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Original Article

Detection of influenza A virus in live bird markets in Kenya, 2009–2011

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Background Surveillance for influenza viruses within live bird markets (LBMs) has been recognized as an effective tool for detecting circulating avian influenza viruses (AIVs). In Sub-Saharan Africa, limited data exist on AIVs in animal hosts, and in Kenya the presence of influenza virus in animal hosts has not been described.

Objectives This surveillance project aimed to detect influenza A virus in poultry traded in five LBMs in Kenya.

Methods We visited each market monthly and collected oropharyngeal and cloacal specimens from poultry and environmental specimens for virological testing for influenza A by real time RT-PCR. On each visit, we collected information on the number and types of birds in each market, health status of the birds, and market practices.

Results During March 24, 2009–February 28, 2011, we collected 5221 cloacal and oropharyngeal swabs. Of the 5199 (99·6%) specimens tested, influenza A virus was detected in 42 (0·8%), including 35/4166 (0·8%) specimens from chickens, 3/381 (0·8%) from turkeys, and 4/335 (1·2%) from geese. None of the 317 duck specimens were positive. Influenza was more commonly detected in oropharyngeal [33 (1·3%)] than in cloacal [9 (0·4%)] specimens. None of the 485 environmental specimens were positive. Virus was detected in all five markets during most (14/22) of the months. Ducks and geese were kept longer at the market (median 30 days) than chickens (median 2 days).

Conclusions Influenza A was detected in a small percentage of poultry traded in LBMs in Kenya. Efforts should be made to promote practices that could limit the maintenance and transmission of AIVs in LBMs.

Keywords Influenza, live bird markets, surveillance.

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Introduction

Influenza A viruses are zoonotic pathogens that infect a variety of domestic poultry such as chickens, turkeys, ducks, and geese.[1–3] From the mid-1970s, investigations have revealed reservoirs of influenza viruses present in wild bird populations and domestic poultry.[2,4,5]

Surveillance for influenza viruses within live bird markets (LBMs) has been recognized as an effective tool for detecting circulating influenza subtypes in the poultry population.[6] Live bird markets are ideal sites for virus mixing and transmission because of their nature of congregating birds from various farms coupled with the practices of mixing newly arrived birds with those that have been in the market for extended periods. Since the 1970s, influenza viruses have been isolated from birds in LBMs in multiple countries. From 7% to 30% of fecal swabs from ducks were positive for circulating H3, H4, H5, H6, H7, and H9 influenza virus subtypes in LBMs in Taiwan, Vietnam, and Hong Kong in the 1980s before the onset of the H5N1, H7N2, and H9N2 poultry epidemics in Southeast Asia.[7,8]

Influenza viruses have also been detected in various environmental specimens collected in contaminated areas in LBMs including drinking water troughs, and surfaces in the delivery, holding and slaughter areas in markets.[9,10] In a study in Hong Kong, influenza A (H9N2) was isolated in 1% of fecal swabs and 7% of drinking water samples collected in eight live poultry markets.[11] Most (56%) of LBMs in Indonesia were found to have ≥6 sites contaminated with avian influenza virus (H5N1) by real-time reverse transcription polymerase chain reaction (rt RT-PCR) with

slaughter and sale areas being the most heavily contaminated.[9]

Although avian influenza viruses (AIVs) in the poultry population have not been described in Kenya, qualitative risk assessment studies carried out in 2007–2008 following the 2005 threat of introduction of highly pathogenic avian influenza (HPAI) H5N1 in the country suggested a significant risk of transmission of AIVs if the virus were introduced into the poultry population.[12] The risk assessment identified complex marketing chains of poultry involving multiple middle men and markets coupled with unsatisfactory levels of biosecurity along the poultry chain as important factors that could contribute to the spread and transmission of influenza viruses through the poultry population and potentially to the human population.[12]

In March 2009, the Kenya Medical Research Institute/US Centers for Disease Control and Prevention – Kenya (KEMRI/CDC-K) in collaboration with the Kenya Department of Veterinary services (DVS) initiated surveillance to assess the presence of avian influenza viruses in birds traded in LBMs in Kenya. Additionally, we investigated market practices that could contribute to mixing and transmission of virus within the market.

