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Surveillance for low pathogenic avian influenza viruses in live-bird markets in Oyo and Ogun States, Nigeria

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PEER REVIEW

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Comments

In the study, the authors indicated AIV antibody prevalence in five LBMs in Oyo and Ogun States, Nigeria. It is a good study.

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ABSTRACT

Objective: To conduct sero-epidemiological and virological surveillance for lowly pathogenic avian influenza virus (LPAIV) in apparently healthy birds slaughtered at live-bird markets (LBMs) in Southwestern Nigeria.

Methods: A competitive ELISA was used for detecting avian influenza virus-specific antibodies in 491 chicken sera obtained from five LBMs in Oyo and Ogun States, Nigeria while haemagglutination inhibiting antibodies against LPAIVs were detected using H5N2, H7N1 and H9N2 subtype-specific antigens. Suspensions prepared from 400 cloacal swabs were inoculated into 9-day-old embryonated chicken eggs and harvested allantoic fluids were tested for the presence of haemagglutinating agents.

Results: An overall avian influenza virus antibody prevalence of 10.4% (51/491) was obtained with mean percentage inhibition of 61.0 (95% confidence interval: 58.2-63.8) and geometric mean of 60.3 (95% confidence interval: 57.7-62.9). Whereas no LPAIV H7N1 antibodies were detected, 47.1% and 52.9% seroprevalence were obtained for H5N2 and H9N2 viruses respectively. Virus isolation in embryonated eggs was unsuccessful.

Conclusions: Considering the propensity of LPAIVs for mutation into high pathogenicity strains, detection of LPAIV H5N2 and H9N2 antibodies in these chickens is of public health significance and warrants continuous surveillance. Interventions to reduce market-based disease transmission including routine cleaning and disinfection, wearing of protective clothing and gloves, and periodic market rest days are advocated in Nigerian LBMs.

KEYWORDS

Low pathogenic avian influenza, Surveillance, Antibodies, Live-bird markets, Nigeria

1. Introduction

Avian influenza (AI) is a poultry disease caused by type A influenza virus, a member of the genus *Influenzavirus A* in the family Orthomyxoviridae[1]. Based on the antigenic characteristics of the immunodominant viral hemagglutinin and neuraminidase proteins, influenza A viruses are presently subtyped into 18 hemagglutinin and 11 neuraminidase subtypes[2,3]. In recent times, AI has become one of the greatest concerns for public health that has emerged from animal reservoirs[4]. The current outbreaks

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detected in poultry and wild birds in many Asian, European and African countries are of concern not only to the poultry industry in which they produce an economically devastating disease, but also to public health[5].

Influenza A viruses are divided into two distinct groups based on their ability to cause disease in chickens: highly pathogenic avian influenza (HPAI) viruses that cause mortality rates up to 100% in several days and low pathogenic avian influenza viruses (LPAIVs) that cause subclinical to mild, primarily respiratory disease, which however can result in a serious disease when complicated by concurrent infections and/or suboptimal environmental conditions[6]. Low pathogenic avian influenza (LPAI) is a second level reportable disease caused by viruses of subtypes H5, H7 and H9 which have become a major concern to the poultry industry worldwide[7]. According to Briand *et al.*[8], LPAIVs of subtype H5 or H7 can easily acquire multiple basic amino acids at the cleavage site which allows them to become highly pathogenic following a few mutations. Outbreaks of HPAI that resulted from circulating LPAI H5, H7 and H9 viruses have been reported in poultry worldwide[9-12]. Moreover, their transmission to humans has been described and this highlights their potential to cause zoonotic disease[10,13]. Due to the subclinical/mild disease that it produces, most poultry producers do not consider LPAI as an important disease and often do not even realize that it is present in their flocks[14].

