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FLOCK-BASED SURVEILLANCE FOR LOW PATHOGENIC AVIAN INFLUENZA VIRUS IN COMMERCIAL BREEDERS AND LAYERS, SOUTHWEST NIGERIA

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Abstract

Background: Flock surveillance systems for avian influenza (AI) virus play a critical role in countries where vaccination is not practiced so as to establish the epidemiological characteristics of AI needed for the development of prevention and control strategies in such countries.
Materials and Methods: As part of routine AI monitoring in southwest Nigeria, a competitive ELISA was used for detecting influenza A virus antibodies in the sera of 461 commercial breeder and layer birds obtained from different flocks in Oyo State, Nigeria while haemagglutination inhibiting antibodies against low pathogenic AI viruses (LPAIVs) were detected using H5N2, H7N7 and H9N2 subtype-specific antigens. Suspensions prepared from cloacal swabs were tested for AI virus RNA using reverse transcriptase-polymerase chain reaction.
Results: Results showed that influenza A virus antibody prevalence was 12.8% and 9.3% for breeders and layers, respectively while HI assay revealed 22.0%, 2.0% and 78.0% prevalence of LPAIV H5N2, H7N7 and H9N2 antibodies respectively. All cloacal swab suspensions were negative for AIV RNA.
Conclusion: Since LPAI infections result in decreased or complete cessation of egg production in breeder and layer birds, increased infection severity due to co-infection with other poultry viruses have occasionally been transmitted to humans, the detection of LPAIV H5N2, H7N7 and H9N2 antibodies in these birds is of both economic and public health significance. These findings underscore the need for continuous flock monitoring as part of early warning measure to facilitate rapid detection and sustainable control of AI in Nigerian poultry.

Keywords: Low pathogenic avian influenza, Surveillance, Antibodies, Breeders, Layers

Introduction

Avian influenza viruses (AIV) continue to be a global problem because they are potential highly infectious, can rapidly spread and cause disease in domestic poultry, and some viruses may also infect other animal hosts, including humans (Feare, 2007). Globally, an enormous number of poultry have died from direct infection with AIV, and countless numbers of poultry flocks at risk have been depopulated as a measure to contain the virus and prevent its further spread (Feare, 2007). Apart from the severe economic losses it causes in commercial poultry, AIV can evolve rapidly and spillover into other species (Perdue and Swayne, 2005; Van Reeth, 2007). In addition, high pathogenic avian influenza (HPAI) viruses in domestic poultry are thought to have evolved from low pathogenic avian influenza (LPAI) viruses through mutations or re-combinations (Alexander, 2007). According to Swayne *et al.* (2011), LPAI is a reportable disease caused by H5, H7 and H9 subtype viruses that have become a major source of concern to the global poultry industry. Most LPAI viruses (LPAIVs) produce mild to moderate disease in commercial rearing settings, especially when complicated by secondary pathogens, immunosuppression, and stress factors in the environment. Drops in egg production have also been observed in chicken breeders and layers infected with these viruses (Lu *et al.*, 2004; Pillai *et al.*, 2009). Furthermore, outbreaks of HPAI that resulted from circulating LPAI H5, H7 and H9 viruses have been reported in poultry worldwide (Iqbal *et al.*, 2009; Snoeck *et al.*, 2011). Additionally, their transmission to humans has been described and this highlights their potential to cause zoonotic disease (Capua and Alexander, 2007a; Wang *et al.*, 2009).