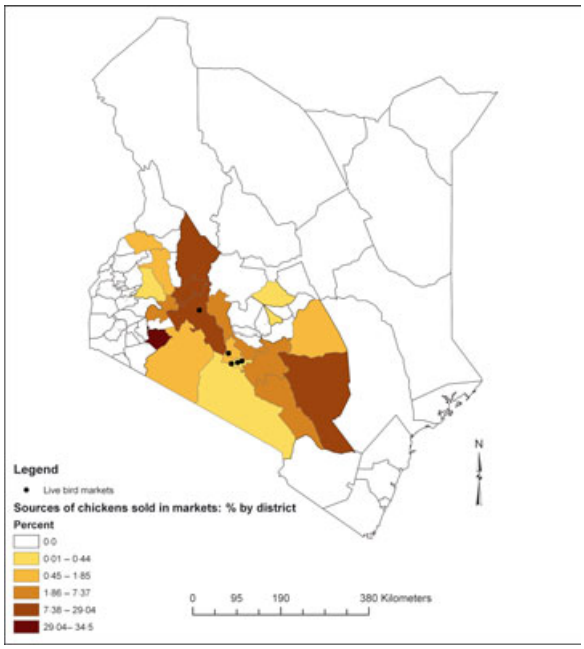

Figure 1. Map of Kenya showing the location of the five live bird markets and the geographic distribution by percentage of the source of chickens sold in the five markets, 2009–2011.

Materials and methods

Live bird markets

Between March 24, 2009 and February 28, 2011, we conducted surveillance in five LBMs in Kenya: Kariokor, Burma, and Kawangware markets, located within the capital city of Nairobi; Nyambari market, located 40 km north of the city along a major highway; and Nakuru market, located in a major urban center in the Rift Valley Province, about 150 km north of Nairobi (Figure 1). We did not collect samples for the months of December because the staff were unavailable to visit the markets. We chose these markets because they are among the largest poultry markets in the country, and they trade primarily in chickens. The Nyambari market trades in multiple avian species, including turkeys, geese, ducks, and doves.

The Kariokor market is housed in an enclosed building, while the other four are outdoor markets. In four of the five markets, birds were kept in wire mesh cages, each housing 40 birds during the day and night. In the fifth market, Nyambari, birds were not kept in cages during the day but they stayed close to the feeding and watering troughs. At night, the birds at Nyambari market were driven to a shelter located 500 m from the market.

The five LBMs receive poultry from districts across the country. The range of birds sold at the markets included chickens (indigenous chickens, spent layers and broilers), ducks, turkeys, and geese. The markets sell live poultry for restocking to farmers and for slaughter to individual homes and hotels. At Kariokor and Kawangware markets, poultry slaughter is carried out within the market premises. No slaughtering occurs at the other three markets.

Sampling and data collection

Each market was visited once a month, and an oropharyngeal (OP) and a cloacal (CL) swabs collected from 25 birds on every visit. Birds that had stayed the longest in the market were preferentially sampled. For the market where there were multiple species, we sampled from all the poultry species. In the chicken markets where the birds were confined in cages, birds from all of the cages were sampled; on average, 3–5 birds were sampled from each cage. In addition, five environmental specimens were collected by swabbing fecal droppings on the floor of the bird cages during each monthly market visit.

Plastic-shafted polyester-tipped swabs were used to collect OP and CL swabs from birds and to collect environmental specimens. The swabs were each placed in cryovials containing 2 ml of freshly prepared viral transport media (VTM) containing bovine serum albumin and veal infusion broth supplemented with amphotericin B and gentamycin (http://www.who.int/csr/resources/publications/surveillance/Annex8.pdf). Specimens were labeled and transported at 4°C to the KEMRI/CDC-K laboratory and frozen at −80°C within 24 hours after collection until testing.

We administered a standardized questionnaire to the poultry traders during each visit. The questionnaire

included questions about the number and types of birds in each market, whether the markets had been cleaned using disinfectants, the presence of rodents and wild birds, the number of days the birds had been in the market, the source of the birds, and the health status of the birds.