Currently, AI surveillance is tilted towards the severe disease (*e.g.* HPAI H5N1) which may bias understanding of the true extent of interspecies transmission caused by less pathogenic strains such as LPAI H5, H7 and H9 viruses. Therefore, surveillance of animal respiratory viruses at the human-animal interface is crucial to identifying potential pandemic and zoonotic threats. Such interface is provided by live-bird markets (LBMs) which present optimal conditions for the zoonotic transfer and evolution of infectious disease agents as they are major contact points between humans and live animals, and also play a major role in facilitating emergence or re-emergence of influenza viruses[15]. In Nigeria, LBMs are common and are located primarily in the urban areas where different bird species produced by multiple suppliers are mixed together to provide a platform for maximum interaction and efficient transfer of infectious agents among the birds and between humans and birds. In this study, we conducted sero-epidemiological and virological surveillance of LPAIVs in apparently healthy birds sold or slaughtered at five LBMs in Oyo and Ogun States, Southwestern Nigeria.

2. Materials and methods

2.1. Study area

The study was conducted in five major LBMs located in Oyo and Ogun States of Southwestern Nigeria which formed the heart of the poultry industry in the country. These markets which serve as focal points for bird trade from different parts of Nigeria include Molete, Mokola and Shasha LBMs (Oyo State) and Kuto and Sabo LBMs (Ogun State). Oyo and Ogun States are located at 8°00' N 4°00' E and 7°00' N 3°35' E, respectively (Figure 1).

Figure 1. Map of Nigeria showing the study area.

2.2. Collection of samples

Using convenience sampling, a total of 491 blood samples and 400 cloacal swabs were collected from birds (commercial broilers and layers, and Nigerian indigenous chickens) at the five LBMs between May and June 2013. With the aid of sterile needles and syringes, about 3 mL of blood was collected from the birds through jugular venipuncture or at slaughter into sterile plain tubes (without anticoagulant) and allowed to clot at room temperature for about 3 h for serum separation. The separated sera were stored at -20 ℃ until analyzed. Cloacal swabs were collected by inserting a sterile swab into the cloaca of the bird and swabbing the wall to remove some faecal material with it. This was immediately transferred into virus transport medium (containing antibiotics and foetal calf serum) in labeled Eppendorf tubes. The swabs were then transported on ice to the laboratory where they were stored at -80 ℃ until processed for virus isolation. The bird sellers were interviewed to obtain information on biosecurity measures practiced in the markets.

2.3. Serology

A competitive ELISA kit (Bionote Inc., Korea) for the quantitative detection of antibodies to avian influenza virus (AIV) was used to screen the sera. All steps were carried out according to the manufacturer's manual and results were read at 450 nm using an ELISA reader (Optic Ivymen® System, Model 2100C, Biotech SL, Madrid, Spain). The percentage inhibition (PI) for each sample was calculated from the absorbance values obtained. Positive samples (PI $\geqslant$ 50) were screened by haemagglutination inhibition (HI) test for AI subtype-specific antibodies using a panel of reference antigens comprising LPAI H5N2, H7N1 and H9N2 viruses and 4 haemagglutinating units of each antigen according to standard protocol[16].

2.4. Attempted virus isolation in embryonated chicken eggs

Suspensions prepared from the cloacal swabs were centrifuged (4 ℃) at 1 000 r/min for 10 min and supernatants decanted into

sterile Eppendorf tubes. Using tuberculin syringes, the suspensions were inoculated via the allantoic route into 9-day-old embryonated specific-antibody-negative chicken eggs obtained from flocks that had not received AI vaccination and which were negative for influenza A virus antibodies at the time the eggs were collected. Two eggs were inoculated per specimen. The holes punched in the eggs were sealed with drop of candle wax and the eggs were incubated at 37 ℃ for 2-3 d. Before harvesting, the eggs were chilled for a minimum of 4 h and harvested allantoic fluids were tested for the presence of any haemagglutinating agent[16]. Samples were considered negative after two passages in eggs.