Highly pathogenic avian influenza has extended from Asia to Europe and Africa since 2003, leading to the death or mass slaughter of millions of birds and significant economic losses. For instance, the 2003 outbreak of HPAI (H7N7 subtype) in the Netherlands, Belgium and Germany led to the destruction of over 33 million birds with the total cost estimated at €750 million while a 2004 outbreak of AI due to H7N3 subtype in Canada resulted in the destruction of 14 million birds and a loss of more than $300 million (Lupiani and Reddy, 2009). These outbreaks which also resulted in transmission of the virus to occupationally exposed people highlight the significance of global surveillance for AIV infections in the natural hosts. The method commonly used in serologic surveillance programs for AI is a test that detects antibodies directed against the cross-reactive nucleoprotein antigen shared by all influenza A viruses (OIE, 2012). In Nigeria where AI vaccine is not currently administered to poultry and where epidemiological and virological data are sparse, the benefits of surveillance may include estimation of the value of influenza prevention through demonstration of the local disease burden associated with influenza. In this way, surveillance can also help establish the epidemiological characteristics of influenza within the country which would inform the development of effective and sustainable prevention strategies (Breese, 2010). Hence, in Nigeria with a poultry population of about 170 million birds (FAOSTAT, 2014) that provides a source of livelihood for thousands of individuals, there is need for continuous surveillance for diseases such as AI which is of immense economic and public health significance. Therefore, in an attempt to further elucidate the epidemiology of AI in southwest Nigeria, we screened birds from commercial breeder and layer flocks in Oyo state for LPAIV antibodies and nucleic acid.

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Materials and methods

Study design and area

Purposive sampling method was used to focus the study on seven local government areas (LGAs) identified as areas of concentration of commercial poultry in Oyo state, namely Afijio, Lagelu, Oluyole, Ido, Egbeda, Akinyele and Ogbomoso South (Figure 1). With a preponderance of hatcheries, commercial breeder, layer, broiler and cockerel farms, backyard poultry, live-bird markets and feedmills, Oyo state is the nerve centre of poultry activities in southwest Nigeria which has previously been described as the hub of poultry production in the country (Owoade *et al.*, 2004; Adene and Oguntade, 2006).