Laboratory testing

All specimens were tested by real-time reverse transcription polymerase chain reaction (rt RT-PCR) at the Biosafety Level 3 KEMRI/CDC-K laboratory in Kisumu using the CDC protocol for influenza A virus detection.[13] Briefly, total RNA was isolated from 100 μl of the oropharyngeal specimens using the QIAamp RNA extraction kit (Qiagen Inc, Valencia, CA, USA) according to the manufacturer's instructions. Total RNA was extracted from 100 μl of each cloacal and environmental specimen using the MagMAX Viral RNA Isolation kit (Ambion Inc, Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. One step rt RT-PCR was carried out using the AgPath-ID rt RT-PCR kit (Applied Biosystems).[13] The rt RT-PCR machine was set to run at 10 minutes at 45°C for reverse transcription, 10 minutes at 95°C to activate the *Taq* polymerase, and a typical 45 cycle PCR with denaturation at 95°C for 15 seconds and annealing/extension at 55°C for 1 minute. Fluorescence was read at the annealing/extension step. The results were collected as cycle threshold (C_T) values. Specimens with C_T values of <40·0 were considered positive.

Data management and analysis

Data were entered and stored in an MS Access database and analyzed using SAS version 9.1 (Cary, NC, USA). Descriptive statistics on number of birds sold, suppliers, and length of stay of birds were calculated. We used chi-square test for all the bivariate analysis.

Results

Market practices

The average number of chickens present at the market on the day of the monthly visit was 641, 629, 381, and 218 for Kawangware, Burma, Nakuru, and Kariokor market, respectively. At the Nyambari market, the average number of ducks, geese, and turkeys was 45, 52, and 60, respectively. Additionally in Nyambari market, doves, rabbits, and guinea fowl were occasionally present for sale and were housed in the same cages.

The source of the poultry traded varied greatly for all species and markets. Overall, birds were sourced by traders and middlemen from districts across five of the eight provinces in Kenya. Over half (55%) of the chickens traded in all the markets originated from Rift Valley Province [Bomet (33%), Baringo (16%), and Kericho (5·5%) District] (Figure 1). Half (51%) of the ducks traded originated from the Western province districts of Bungoma, Busia, Kakamega, and Malaba, while 16% originated from the neighboring country of Uganda. Forty-three percent of the geese originated from the Rift Valley province (Nakuru district) and 22% from Central province (Nyandarua district). A majority (68%) of the turkeys originated from the Western province [Bungoma (25%), Kakamega (17%), Malaba (9·4%), Busia (14%), and Teso (2·6%) districts].

The five markets were open for trading for 7 days every week. Rodents were reported to be present in Kariokor, Burma, Kawangware, and Nakuru markets, and disinfection was rarely carried out in any of the five markets. In all five markets, wild birds were observed mixing and feeding with the poultry.

The majority of the birds sampled [2322 (88·9%)] were supplied to the market traders by middlemen (Table 1). A small percentage (5·0%) was bought directly from a farm by the traders, and 2·5% of the poultry were bought from other markets (Table 1).

Ducks, geese, and turkeys stayed on average 15 times longer than chickens in the market (Table 2). Over half of the ducks, geese and turkeys had been in the markets for 30 days at the time of sampling.

Laboratory results

We collected 5221 cloacal and oropharyngeal swabs. Of these, 4176 (80%) were from chickens, 321 (6·1%) from ducks, 382 (7·3%) from turkeys, and 342 (6·6%) from geese (Table 3). Most (99·5%) of the specimens were collected from healthy birds, but 0·5% of samples were collected from clinically sick birds that mainly had diarrhea, difficulty in breathing, and nasal discharges.

Of the 5199 (99·6%) specimens tested that could be linked to individual bird data, influenza A virus was detected in 42 (0·8%). Influenza was detected in 35/4166 (0·8%) chicken OP/CL specimens, 3/381 (0·8%) turkey OP/CL specimens, and 4/335 (1·2%) geese OP/CL specimens (Table 3). No virus was detected in 317 duck OP/CL specimens [2·3% upper limit at 95% confidence level (CI)] (Table 3). The mean C_T value of the 42 specimens that were positive for influenza A by rt RT-PCR was 37·2 (Standard error 0·29); the median C_T value was 38·1 (Range 33·1–39·6). Test results for 22 (0·4%) specimens could not be linked to individual bird data and were excluded from further analysis. All of these 22 specimens were negative for influenza A.