3. Results

Based on interviews conducted at the LBMs, the only biosecurity measure being practiced by the traders and butchers was daily sweeping of the market. It was observed that the LBMs were located within the main markets and interactions between birds of different species were a common occurrence (Figure 2). In addition, the butchers, who were mostly girls and women, carried out bird slaughtering, evisceration and processing of raw poultry meat with their bare hands and without any protective apparel (Figures 3 and 4). Using the ELISA, an overall AIV antibody prevalence of 10.4% (51/491) was obtained with mean PI value of 61.0 (95% confidence interval: 58.2-63.8) and geometric mean of 60.3 (95% confidence interval: 57.7-62.9). Based on state of sample collection, 8.3% (24/291) and 13.5% (27/200) prevalence of AIV antibodies were obtained for Oyo and Ogun States respectively while on individual market basis, the highest prevalence of 15.5% was obtained in Kuto LBM with no detection of AIV antibodies in sera from Shasha and Sabo LBMs (Table 1). The HI test to confirm the presence of AIV subtype-specific antibodies in the ELISA-positive sera revealed 47.1% and 52.9% prevalence of H5N2 and H9N2 LPAIV antibodies respectively while no H7N1-specific antibodies were detected in all the tested sera (Table 2). The ranges of HI titres obtained were 1:32 to 1:512 and 1:64 to 1:2048 for LPAIV subtypes H5N2 and H9N2 respectively. Following inoculation of 9-day-old embryonated chicken eggs with the cloacal swab suspensions, no haemagglutinating agent was isolated.

Figure 2. Cages housing different bird species at a LBM.

Figure 3. Bird slaughtering with bare hands at a LBM.
The lady in white overall and gloved hands is collecting blood sample.

Figure 4. Women handling raw poultry meat with ungloved hands at a LBM.

Table 1
Prevalence of AIV antibodies using ELISA.

LBM	Number positive/Number tested (%)	Mean PI ± standard deviation
Molete	15/194 (7.7)	61.9 ± 13.1
Mokola	9/91 (9.9)	57.4 ± 9.1
Shasha	0/6 (0.0)	0.0 ± 0.0
Kuto	27/174 (15.5)	61.6 ± 8.2
Sabo	0/26 (0.0)	0.0 ± 0.0
Total	51/491 (10.4)	61.0 ± 10.0

Table 2
Prevalence of LPAIV H5N2, H7N1 and H9N2 antibodies by HI.

LBM	Number positive by ELISA	Number positive by HI		
		H5N2	H7N1	H9N2
Molete	15	5	0	10
Mokola	9	4	0	5
Kuto	27	15	0	12
Total	51	24 (47.1%)	0 (0.0%)	27 (52.9%)

4. Discussion

LPAI viruses have attracted increasing interest by virtue of their insidious spread, potential to mutate to high pathogenicity strains

and concerns about their zoonotic transmission[17,18]. They have been isolated from poultry from LBM systems worldwide[11,19-21]. In the present study, which is part of ongoing routine surveillance of LPAI in Southwestern Nigeria, AIV antibody prevalence of 8.3% and 13.5% respectively were obtained by ELISA technique in the LBMs surveyed in Oyo and Ogun States, an indication that the infection was more prevalent in Ogun State than in Oyo State. Since the two states form the heart of the poultry industry in Southwestern Nigeria, it can be assumed that the infection is widespread over this region of the country. Additionally, HI antibody prevalence of 47.1% and 52.9% were obtained for LPAI H5N2 and H9N2 respectively in the sampled birds. In Nigeria where vaccination of birds against AI is currently not allowed, detection of H5N2 and H9N2 LPAIV-specific antibodies in the sera of birds from five major LBMs in the absence of overt disease is an indication that they had been naturally exposed to these viruses. Thus, the birds could serve as reservoirs shedding the viruses into the environment, thereby playing a crucial role in the epidemiology of the disease. This finding is consistent with previous reports of LPAI H5 and H9 subtypes in poultry elsewhere and supports the fact that a significant part of transmission of AI is limited to commercial trade of poultry and derived products[10,11,14,22,23].