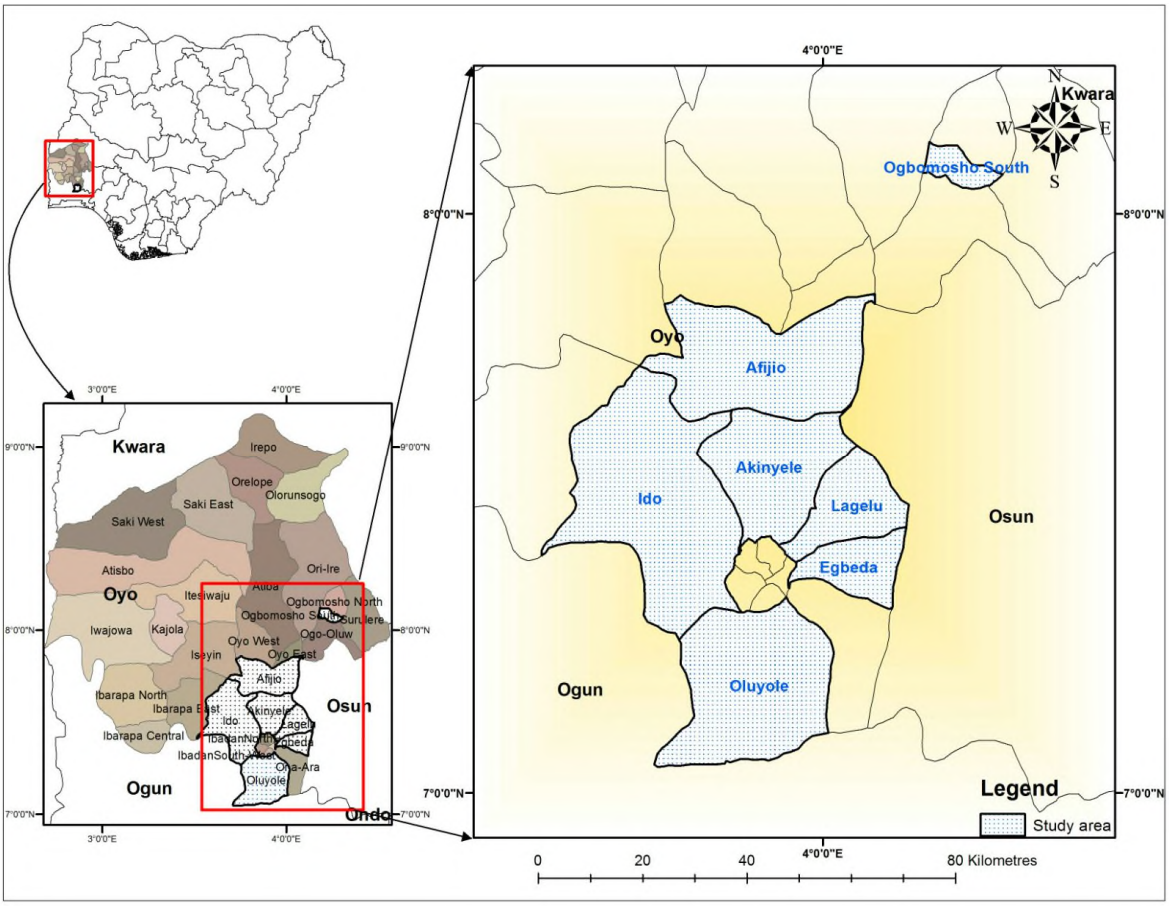

Figure 1: Map showing the study area in Oyo state, Nigeria

In addition, the poultry farmers/personnel were interviewed on AI vaccination practice in their farms. Samples were collected between March and October 2013 from 20 commercial poultry flocks located in these seven LGAs. The survey was targeted at breeder and layer flocks since these chicken types are regarded as the frontliners in poultry farming producing day-old chicks, table eggs and poultry meat for human use and consumption in the study area.

Sample collection

A total of 461 blood and cloacal swab samples were collected for this study. Of this, 180 were obtained from six breeder flocks located in Egbeda and Oluyole LGAs and 281 from 14 commercial layer flocks in the remaining 5 LGAs (Table 1). Two milliliters of blood collected from each bird via the brachial vein was allowed to clot at room temperature for about 3-4 hours. The separated sera were collected in labeled Eppendorf tubes and stored at -20ºC until tested.

Cloacal swab samples were collected by inserting sterile cotton swab in the cloaca of each bird and swabbing it against the mucosal wall until some faecal materials was obtained. This was immediately transferred into virus transport medium containing antibiotics and fetal bovine serum in labeled cryovials. The swabs were then transported on ice to the laboratory where they were stored at -80°C until processed for testing. None of the flocks showed clinical signs of AI at the time of sampling.

Serologic testing

The serum samples were tested for antibodies to influenza A virus with a commercial competitive enzyme-linked immunosorbent assay (ELISA) (Bionote Inc., Korea) following the manufacturer's protocol. According to the manufacturer, the kit was developed to detect antibodies against LPAIV subtypes (H1-15, N1-6). Results were read at 450 nm using a microplate ELISA reader and positive samples (PI ≥ 50) were thereafter screened by haemagglutination inhibition (HI) assays for AIV subtype-specific

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antibodies using a panel of reference antigens comprising LPAI H5N2, H7N7 and H9N2 viruses, 4 haemagglutinating units of each antigen, and positive- and negative-control serum as recommended by the World Organization for Animal Health (OIE, 2014).

