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Avian influenza virus infection in apparently healthy domestic birds in Sokoto, Nigeria

Innocent Okwundu Nwankwo[1], Olufemi Oladayo Faleke[1] & John Garba[2]

Summary

The study was conducted among apparently healthy birds brought from different local government areas, neighbouring states and across international boundaries to the Sokoto central live bird market between October 2008 and March 2009. Tracheal and cloacal swabs were collected from 221 apparently healthy birds comprising 182 chickens, 3 turkeys, 11 guineafowl, 17 ducks and 8 pigeons. These samples were analysed using nested polymerase chain reaction (nPCR) to check for the presence of avian influenza virus. An overall prevalence of 1.4% (3 positive cases) was detected with two cases observed in chickens and one in a pigeon. The findings indicate the circulation of avian influenza in the study area. This raises concern for human and animal health due to zoonotic and economic implications of this virus.

Keywords

Apparently healthy birds, Avian influenza, Bird, Nested polymerase chain reaction, Nigeria, nPCR, Sokoto, Virus.

Influenza aviaria in volatili domestici apparentemente sani in Sokoto, Nigeria

Riassunto

Lo studio è stato condotto su uccelli apparentemente sani provenienti da diverse aree del governo locale, da Paesi vicini e attraverso i confini internazionali e destinati al mercato centrale degli uccelli vivi di Sokoto nel periodo compreso tra ottobre 2008 e marzo 2009. Sono stati prelevati tamponi tracheali e cloacali da 221 uccelli apparentemente sani: 182 polli, 3 tacchini, 11 faraone, 17 anatre e 8 piccioni. I campioni sono stati analizzati utilizzando nested polymerase chain reaction *(nPCR) per verificare la presenza del virus dell'influenza aviaria. E' stata rilevata una prevalenza complessiva dell' 1,4% (3 casi positivi) con due casi nei polli e uno in un piccione. I risultati indicano la circolazione dell'influenza aviaria nell'area di studio che solleva problematiche per la salute umana e degli animali a causa delle implicazioni zoonosiche ed economiche del virus.*

Parole chiave

Influenza aviaria, *Nested polymerase chain reaction*, Nigeria, nPCR, Sokoto, Uccelli apparentemente sani, Uccello, Virus.

Introduction

Avian influenza is an illness caused by strains of influenza viruses that have adapted to birds which serve as their host. It is highly contagious among birds and can mutate into a

(1) Department of Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, P.M.B. 2254, Sokoto, Nigeria
kinginnoma@yahoo.com
(2) Department of Veterinary Services, Ministry of Animal and Fisheries Development, No. 2, Maiduguri Road, IBB Secretariat Complex, P.M.B. 1155, Damaturu, 620001 Yobe, Nigeria

form that can spread and kill over 90% of entire flock at a given time. Infected birds shed the virus in saliva, nasal secretions and faeces (4). The spread of influenza virus appears to be principally related to the movement of poultry and poultry products (6, 7). Infection with avian influenza in domesticated poultry causes two main forms of the disease that are distinguished by low and high extremes of virulence (3). The low pathogenic form may pass unnoticed and usually causes only mild symptoms, such as ruffled feathers and reduced egg production. However, the highly pathogenic form may cause disease that affects multiple internal organs.

Avian influenza was first reported in Nigeria in February 2006 (12) and, since then, there have been reports from a number of commercial poultry houses (9, 11). One case of avian influenza was reported in a woman from Lagos, a southern state of Nigeria, in February 2007. Thus, the extensive circulation of influenza virus in Nigeria raises concern for both human and animal health in the country (10).

Materials and methods

Our study was conducted between October 2008 and March 2009 at the main live bird market in Sokoto which is located in north-western Nigeria and lies between the latitude 12°N to 58°N and longitude 4.8°E to 6.54°E.

A census of birds brought for sale at the live bird market was conducted through direct counting of birds in each cage at the point of sample collection. The representative sample size was obtained using an estimated prevalence rate of 20% and the formula: $n = Z^2PQ/L^2$ (8), while systematic sampling techniques were used to select the sampled birds (14).