The non-detection of LPAI H7N1 virus-specific antibodies in all tested sera is noteworthy and confirms earlier works which showed that LPAI outbreaks in poultry were dominated by the H5N2 and H9N2 virus subtypes[10,12,22]. Also, the absence of AIV antibodies in sera from Shasha and Sabo LBMs in Oyo and Ogun States respectively could be due to the small number of samples obtained from the two markets. This is attributable to the uncooperative, and sometimes hostile, attitude of the traders at these markets towards sample collection. Moreover, the unsuccessful attempt at virus isolation is similar to the findings of Parker *et al.* in England and suggests absence of an acute/on-going infection in the birds or that the titre of AIV in the swab suspensions was too low to cause any infection or pathology in the embryos[22]. Alternatively, the virus may also not have been isolated if it was being shed via the respiratory tract. However, this possibility could not be proved further as tracheal swabs were not collected in this study. We therefore recommend the collection of both tracheal and cloacal swabs for future AI surveillance studies.

Factors such as continual movement of birds into, through, and out of LBMs, as well as attempts to sell infected dead or dying birds have been reported to provide opportunity for the introduction, entrenchment, and dissemination of AIVs[24,25]. These practices coupled with the tradition of keeping different species of birds, and sick and healthy birds in the same cages and/or in close proximity, were common occurrences in the five LBMs surveyed in this study. Other risky practices observed in these LBMs include bird slaughter, evisceration and processing of raw poultry meat using bare hands as well as lack of protective apparel. Indeed, slaughtering has been reported to generate droplets that may contain viral particles and expose internal organs with potentially high viral loads[25]. Therefore, these marketing practices, which are of public health significance, make the LBMs to be high-risk environments that provide excellent prospects for transmission of infection from birds to humans and other animals in the markets. Previous studies have reported the transmission of AI from birds to humans at LBMs[10,11,13].

The findings of this study reveal that LPAI H5N2 and H9N2 viruses presently circulate in LBMs in Oyo and Ogun States of Southwestern Nigeria, making these markets potential risk environments for transmission of infection to humans and other animals. The risky practices of the traders and butchers in the surveyed markets, which are typical of LBMs across Nigeria, highlight the need for routine nationwide sero-epidemiologic and virologic surveillance of birds and occupationally exposed persons in these markets as an early-warning system. Furthermore, interventions to reduce market-based disease transmission such as routine cleaning and disinfection to decontaminate surfaces, daily disposal and removal of waste from the market to eliminate AIV reservoirs, segregation of poultry-related activities into zones to limit virus spread as well as periodic market rest days with thorough cleaning, all of which have been practised successfully elsewhere[11,20,26,27], should be adopted for implementation in Nigerian LBMs. Also, it is advisable that butchers at these markets wear protective clothing and avoid handling raw bird meats with their bare hands by putting on gloves during bird slaughtering and evisceration. Lastly, increased public education about the risks for influenza virus in association with LBMs is advocated as this will help prevent both LPAI and HPAI infections in humans.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Currently, AI surveillance is tilted towards the severe disease (*e.g.* HPAI H5N1) which may bias understanding of the true extent of interspecies transmission caused by less pathogenic strains such as LPAI H5, H7 and H9 viruses. Therefore, surveillance of animal respiratory viruses at the human-animal interface is crucial to identifying potential pandemic and zoonotic threats.

Research frontiers

In Nigeria, LBMs are common and are located primarily in the urban areas where different bird species produced by multiple suppliers are mixed together to provide a platform for maximum interaction and efficient transfer of infectious agents among the birds and between humans and birds. The paper conducts sero-epidemiological and virological surveillance for LPAIVs in apparently healthy birds slaughtered at LBMs in Southwestern Nigeria.

Innovations & breakthroughs

The findings of this study reveal that LPAI H5N2 and H9N2 viruses presently circulate in LBMs in Oyo and Ogun States of southwestern Nigeria, making these markets potential risk environments for transmission of infection to humans and other animals.

Applications

Considering the propensity of LPAIVs for mutation into high pathogenicity strains, detection of LPAIV H5N2 and H9N2 antibodies in these chickens is of public health significance and warrants continuous surveillance. Interventions to reduce market-based disease transmission including routine cleaning and disinfection, wearing of protective clothing and gloves, and periodic market rest days are advocated in Nigerian LBMs.

Peer review

In the study, the authors indicated AIV antibody prevalence in five LBMs in Oyo and Ogun States, Nigeria. It is a good study.

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