Molecular testing

Suspensions prepared from the collected cloacal swabs were centrifuged (4°C) at 1000 rpm for 10 minutes to obtain a clear supernatant. Extraction of each supernatant was done using the QIAmp viral RNA extraction kit (QIAGEN GmbH, Germany) according to the manufacturer's manual. The extracts were amplified by conventional reverse transcriptase-polymerase chain reaction (RT-PCR) using the QIAGEN One-Step RT-PCR kit (Hilden, Germany) in a 50µl reaction volume containing 2µl of enzyme mixture (including Omniscript reverse transcriptase and hot-start Taq polymerase), 10µl of 1X RT-PCR buffer, 2µl dNTPs, 5µl of each primer, 0.25µl RNase inhibitor, 20.75µl nuclease-free water and 5µl of the extracted RNA. Each amplification run contained one negative and one positive control. The negative control was nuclease-free water while for the positive control, nucleic acid extracted from archived virus stock was used. Gene-specific primers designed for the AIV nucleoprotein: 5' – AGC AGC ACA AAG AGC AAT GA - 3' (forward) and 5' – ACT CAT GTC AAA GGA GGG CAC GAT – 3' (reverse) were used (Lu *et al.*, 2013). The thermal profile was as follows: reverse transcription at 50°C for 30 min, a single cycle initial PCR activation step at 95°C for 15 min, followed by 35 cycles of 94°C for 1 min, 56°C for 30 sec and 72°C for 1 min. Final extension was done at 72°C for 10 min.

Statistical analysis

Data obtained were analysed with Fisher's exact test (2-tailed) using Graph Pad prism version 5.0 (Graph Pad software, San Diego, CA, USA) at $\alpha_{0.05}$.

Results

The responses obtained from interviewing the poultry farmers and personnel revealed that they did not vaccinate their birds against AI. Using the ELISA, an overall influenza A virus antibody prevalence of 10.6% (49/461) was obtained. A higher (12.8%) seroprevalence was obtained for breeder flocks compared to 9.3% for layers (Table 1).

Table 1: Prevalence of influenza A virus antibodies based on flock type

Flock type	Location	No. Positive/No. tested	Prevalence (%)
Breeders			
	Egbeda	9/40	22.5
	Oluyole	14/140	10.0
		23/180	12.8
Layers			
	Afijio	8/101	7.9
	Lagelu	1/41	2.4
	Ido	8/37	21.6
	Akinyele	1/60	1.7
	Ogbomoso south	8/42	19.0
		26/281	9.3
Total		**49/461**	**10.6**

However, using the Fisher's exact test (two-sided), there was no statistically significant difference between both flock types sampled. The HI assay to confirm the presence of AIV subtype-specific antibodies in the ELISA-positive sera revealed 22.0%, 2.0% and 78.0% prevalence of H5N2, H7N7 and H9N2 LPAIV antibodies, respectively (Table 2).

Table 2: Prevalence of LPAIV antibodies by HI test

		No. positive by HI		
Flock type	Location	H5N2	H7N7	H9N2
Breeders				
	Egbeda	2	0	7
	Oluyole	1	1	12
Layers				
	Afijio	2	0	7
	Lagelu	1	0	1
	Ido	3	0	5
	Akinyele	0	0	1
	Ogbomoso South	2	0	6
Total		**11 (22.0%)**	**1 (2.0%)**	**39 (78.0%)**

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Testing of the cloacal swabs by RT-PCR gave negative results as none yielded the expected band size of 750 bp following RT-PCR and agarose gel electrophoresis of the amplified products.

Discussion

The importance of continuous surveillance for AI viruses in order to prevent outbreaks and possibly identify potential carriers and reservoirs of the virus that can be included in future surveillance programmes cannot be overemphasized (Coker *et al.*, 2014). Considering that AI viruses have been reported to impact negatively on food supply and the economy through their effects on the poultry industry (Peiris *et al*., 2007), flock surveillance can help establish the epidemiological characteristics of AIV infection that would inform development of prevention and control strategies, especially in countries like Nigeria where vaccination against AI is currently prohibited. As part of routine screening for AIVs in southwest Nigeria (Aiki-Raji *et al.*, 2015; Oluwayelu *et al.*, 2015), serologic and molecular surveillance for LPAIVs was conducted in birds from commercial breeder and layer flocks in Oyo state. It has been reported that serologic screening provides insight into the infection history of an animal's entire life (Couacy-Hymann *et al*., 2012) while RT-PCR detection of the shedding of viral RNA is often performed to monitor the current influenza status in birds (Wallerström *et al*., 2014).