Both cloacal and tracheal swabs were collected aseptically from one in every five birds using a sterile cotton swab for each bird. The buds were cut into 1.5 ml labelled Eppendorf tubes containing Hank's viral transport medium. The tubes were placed on ice packs and transported within 48 h to the Avian Diseases Biotechnology Research Laboratory at the Faculty of Veterinary Medicine, University of Ibadan, Nigeria, for the viral genome extraction and polymerase chain reaction (PCR) analysis. Some samples were also selected for analysis at the Institute of Immunology, National Public Health Laboratory in Luxembourg.

The samples were prepared for viral genome extraction using the Qiagen viral RNA mini kit (Qiagen, Hilden).

The extracted RNA was subjected to reverse transcription using Chen F(M52) 5′CTTCTAACC GAGGTCGAAA CG3′ Chen R(M253) 5′AGGGCATTTT GGACAAATCG TCTA 3′ primers (5), and superscript III reverse transcriptase (Life Technologies, Carlsbad, California). The product from the reaction was complementary DNA (cDNA). cDNA was then converted to PCR products using avian influenza-specific oligonucleotide primers (forward and reverse) after the preparation of PCR mixes (5).

Gel electrophoresis of 5 µl of each amplified PCR product was performed using 1.5% agarose gel in 1× TAE buffer stained with 2 µl of ethidium bromide. A 1 kb marker Invitrogen® (Life Technologies, California) was used as a loading buffer to determine the DNA segment size. Electrophoresis was conducted for 30 min at a constant voltage of 90 volts. Finally, the gel was visualised under ultraviolet (UV) light and photographed to capture the marker and PCR product bands using a Kodak camera connected to the computer. The size of the amplified fragment was compared to the positive control or the expected known band size of the organism (17).

Results

Single discrete and specific bands of expected size (250 bp) were positive. Of 182 samples from chicken, 2 (1.1%) were positive. Other species presented no positive cases except for the representative samples sent to Institute of Immunology, National Public Health Laboratory in Luxembourg where 1 (1.2%) of 19 samples from pigeons was positive. None of

the two positive cases at Ibadan gave a positive result in the Luxembourg laboratory.

Discussion

Birds at the main live-birds markets in Sokoto originate from diverse areas and are transported together in cages and occasionally on the floor of vehicle, in close proximity to humans as co-passengers. Cheap, fast and extensive transportation with ease of movement across borders have been identified as major factors in the risk of transmission of zoonotic diseases (1, 2, 9, 15).

It has been observed that birds that are raised in backyard flocks without an adequate history or veterinary care are being exchanged between farmers and marketers during sales at live bird markets. Some of the birds purchased at the market are introduced to homesteads for keep, without any verification of their health status. This practice can lead to the transfer and establishment of diseases among the resident bird flocks to which the new birds are introduced. It is worth noting that birds from live bird markets in Nigeria are known to harbour one disease or another (13).

Avian influenza has been reported in northern Nigeria and the detection of the virus in apparently healthy birds in this study attest to its continuous presence in the study area (10). The persistence of the virus in an area may be aided by reservoir hosts that show no clinical signs but that shed the virus, thereby contaminating the environment. The few positive cases observed in chicken and pigeons were identified using PCR techniques which facilitated the rapid identification of virus genomes in large numbers of specimens, even when present at very low levels. The inability to detect avian influenza in any of the chicken samples sent to Luxembourg may be due to viral depletion. It might also be due to sample conservation as several freezing and thawing cycles may have lead to the loss of genetic epitopes which hinders identification.

On the other hand, the factors responsible for the continued viability of the virus in pigeons, even after prolonged conservation, may suggest the ability of pigeons to harbour this virus in a very high concentration and still became refractory to the infection.

There is a need for continuous surveillance as avian influenza infection has the ability for antigenic drift and antigenic shift which can lead to an increase in the number of flu infections or can lead to major epidemics due to reassortment (16).

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References

1. Adene D.F., Wakama A.M., Abdu P.A., Lombin L.H., Kazeem H.M., Saidu L., Fatihu M.Y., Joannis T.M., Adeyefa C.A.O. & Obi U.I. 2006. Clinical-pathological and husbandry features associated with the maiden diagnosis of avian influenza in Nigeria. *Nig Vet J*, **27** (1), 32-38.
2. Burgos S. & Burgos S.A. 2007. Influence of exotic birds and wildlife trade on avian influenza transmission dynamics: animal-human interface. *Int J Poult Sci*, **6** (7), 535-538.
3. Centres for Disease Control and Prevention (CDC) 2004. Interim recommendations for infection control in health-care facilities caring for patients with known or suspected avian influenza. CDC, Atlanta, 3 pp (www.cdc.gov/flu/avian/professional/pdf/infectcontrol.pdf accessed on 4 September 2012).

