in embryonated egg while 16 (37.2%) were positive for influenza A virus by real time PCR. Only 1 (2.3%) was confirmed by the three methods used for detection of influenza A virus in this study.
Conclusion: The occurrence of influenza A virus particles in the samples obtained from live bird handlers confirmed by the methods employed in this study revealed the possibility of cross infection by the virus.

Keywords: Influenza A virus; surveillance; live bird handlers; MDCK; real time PCR.

1. INTRODUCTION

The influenza disease is caused by influenza viruses (family: Orthomyxoviridae). Influenza is an acute respiratory disease that has caused global epidermics and pandemics [1]. It is associated with fever, headache, cough, nasal congestion, sneezing and whole-body aches. Influenza epidermic continues to infect large numbers of people worldwide, despite the availability of inactivated vaccines derived from current circulating strains, because of frequent natural variation of the hamagglutinin (HA) and neuraminidase (NA) envelope proteins of the virus [2]. This variation allows the virus to escape neutralization by preexisting circulating antibodies in the blood stream, present as a result of either previous natural infection or immunization [3].

Avian influenza (AI) is a highly infectious disease primarily of birds and caused by influenza A virus. It constitutes one of the greatest concerns for public health because of the emergence from the animal reservoir [4,5]. The spread of the highly pathogenic avian influenza (HPAI) to countries where hygienic standards are lacking increases potential for the pandemic of the virus and thereby raises concerns about food security particularly in rural villages [5] and safety of poultry workers. Aquatic birds are considered to be the sources of AI viruses [6,7].

In Nigeria, the highly pathogenic avian influenza (HPAI) viruses of the H5N1 subtype was first detected in February 2006, in chickens at a commercial poultry farm in Kaduna state, Northern Nigeria [8]. This was the first Africa's confirmed HPAI (H5N1) outbreak. The infection spread and persisted for 21 months, and a fatal human case was reported [9]. Also, molecular detection of AI viruses of the H5N2 subtypes had been reported from free-living and apparently healthy White-faced Whistling-duck (*Dendrocygna viduata*) and Spur-winged Goose (*Plectropterus gambensis*) [10] in Nigeria. Migrating birds had been suggested to be playing an important role in the introduction of HPAI (H5N1) viruses into Nigeria [11]. Also, introduction and spread of the virus through illegal trade in poultry and poultry products can not be ruled out [11]. Karesh et al. [12] mentioned that transaction of wildlife commonly provides means of disease-transmission that can not only cause human disease outbreaks but also threaten livestock, international trade, rural livelihoods, native wildlife populations, and even entire ecosystems. In July 2008, new HPAI-virus isolates obtained from Kano and Katsina states belonged to clade 2.2, which had been previously isolated in Nigeria, however isolates obtained from Gombe and Kebbi states were of a new sublineage of clade 2.2.1, which was new to the African continent [11,13].

Surveillance of avian influenza viruses in birds requires special consideration by targeting both sick and recently dead birds. Also, active surveillance for avian influenza viruses is essential in providing important information on LPAI viruses' circulation, including H5 and H7 subtypes that have the potential to become highly pathogenic in poultry [8]. Therefore, this study was designed to investigate the occurrence and prevalence of influenza A virus in the orophargneal swabs of the Live Bird Handlers (LBM) in Ibadan.

2. MATERIALS AND METHODS

2.1 Study Area

Samples were collected from three large bird markets and three Poultry Farms from two out of eleven Local Governments areas within Ibadan metropolis. Ibadan lies at latitude 7°23' N and Longitude 3°56'E and is a transition zone between the forest and grassland areas of Nigeria. All Laboratory investigations were carried out in the Department of Virology, College of Medicine, University College Hospital, Ibadan.

2.2 Study Population

Life bird handlers that are directly selling or working in poultry farms with clinical presentation, signs and symptoms of Upper Respiratory Tract (URT) infection irrespective of their age and sex were eligible for the sampling.