Our findings revealed a low prevalence (10.6%) of antibodies against influenza A virus in asymptomatic, unvaccinated commercial breeder and layer birds in Oyo state, southwest Nigeria. This finding is consistent with previous reports of low seroprevalence of AI in commercial chickens elsewhere: 20% in Bangladesh (Biswas *et al.*, 2009), 11.7% in Grenada (Sabarinath *et al*., 2011) and 12.9% in Kano state, Nigeria (Wakawa *et al.*, 2012). Since the birds were not vaccinated, the further detection of HI antibodies against LPAIV H5N2, H7N7 and H9N2 in them is an indication of response to natural infection with LPAIVs in the environment and suggests that these viruses were present in the sampled farms even in the absence of overt disease. It has been reported that antibodies to influenza A virus can be detected long after viral shedding has ceased (Spackman *et al*., 2009). Interestingly, HI antibodies against two LPAIV subtypes (H5N2 and H9N2) were detected in two layer (Afijio and Lagelu) flocks (Table 2) in this study. This is an indication that birds in these flocks were exposed to more than one LPAIV subtype at the same time, a situation that could potentially lead to genetic reassortment between the two viruses and possibility of emergence of novel virus strains with zoonotic tendencies as previously documented (Smolinski *et al.*, 2004).

Other workers have shown that LPAIV infections may affect feed and water consumption sufficiently to cause distressed egg lay with a resultant decrease in or complete cessation of egg production in both breeder and layer birds (Pillai *et al.*, 2009; Pantin-Jackwood *et al.*, 2012). Moreover, LPAIVs have been associated with increased severity because of co-infection with other poultry viruses, thus they cause economic losses to the poultry industry and negatively impact food supply (Peiris *et al.*, 2007; Samaha *et al.*, 2015). Therefore, considering that the subclinical to mild illness caused by LPAIVs can result in serious disease outbreaks especially when complicated by concurrent infections and/or suboptimal environmental conditions (Seifi *et al*., 2010), the seropositive birds detected in this study could constitute an economic threat to the poultry industry in Oyo state, and possibly Nigeria if interstate spread occurs. Furthermore, environmental exposure to AI viruses by the cloacal and intraocular routes has been shown to be a possibility in chicken breeders if the reproductive organs of the males are infected with the virus or via transmission by semen as previously documented for turkeys (Samadieh and Bankowski, 1970). Hence, these birds could serve as reservoirs shedding the viruses into the environment.

Additionally, the detection of antibodies to LPAIVs in this study corroborates earlier reports of occurrence of LPAI H5, H7 and H9 subtypes in poultry (Nguyen-Van-Tam *et al*., 2006; Cheng *et al*., 2010; Parker *et al*., 2012; Aiki-Raji *et al*., 2015). This finding is significant as it underscores the possibility of a future outbreak of HPAI in Oyo state especially considering previous reports (Swayne and Halvorson, 2003; Werner and Harder, 2006; Briand *et al*., 2010) of the possibility of a field LPAIV mutating into a highly pathogenic strain after circulating in susceptible poultry. Such mutation probably occurs after the viruses have moved from their natural wild bird host to poultry.

The non-detection of AIV RNA by RT-PCR in the cloacal swabs collected from commercial breeder and layer birds in this study suggests that the birds were not actively shedding the virus at the time of sample collection. Since influenza virus is excreted continuously in faeces for only about 12 days in birds (Henaux and Samuel, 2011; Couacy-Hymann *et al*., 2012), it is possible that the period of virus shedding could have been missed in the birds sampled. It could also be due to too low titres of the virus in faeces which makes it undetectable by RT-PCR. Similar findings of absence of AIV RNA in seropositive birds have been reported previously (Obon *et al.*, 2009; Molia *et al.*, 2010; Sabarinath *et al.*, 2011; Wakawa *et al.*, 2012). Likewise, the evidence of LPAIV in commercial poultry in this study suggests that the birds could serve as carriers shedding the virus into the environment when immunity against AI is no longer sufficient, thereby playing a crucial role in the epidemiology of the disease and posing a potential public health risk especially to occupationally exposed humans in the study area. This possibility is highlighted by several reports which have implicated H5, H7 and H9 subtype AIVs in previous cases of human infections (Fouchier *et al*., 2004; Koopmans *et al*., 2004; Capua and Alexander, 2007b; Wang *et al*., 2009).