None of 485 environmental specimens collected and tested for influenza A virus were positive (upper limit 95% CI 0·8%) (Table 4).

In total, we collected specimens for 22 months and influenza virus was detected in the poultry in 14 (63·64%) of

Table 1. Poultry suppliers to five live bird markets in Kenya, March 24, 2009–February 28, 2011

Source of poultry in the market	*n* (%)
Self-owned	130 (5·0)
Farm by farmer	40 (1·5)
Middlemen	2322 (88·9)
Other markets	66 (2·5)
Unknown	3 (0·1)
Missing	51 (2·0)
n	2612 (100)

Table 2. Days poultry spent in the market from delivery by suppliers to day of sampling in the five live bird markets in Kenya, March 24, 2009–February 28, 2011

Poultry type	*n*	Days mean (SD)	Days median	Days range (min–max)
Chicken	4176	2·54 (2·0)	2	29 (1–30)
Ducks	321	36·79 (25·6)	30	89 (1–90)
Geese	342	29·70 (21·1)	30	88 (2–90)
Turkey	382	28·16 (21·5)	28	89 (1–90)

these months. We did not observe any seasonal or monthly differences in influenza detection (Figure 2). The median monthly detection rate was 0·4%; the highest detection rate (4·1%) was in January 2011.

Influenza virus was detected in both OP and CL specimens in chickens (Table 3). In turkeys and geese, influenza A was detected in OP but not in CL specimens (Table 3). In all species, virus detection was significantly higher in OP [33 (1·2%)] than in cloacal [9 (0·4%)] specimens (P-value < 0·01). Overall, influenza virus prevalence was highest in geese 4/168 (2·4%) and lowest in turkeys 3/191 (1·6%). There was no significant difference in the observed prevalence of influenza among chickens, turkeys, geese, and ducks (P-value 0·29). All the influenza positive specimens were from healthy birds.

Influenza A was detected in all the five markets (Table 4). In the four markets trading primarily in chickens, the detection rate varied from 0·5% in Nakuru to 1·2% in Kariokor. The detection rate in Nyambari market, which traded in mixed species (turkeys, geese and ducks), was 0·7%. There was no significant difference in the influenza detection rate in the five markets during this period. In total, the 35 influenza A-positive specimens in chicken were distributed in 13 administrative districts. Ten of 35 (28·6%) influenza A-positive chicken specimens were from birds sourced from one district (Bomet district) where 1/3 of the chickens sold in the four markets originated from. Influenza A detection by district was variable and ranged from 0·3% to 12·5% for this period. However, there was no significant difference in the influenza A detection rate of the chicken, geese, or turkey specimens by district or source of the birds.

Discussion

We detected influenza A viruses in poultry traded in all five LBMs in Kenya. The influenza A viral RNA was detected in geese, turkeys, and chicken. To our knowledge, this is the first time influenza A RNA has been detected in poultry traded in LBMs in Kenya. In our study, the overall influenza A RNA detection rate among the birds sampled was 1·6%. Surveillance studies on influenza viruses have recorded variable prevalence of AIVs in poultry traded in

Table 3. RT PCR test results for oropharyngeal (OP) and cloacal (CL) specimens collected during influenza surveillance in live bird markets by host species, Kenya, March 2009–February 2011

Species	Specimen type	Specimens collected	No. of specimens tested	Influenza A positive by specimen type *n* (%)	Influenza A positive by specimen type 95% CI	No. of birds positive *n* (%)
Chicken	OP	2138	2129	26 (1·2)	0·8, 1·8	35 (1·7)
	CL	2038	2037	9 (0·4)	0·2, 0·8	
Turkey	OP	187	186	3 (1·6)	0·3, 4·6	3 (1·6)
	CL	195	195	0 (0·0)	0·0, 1·9	
Goose	OP	165	162	4 (2·5)	0·7, 6·1	4 (2·4)
	CL	177	173	0 (0·0)	0·0, 2·1	
Duck	OP	158	156	0 (0·0)	0·0, 2·3	0
	CL	163	161	0 (0·0)	0·0, 2·2	
Total (%)		5221	5199	42 (0·8)	0·6, 1·1	42 (1·6)