2.3 Sample Collection

Consent was obtained from bird handlers prior to sample collection. Throat swabs were used to rub the posterior of the tonsils, the soft palate and back wall of the lower pharynx. The swab was then carefully removed; the cotton tip of swab was broken off into a labeled screw-cap vial containing Virus Transport Medium (VTM). Specimens were quickly transferred in a box containing ice packs to maintain cold-chain during the course of transportation to the laboratory where they were inoculated immediately or stored under mechanical refrigeration at -86℃ pending further inoculation.

2.4 Sample Processing

Prior to inoculation, the throat specimens in transport media were treated with 0.1 ml of antibiotics made up of penicillin and streptomycin in a laminar flow hood. This procedure ensures the elimination of non-viral agents like bacteria in the sample. This was left in a temperature of 4℃ for 1 hour. The mixture was then clarified by centrifugation in the cold at approximately 1,500 rpm to ensure separation of debris from the fluid. The treated samples were then used for inoculation.

2.5 Sample Inoculation into Cell Cultures

Samples were inoculated into cell line (MDCK) in duplicates and control was also set up. These procedures were carried out under biosafety cabinet and daily observation for Cytopathic Effect was carried out for 7 – 8 days and observation recorded accordingly. Flasks with no visible CPE at the end of 7^{th} day were also retrieved for blind passage samples and inability to show CPE after second passage was considered to be negative.

2.6 Inoculation of Embryonated Eggs

Samples were inoculated into ten to eleven days old embryonated eggs in duplicates and control was also set up. Incubation was done at 37℃ for 3 days. Eggs were chilled at 4℃ for 4 – 24 hrs to minimize bleeding during harvesting. The allantoic fluid was then harvested with the aid of Pasteur pipette.

2.7 Real Time (RT) PCR

Ribonucleic Acid (RNA) was extracted according to manufacturer's instruction for the amplification. The reagents for PCR mix were RNA free water, PCR buffer, forward primer, reverse primer, probe and enzyme mix. The reaction mix was prepared according to the manufacturer's protocol. Twenty microtitre of the PCR mix was transfer into the labeled wells of the 96 well microtitre plate designed for the real time cycler after which 5 µl of the extracted RNA from the throat swab solution was dispensed into the corresponding microwells. The sealed microtitre plate was later placed on the carriage of the real time cycler already switched on and programmed for the amplification. The results were later harvested from the graphical display on the monitor.

2.8 Statistical Analysis

The results of this study were statistically analyzed using percentages and Chi Square test at 5% confidence interval and 1 degree of freedom. The data were presented using tables and multiple bar chart with the aid of Microsoft excel 2007 version.

3. RESULTS

Fever, malaise, sore throat, headache, cough and fatigue were common clinical manifestations observed in the bird handlers enrolled for the study. It was observed that majority of the people presented muscles ache (85%) while only 6 (28.6%) of the subjects had chill. Also, manifestations of neurological disorder were not observed.

Out of 43 samples inoculated into MDCK cell line only 3 (6.9%) samples showed distinct cytopathic effect (CPE), while 2 (4.7%) samples showed CPE but not distinct with a total of 5 (11.6%) samples positive in MDCK cell line. Cytopathic effect observed in cell culture include: cell granulation, swelling, fragmentation of the cell and dislodgement of the cell monolayer. Embryonated egg was able to detect 2 (4.7%) from the total sample collected. Molecular detection by real time PCR confirmed 16 (37.2%) out of the 43 oropharyneal swabs collected.

However, only 1 (2.3%) of the oropharyneal swabs was confirmed by the three methods of detection examined in this study. The statistical analysis (χ^2 = 0.17, P=.05) revealed that the relationship between the sex difference and incidence of influenza A virus is not significant. However, the spread and occurrence of this infection is significantly dependent on other factors like season variation but not on the sex difference.