Conclusions

The findings of this study show that LPAI H5N2, H7N7 and H9N2 viruses presently circulate without producing overt disease in commercial breeder and layer flocks in Oyo state, Nigeria. This highlights the possibility of a future outbreak of HPAI in the state. There is therefore a need for continuous active, flock-based serological, virological and molecular surveillance for AIV as part of early warning measure to achieve prompt detection of the virus and its sustainable control in Nigeria.

References

1. Adene, D.F. and Oguntade, A.E., (2006). Poultry Sector Country Review, FAO Animal Production and Health Division.
2. Aiki-Raji, C.O., Adebiyi, A.I., Agbajelola, V.I., Adetunji, S. A., Lameed, Q., Adesina, M., et al., (2015). Surveillance for Low Pathogenic Avian Influenza (LPAI) Virus in Live Bird Markets in Oyo and Ogun States, Nigeria. Asian Pac. J. Trop. Dis., 5(5): 369-373.
3. Alexander, D.J., (2007). An overview of the epidemiology of avian influenza. Vacc., 25(30): 5637-5644.

4. Biswas, P.K., Barua, H., Uddin, G.M., Biswas, D., Ahad A. and Debnath, N.C., (2009). Serosurvey of five viruses in chickens on smallholdings in Bangladesh. Prev. Vet. Med., 88: 67-71.
5. Breese, J. (2010). Seasonal influenza surveillance- what are the benefits. Infl. Resp. Vir., 4 (Suppl. 2): 3-9.
6. Briand, F.X., Le Gall-Recule, G., Guillou-Cloarec, C., Ogor, K. and Jestin, V.,(2010). Phylogeny and genotyping of recent avian low-pathogenic H5 subtype influenza viruses from French ducks. J. Gen. Virol., 91: 960-970.
7. Capua, I. and Alexander, D.J., (2007a). Avian influenza infections in birds - a moving target. Infl. Resp. Vir., 1: 11-18.
8. Capua, I. and Alexander, D. J. (2007b). Animal and human health implications of avian influenza Infect. Bio. Rep. 27: 359–372.
9. Cheng, M.C., Soda, K., Lee, M.S., Lee, S.H., Sakoda, Y., Kida, H. and Wang, C.H., (2010). Isolation and characterization of potentially pathogenic H5N2 influenza virus from a chicken in Taiwan in 2008. Avian Dis. 54: 885–893.
10. Coker, T., Meseko, C., Odaibo, G., and Olaleye, D., (2014). Circulation of the low pathogenic avian influenza subtype H5N2 virus in ducks at a live bird market in Ibadan, Nigeria. Infect. Dis. Pov., 3:38.
11. Couacy-Hymann, E., Kouakou, V.A., Aplogan, G.L., Awoume, F., Kouakou, C.K., Kakpo, L., et al, (2012). Surveillance for Influenza Viruses in Poultry and Swine, West Africa, 2006–2008. Emerg. Infect. Dis., 18 (9): 1446-1452.
12. FAO, (2007). Questions and answers: The facts of bird flu, Food and Agriculture Organization (FAO). http://www.fao.org/avianflu/en/qanda.html
13. FAOSTAT (2014). http://faostat3.fao.org/download/Q/QA/E. Accessed 15 May, 2016.
14. FDLPCS, (2007). Current Issues in the Control of Avian Influenza in Nigeria. Presentation by M.D. Saidu, Stakeholders Meeting with Poultry Association, Federal Department of Livestock and Pest Control Services (FDLPCS). Abuja.
15. Feare, C. J., (2007). The spread of avian influenza. Ibis, 149(2): 424–425.DOI: 10.1111/j.1474-919X.2007.00711.x.
16. Fouchier, R.A., Schneeberger, P.M., Rozendaal, F.W., Broekman, J.M., Kemink, S.A., Munster, V., et al. (2004). Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. Proc. Natl. Acad. Sci. USA, 101: 1356–1361.
17. Henaux, V. and Samuel, M.D., (2011). Avian influenza shedding patterns in waterfowl: implications for surveillance, environmental transmission, and disease spread. J. Wildl. Dis., 47: 566-578.
18. Iqbal, M., Yaqub, T., Reddy, K. and McCauley, J.W. (2009). Novel genotypes of H9N2 influenza A viruses isolated from poultry in Pakistan containing NS genes similar to highly pathogenic H7N3 and H5N1 viruses. PLoS One, 4: e5788.
19. Koopmans, M., Wilbrink, B., Conyn, M., Natrop, G., van der Nat, H., Vennema, H., et al. (2004). Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. Lancet, 363: 587–593.
20. Lu, H., Dunn, P. A., Wallner-Pendleton, E. A., Henzler, D. J., Kradel, D. C., Liu, J. et al. (2004). Investigation of H7N2 avian influenza outbreaks in two broiler breeder flocks in Pennsylvania, 2001–2002. Avian Dis., 48: 26–33.