4. Centres for Disease Control and Prevention (CDC) 2007. Key facts about avian influenza (bird flu) and highly pathogenic avian influenza (H5N1) virus. CDC, Atlanta (www.cdc.gov/flu/avian/gen-info/facts.htm accessed on 4 September 2012).
5. Fouchier R.A.M., Munster V., Wallensten A., Bestebroer T.M., Herfst S., Smith D., Rimmelzwaan G.F., Olsen B. & Osterhaus A.D.M.E 2005. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. *J Virol*, **79** (5), 2814-2822.
6. Gauthier-Clerc M., Lebarbenchon C. & Thomas F. 2007. Recent expansion of highly pathogenic avian influenza. A critical review. *Ibis*, **149**, 202-214.
7. Kilpatrick A.M., Chmura A.A., Gibbons D.W., Fleisher R.C., Marra P.P & Daszak P. 2006. Predicting the global spread of H5N1 avian influenza. *Proc Natl Acad Sci USA*, **103** (51), 19368-19373.
8. Martin S.W., Meek A.H. & Willeberg P. 1987. Veterinary epidemiology principles and methods. Iowa State University, Ames, Iowa, 331-346.
9. Meseko C.A., Oladokun A.T. & Shehu B. 2007. An outbreak of highly pathogenic avian influenza (HPAI) in a mixed farm by the introduction of a water fowl. *Nig Vet J*, **28** (3), 67-68.
10. Monne I. Joannis T.M., Fusaro A., De Benedictis P., Lombin L.H., Uluaramu H. Egbuji A., Solomon P., Obi T.U., Cattoli G. & Capua I. 2008. Reassortant avian influenza virus (H5N1) in poultry, Nigeria, 2007. *Emerg Infect Dis*, **14** (4), 1-3 (wwwnc.cdc.gov/eid/article/14/4/07-1178_article.htm accessed on 5 August 2012).
11. Owoade A.A, Ducatez M.F. & Muller C.P. 2005. Seroprevalence of avian influenza virus infectious bronchitis virus, reovirus, avian pneumovirus, infectious laryngotracheitis virus, and avian leukosis virus in Nigerian poultry. *Avian Dis*, **50** (2), 222-227.
12. Owoade A.A., Gerloff N.A., Ducatez M.F., Taiwo J.O., Kremer J.R. & Muller C.P. 2008. Replacement of sublineages of avian influenza (H5N1) by reassortment in sub-Saharan Africa. *Emerg Infect Dis*, **14** (11), 1731-1735.
13. Permin A. & Pedersen G. 2002. The need for a holistic view on disease problem in free-range chickens. *In* Characteristics and parameters of family poultry production in Africa. Results of a Food and Agriculture Organization (FAO)/International Atomic Energy Agency (IAEA) Co-ordinated Research Programme on Assessment of the effectiveness of vaccination strategies against Newcastle disease and Gumboro disease using immunoassay-based technologies for increasing farmyard poultry production in Africa. Food and Agriculture Organization (FAO)/International Atomic Energy Agency (IAEA), Vienna, 9-13 (www-naweb.iaea.org/nafa/aph/public/1-the-need-permin.pdf accessed on 5 August 2012).
14. Putt S.N.H., Shaw A.P.M., Woods A.J., Tyler L. & James A.D. 1992. Veterinary epidemiology and economics in Africa. Veterinary Epidemiology and Economics Research Unit, Department of Agriculture, University of Reading, Reading, 30-34.
15. Van Reeth K. 2007. Avian and swine influenza viruses: our current understanding of the zoonotic risk. *Vet Res*, **38**, 243-260 (www.vetres.org/index.php?option=com_article&access=doi&doi=10.1051/vetres:2006062&Itemid=129 accessed on 26 August 2012).
16. Willey J.M., Sherwood L.M. & Woolverton C.J. 2008. Prescott, Harley, and Klein's microbiology, 7th Ed. McGraw-Hill, New York, 915-917.
17. World Health Organization (WHO) 2002. Manual on animal influenza diagnosis and surveillance. Department of Communicable Diseases Surveillance and Response, WHO Global Influenza Programme. WHO, Geneva, 55-60.