Table 1. Frequency of occurrence of observed clinical manifestation in patients whose samples were analyzed

S/No	Clinical signs	Frequency (%)
1	Fever	35 (81.4%)
2	Cough	22 (51.2%)
3	Chills	21 (48.8%)
4	Sore throats	13 (30.20%)
5	Muscles ache	32 (74.4%)
6	Malaise	30 (69.8%)
7	Vomiting	19 (44.2%)
8	Abdominal pains	27 (62.8%)
9	Nasal stiffness	30 (69.8%)
10	Running Nose	22 (51.2%)
11	Sputum production	18 (41. 9%)
12	Headache	33 (76.7%)
13	Fatigue	35 (81.4%)
14	Diarrhea	8 (18.6%)
15	Neurological manifestation	

Table 2. Gender distribution of influenza virus infection by sex based on MDCK detection

Sex	No tested	No (%) of positive	No (%) of Negative
Male	6	1 (16.7%)	5 (83.3%)
Female	37	4 (10.8%)	33 (89.2%)
Total	43	5 (11.6%)	38 (88.4%)

4. DISCUSSION

Influenza virus is notoriously known for its unique ability to cause recurrent epidemics and pandemics during which acute febrile respiratory illness usually occurs explosively in all age-groups. In this study, it was discovered that many people under this investigation complain of frequent fever with headache and this was consistent. This is similar to the report by Bresson et al. [14] that fever is common to influenza infection.

It was observed in this study that the embryo death in the embryonated egg was rapid and this responsible for the inability to raise the virus titre during the course of this study which confirms the report of Puzelli et al. [15] that HPAI viruses rapidly kill the embryonated egg production before good viral antigen titre. Also, Lu et al. [16] reported that natural infection with avian influenza induces poor HA inhibition titre but better neuralisation titre.

In the study, two isolates obtained from two samples were investigated by real time PCR technique for the possibility of being influenza A virus but the viral genome is yet to be sequenced for detailed confirmation. However, incidence of two cases of influenza viruses is epidemiological important and this correlates with an outbreak of HPAI H5N1 virus in poultry farms in British Colombia, Canada which led to self-limited conjunctivitis [17]. It is also reported that detection of a human case in a region is the first indication of the presence of poultry infection in that locality [18]. Orophargneal swabs tested positive for influenza virus were those samples collected between May and July as reported by WHO [18] that human case of influenza A virus appear to increase during winter and spring month. In this study also, there was consistent rain and apparently excessive cold during the period of sample collection.

One of the samples confirmed by three methods (MDCK, embryonated egg and real time PCR) to be influenza A virus in this study was obtained from bird sellers who exhibited clinical manifestations like headache, sore throat, runny nose, malaise, muscle ache and conjunctivitis. There was no account of the use of PPE by handlers from Life Bird Markets enrolled in this study. Studies have shown that exposure of Live Bird Handlers to birds without use of PPE predisposes the bird handlers to influenza A virus infection [19]. None of the samples collected from poultry workers was positive for influenza A virus. However, practice of using PPE by poultry workers was nearly adequate but not holistic as observed in all the farms employed in this study.

In other to curb the emergence of a new strain which might result into a new strain of influenza of which people might not have immunity therefore there is needed to emphasize the campaign on the use of Personal Protective Equipment (PPE) and continuous surveillance of influenza virus infection by health authority and veterinary practice. The continuous surveillance should be regular and active through isolation, characterization and possible molecular sequencing, so that the occurrence of a mutant influenza virus can be detected. This will make vaccine formulation and modification effective.