21. Lupiani, B., and Reddy, S.M., (2009). The history of avian influenza. Comp. Immunol., Microbiol. Infect. Dis., 32: 311-323.
22. Molia, S., Traore, A., Gil, P., Hammoumi, S., Lesceu, S., Servan de Almeida, R, et al., (2010). Avian influenza in backyard poultry of the Mopti region, Mali. Trop. Anim. Hlth. Prod., 42(5): 807-809.
23. Nguyen-Van-Tam, J.S., Nair, P., Acheson, P., Baker, A., Barker, M., Bracebridge, S, et al. (2006). Outbreak of low pathogenicity H7N3 avian influenza in UK, including associated case of human conjunctivitis. EuroSurveill., 11:E060504.2.
24. Obon, E., Bailey, T.A., Silvanose, C., O'Donovan, D., McKeown, S., Joseph, S, et al., (2009). Seroprevalence of H5 avian influenza virus in birds in the United Arab Emirates. Vet. Rec.,165 (25): 752-754.
25. OIE, (2012). Avian influenza. In: OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, chapter 2.3.4, Office Internationale des Epizooties (OIE), Paris, 7th edition.
26. OIE, (2014). Manual of diagnostic tests and vaccines for terrestrial animals. Office International des Epizooties (OIE), Paris, 2014. http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/
27. Oluwayelu, D.O., Aiki-Raji, C.O., Adigun, O.T., Olofintuyi, O.K., and Adebiyi, A.I.,(2015). Serological survey of avian influenza in turkeys in three states of southwest Nigeria. Infl. Res. Treat. 2015. Doi:10.1155/2015/787890.
28. Owoade, A.A., Oluwayelu, D.O., Fagbohun, O.A., Ammerlaan, W., Mulders, M.N. and Muller, C.P., (2004). Serological evidence of chicken infectious anemia in commercial chicken flocks in Southwest Nigeria. Avian Dis., 48: 202-205.
29. Pantin-Jackwood, M. J., Smith, D. M., Wasilenko, J. L., and Spackman, E., (2012). Low Pathogenicity Avian Influenza Viruses Infect Chicken Layers by Different Routes of Inoculation. Avian Dis., 56(2): 276-281.
30. Parker, C.D., Reid, S.M., Ball, A., Cox, W.J., Essen, S.C., Hanna, A, et al. (2012). First reported detection of a low pathogenicity avian influenza virus subtype H9 infection in domestic fowl in England. Vet. Rec., 171: 372.
31. Peiris, J.S.M., De Jong, M.D. and Guan, Y., (2007). Avian influenza (H5N1): a threat to human health. Clin. Microbiol. Rev., 20: 243-267.
32. Perdue, M.L., and Swayne, D.E., (2005). Public health risk from avian influenza viruses. Avian Dis., 49(3): 317-327.
33. Pillai, S.P., Pantin-Jackwood, M., Jadhao, S. J., Suarez, D.L. and Swayne, D.E. (2009). Pathobiology of triple reassortant H3N2 influenza viruses in breeder turkeys and its potential implication for vaccine studies in turkeys. Vacc., 27: 819–824.
34. Sabarinath, A., Galakrishnan, P. S., Keshaw, P. T., Sachin, M. K., Derek, T., and Ravindra, N. S., (2011). Virological and Serological Surveillance of Avian Influenza Virus in the Birds of Grenada. Int. J. Poultry Sci., 10(8): 579-582.
35. Samadieh, B., and Bankowski, R. A., (1970). Effect of avian influenza-A viruses upon egg production and fertility of turkeys. Avian Dis., 14: 715–722.
36. Samaha, H., Ibrahim, M.S., Ayoub, M., and Shaaban, S.I., (2015). Seroepidemiology of avian influenza viruses H5 and H9 in Beheira Governorate. Alex. J. Vet. Sci., 44: 86-92.
37. Seifi, S., Asasi, K. and Mohammadi, A., (2010). Natural co-infection caused by avian influenza H9 subtype and infectious bronchitis viruses in broiler chicken farms. Vet. Arh., 80: 269-281.
38. Smolinski, M.S., Hamburg, M.A., and Lederberg, J., (2004). Microbial threats to health: Emergence, detection, and response. National Academics Press, Washington DC, pp. 398.
39. Snoeck, C.J., Adeyanju, A.T., De Landtsheer, S. et al. (2011). Reassortant low-pathogenic avian influenza H5N2 viruses in African wild birds. J. Gen. Virol., 92: 1172-1183.
40. Spackman, E., Pantin-Jackwood, M.J., Swayne, D.E. and Suarez, D.L., (2009). An evaluation of avian influenza diagnostic methods with domestic duck specimens. Avian Dis., 53: 276-280.