Table 4. Influenza viruses detected in five live bird markets (LBMs) during influenza surveillance in LBMs in Kenya, March 2009–February 2011

Market	OP/CL specimens			Environmental specimens		
	Samples collected	No. of influenza A viruses detected/ tested (%)	No. of influenza A viruses positive (95% CI)	Samples collected	No. of influenza A viruses detected/ tested (%)	No. of influenza A viruses positive (95% CI)
Nyambari	1045	7/1033 (0·7)	0·3, 1·4	91	0/91 (0·0)	0·0, 4·2
Burma	1016	10/1014 (1·0)	0·5, 1·8	90	0/90 (0·0)	0·0, 4·3
Kariokor	1030	12/1027 (1·2)	0·6, 2·0	97	0/97 (0·0)	0·0, 4·2
Kawangware	1051	8/1049 (0·8)	0·3, 1·5	102	0/102 (0·0)	0·0, 4·2
Nakuru	1079	5/1076 (0·5)	0·2, 1·1	105	0/105 (0·0)	0·0, 3·7
Total	5221	42/5199 (0·8)	0·6, 1·1	485	0/485 (0·0)	0·0, 0·8

OP, oropharyngeal; CL, cloacal.

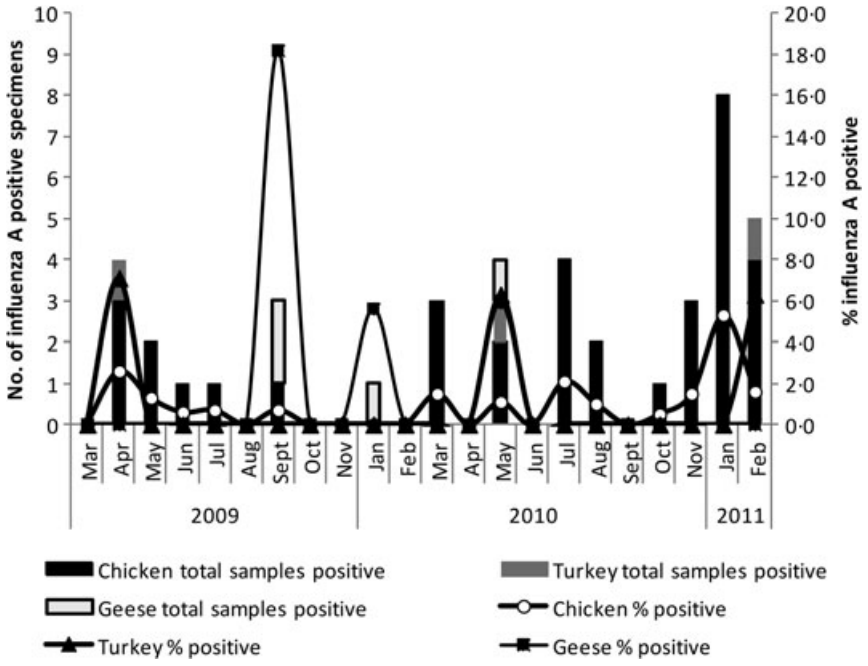

Figure 2. Temporal distribution of influenza A-positive specimens from five live bird markets in Kenya by species, March 2009–February 2011.

LBMs in different countries around the world. In a study conducted in Korea in 2003, 6% of chicken specimens were positive for AIVs,[14] whereas 31% and 6·1% of duck and geese specimens, respectively, were positive for AIVs in Vietnamese markets in 2001.[7] In the Vietnam study, AIVs were not detected from chicken specimens.[7]

We did not detect influenza A in any of the duck specimens. This finding was unexpected, because ducks are associated with maintenance of influenza virus in domestic birds. In fact, many studies conducted in LBMs and farms reported a higher prevalence of influenza in ducks compared with other poultry species.[7,15–17] It is not clear why influenza A virus was not detected in ducks in the markets in Kenya, particularly in light of the fact that the ducks stayed for longer periods of time in the market and were housed in the market together with geese and turkeys. However, all of the ducks sampled were adults of market age, and it is possible that they had already been exposed to influenza viruses early in life and therefore may have developed some immunity to the circulating viruses.