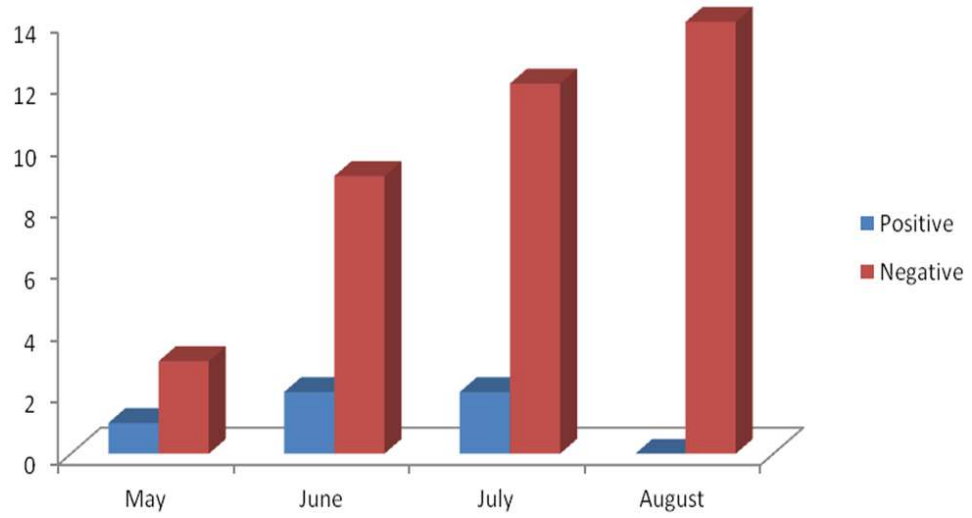

Fig. 1. Percentage distribution of influenza virus infection based on MDCK detection by Month

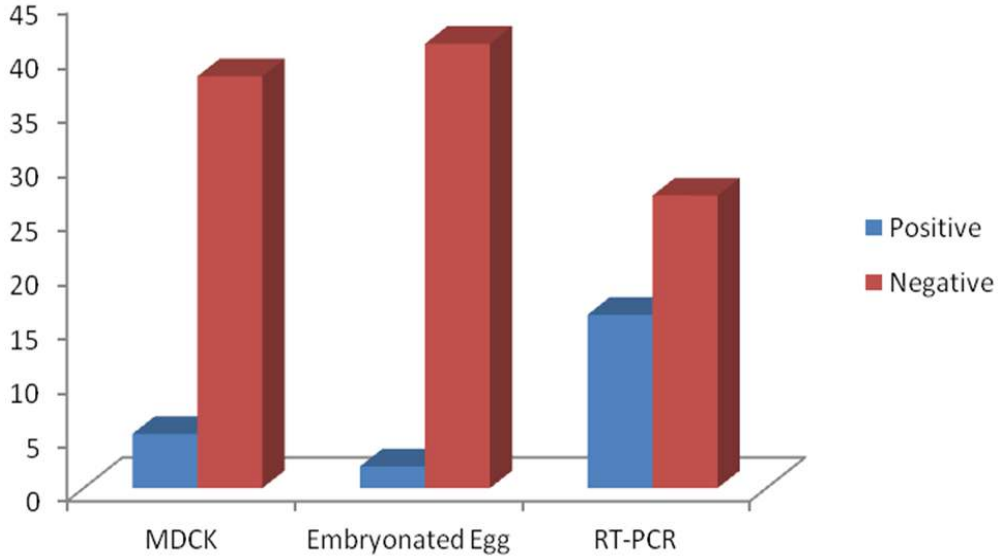

Fig. 2. Percentage detecting capacity of influenza virus by three different methods

5. CONCLUSION

The study focused on live bird handlers because of epidemiological roles played by birds in the transmission of avian influenza viruses. However, the use of protective materials were not practiced by the bird handlers during the process of keeping and handling of birds in the bird markets and poultry farms therefore this could account for the detection of influenza A virus among live bird handlers in this study. There is need for awareness on the significance of the use of personal protective equipment among bird handlers so as to forestall future outbreaks of avian influenza viruses in the human population.

ETHICAL ISSUES

The concept of the research was properly explained to all the subjects (Life Bird Handlers) and they were given the liberty to give their consent by interviewing them. The risk of taking oropharyneal swab (sample) was explained to them like irritation and they were given the freedom to disengage from the research anytime they felt uncomfortable with the process. Also, the benefit of the research was duly explained to