http://dx.doi.org/10.21010/ajid.v11i1.5
41. Swayne, D.E., and Halvorson, D.A., (2003). Influenza. In: Saif, Y.M. Barnes, H.J. Fadly, A.M. Glisson, J.R. McDougald, L.R., Swayne, D.E.(Eds.), Diseases of Poultry, 11th edition, Ames: Iowa State University Press, pp. 135-160.
42. Swayne, D.E., Pavade, G., Hamilton, K., Vallat, B. and Miyagishima, K. (2011). Assessment of national strategies for control of high-pathogenicity avian influenza and low-pathogenicity notifiable avian influenza, with emphasis on vaccines and vaccination. Revue Sci. Tech., 30(3): 839-870.
43. Van Reeth, K., (2007). Avian and swine influenza viruses: our current understanding of the zoonotic risk. Vet. Res., 38(2): 243-260.
44. Wakawa, A.M., Abdu, P.A., Oladele, S.B., Sa'idu, L., and Owoade, A.A., (2012). Surveillance for Avian Influenza H5 Antibodies and Viruses in Commercial Chicken Farms in Kano State, Nigeria. Int. J. Anim. Vet. Adv., 4(5): 321-325.
45. Wallerström, S., Lagerqvist, N., Temperton, N.J., Cassmer, M., Moreno, A., Karlsson, M, et al., (2014). Detection of antibodies against H5 and H7 strains in birds: evaluation of influenza pseudovirus particle neutralization tests. Infect. Ecol. Epidem., 4: 23011.
46. Wang, M., Fu, C.X. and Zheng, B.J., (2009). Antibodies against H5 and H9 avian influenza among poultry workers in China. New Engl. J. Med., 360: 2583-2584.
47. Werner, O., and Harder, T.C., (2006). Avian influenza. In: Kamps, B.S., Hoffman, C., Preiser, W. (Eds.), Influenza report, chapter 2, Paris: Flying Publisher, pp.48-86.