Screening for anti-influenza A antibodies in these species would have helped to clarify this, but this was beyond the scope of our surveillance project.

In our surveillance, in all avian species, influenza A virus was more commonly detected in oropharyngeal specimens than in cloacal specimens by rt RT-PCR. In a study of AI in backyard poultry in Mali in 2007, 2·2% of tracheal swabs and 1·3% of cloacal swabs tested positive for influenza A by rt RT-PCR.[16] Experimental studies in ducks showed that viruses replicated to higher levels in the trachea than in the cloaca of both inoculated and contact birds, suggesting that the digestive tract is not the main site of H5N1 influenza virus replication in ducks.[18,19] Likewise, experimentally inoculated geese and chicken shed higher virus titers in oropharyngeal swabs than in cloacal swabs.[20,21] Naturally occurring inhibitors present in cloacal and environmental swabs have been shown to limit the sensitivity of rt RT-PCR in detection of influenza A.[22] To minimize this effect in our study, we used the MagMAX extraction kit, which has been shown to be more effective in removing inhibitors, though the effect of inhibitors cannot be completely ruled out.[22]

In the five markets, we observed several practices that could promote influenza transmission among birds. These included keeping markets open for 7 days a week, limited cleaning and disinfection of the market, mixing of new and old birds, trading multiple poultry species in the same market, and mixing with wild birds. These factors were found to be associated with transmission of low pathogenic avian influenza viruses in markets in North America.[23]

Environmental sampling, where specimens are collected from contaminated areas of the market, has been suggested as an effective surveillance method for influenza virus circulation. As part of our surveillance, we collected specimens from fecal droppings on the ground in the markets. However, we did not detect influenza A viruses in any of the specimens. One study in Hong Kong detected AIVs in up to 1% of fecal swab specimens.[11] The reason for lack of detection in the environmental specimens is not clear, but we suspect that the high environmental temperatures in Kenya may limit the survival of any virus shed in feces by the birds. Reduced viability of several AIV subtypes has been shown to be associated with increases in temperature.[24–26] The presence of rt RT-PCR inhibitors could also have limited influenza A viral RNA detection in these samples.[22]

Our surveillance was subject to certain limitations. We did not carry out subtyping of the influenza A specimens or virus isolation; hence, we are not able to report influenza subtypes from the birds sampled. We used rt RT-PCR for screening of the specimens for influenza A virus to determine positivity. Although this method has high sensitivity and specificity for detection of type A influenza matrix gene, we may have missed some infections; in one study, virus isolation in embryonated chicken eggs was found to detect an additional 2·3% of specimens that were negative by rt RT-PCR.[27] The authors attributed the reduced sensitivity of rt RT-PCR in part to the presence of rt RT-PCR inhibitory substances in the samples and the less volume used in rt RT-PCR assays compared with virus isolation.[27] Additionally, virological studies only establish the prevalence of active infections. Serology testing would have provided more information about the extent of previous exposure at the farms and markets. However, in our case, we sampled poultry in the market destined for sale, and bleeding of the birds would have been undesirable for the traders.

Our results show that influenza A viruses circulate regularly in LBMs in Kenya. Continued monitoring of influenza viruses in poultry in LBMs would help in detecting new introductions of AIVs in the poultry population that would be of public health and socioeconomic significance to the poultry industry in the country. Early detection of new potentially dangerous influenza viruses could lead to early application of control measures that could minimize the public health impact of outbreaks of HPAI viruses and decrease the impact on the livelihoods along the poultry value chain.

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Addendum

P. Munyua contributed to the overall design of the study, coordinated the field work, analyzed the data, and wrote the manuscript. L. Waiboci and L. Mwasi were responsible for all the RNA extractions and testing and reviewed the manuscript. J. Githinji, L. Njagi, R. Murithi and J. Macharia were responsible for the field work, approval for the surveillance work, and reviewed the manuscript. G. Arunga coordinated the data entry, analyzed the data, and reviewed the manuscript. R. Breiman, K. Njenga, M. Katz contributed to the overall design of the study and reviewed the manuscript.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official

position of the US Centers for Diseases Control and Prevention.

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