them like advocating for the use of protective equipment. None of the subjects was placed under coercion. Samples were coded so as to ensure protection of privacy.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Taubenberger JK. The origin and virulence of the 1918 “Spanish” influenza virus. Proc. Am. Philos. Soc. 2006;150:86–112.
2. Fouchier RA, Munster V, Wallensten A, Bestebroer TM, Herfst S, Smith D, Rimmelzwaan GF, Olsen B, Osterhaus AD. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. J. Virol. 2005;79:2814-2822.
3. Russell RJ, Haire LF, Stevens DJ, Collins PJ, Lin YP, Blackburn GM, Hay AJ, Gamblin SJ, Skehel JJ. The structure of H5N1 avian influenza neuraminidase suggests new opportunities for drug design. Nature. 2006;443:45-49.
4. Brown JD, Stallknecht D, EBeck JR, Suarez DL and Swayne DE. Susceptibility of North American ducks and gulls to H5N1 highly pathogenic avian influenza viruses, Emerg. Infect. Dis. 2006;12(11): 1663–1670.
5. Capua I, Marangon S. Control and prevention of avian influenza in an evolving scenario. Vaccine. 2007;25(30): 5645–5652.
6. Krauss S, Webster RG. Avian influenza virus surveillance and wild birds: past and present. Avian Dis. 2010;54(1):394–398.
7. Pantin-Jackwood MJ, Swayne DE. Pathogenesis and pathobiology of avian influenza virus infection in birds. Revue Scientifique et Technique. 2009;28(1): 113-136.
8. Vakuru CT, Shiiwua AM, Gashash IA, Kabir J, Scott N, Joseph N, Vivian NI, Gideon MM, Joannis T, Junaidu AM, Paul TA. Situation-based survey of avian influenza viruses in possible “Bridge” Species of Wild and Domestic Birds in Nigeria; 2012. Article ID 567601, 8 pages. DOI: 10.1155/2012/567601.
9. Fusaro A, Joannis T, Monne I. Introduction into Nigeria of a distinct genotype of avian influenza virus (H5N1). Emerging Infectious Diseases. 2009;15(3):445–447.
10. Gaidet N, Cattoli G, Hammoumi S. Evidence of infection by H5N2 highly pathogenic avian influenza viruses in healthy wild waterfowl. PLoS Pathogens. 2008;4:8. Article ID e1000127.
11. Fusaro A, Nelson MI, Joannis T. Evolutionary dynamics of multiple sublineages of H5N1 influenza viruses in Nigeria from 2006 to 2008. Journal of Virology. 2010;84(7):3239–3247.
12. Karesh WB, Cook RA, Gilbert M, Newcomb J. Implications of wildlife trade on the movement of avian influenza and other infectious diseases. Journal of Wildlife Disease. 2007;43:S55-59.
13. Joannis TM, Meseko CA, Oladokun AT. Serologic and virologic surveillance of avian influenza in Nigeria, 2006-7. Euro Surveillance. 2008;13:article 42.
14. Bresson JL, Perronne C, Launay O, Gerdil C, Saville M, Wood J, Hoschler K, Zambon MC. Safety and immunogenicity of an inactivated split-virion influenza A/Vietnam/ 1194/2004 (H5N1) vaccine: phase I randomised trial. Lancet. 2006;367: 1657-1664.
15. Puzelli S, Di Trani L, Fabiani C, Campitelli L, De Marco MA, Capua I, Aguilera JF, Zambon M, Donatelli I. Serological analysis of serum samples from humans exposed to avian H7 influenza viruses in Italy between 1999 and 2003. J. Infect. Dis. 2005;192:1318-1322.
16. Lu X, Edwards LE, Desheva JA, Nguyen DC, Rekstin A, Stephenson I, Szretter K, Cox NJ, Rudenko LG, Klimov A, Katz JM. Cross-protective immunity in mice induced by live-attenuated or inactivated vaccines against highly pathogenic influenza A (H5N1) viruses. Vaccine. 2006;24: 6588-6593.
17. Sambhara S, Kurichh A, Miranda R, Tumpey T, Rowe T, Renshaw M, Arpino R, Tamane A, Kandil A, James O, Underdown B, Klein M, Katz J, Burt D. Heterosubtypic immunity against human influenza A viruses, including recently emerged avian H5 and H9 viruses, induced by FLU-ISCOM vaccine in mice requires both cytotoxic T-lymphocyte and macrophage function. Cell. Immunol. 2001;211:143–153.
18. World Health Organization. Update: WHO-confirmed human cases of avian influenza

A (H5N1) infection, 25 November 2003-24 November 2006. Wkly. Epidemiol. Rec. 2007;82:41-47.

19. Avian Influenza *Fowl Plague, Grippe Aviaire* Last Updated: September 2014. An enhanced version of this factsheet, with citations is Available:http://www.cfsph.iastate.edu/Factsheets/pdfs/highly_pathogenic_avian_influenza-citations

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