Full Length Research Paper

Poor biosecurity in live bird markets in Uganda: A potential risk for highly pathogenic avian influenza disease outbreak in poultry and spread to humans

Halid Kirunda^{1*}, Hannah Kibuuka^{2†}, Achilles Byaruhanga^{3†}, Edison Mworozi^{4†}, Josephine Bwogi^{5†}, Lukwago Luswa^{6†}, Millard Millard^{2†}, Fred Wabwire-Mangen⁷ and Denis K Byarugaba^{8*}

¹National Livestock Resources Research Institute, P.O Box 96, Tororo, Uganda.
²Makerere University Walter Reed Project, P.O. Box 16524, Kampala, Uganda.
³NatureUganda, The East Africa Natural History Society, P.O. Box 27034, Kampala, Uganda.
⁴Mulago National Referral Hospital, Department of Paediatrics and Child Health, College of Health Sciences, Makerere University, P.O. Box 7072,Kampala, Uganda.
⁵Uganda Virus Research Institute, Ministry of Health, P.O. Box 49, Entebbe, Uganda.
⁶Epidemiology and Surveillance Division,Ministry of Health, P.O. Box 7272, Kampala, Uganda.
⁷School of Public Health, College of Health Sciences, Makerere University, P.O. Box 7072, Kampala, Uganda.
College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, P.O. Box 7062, Kampala, Uganda.

Accepted 2, June 2014

Live bird markets (LBMs) are essential for marketing of poultry, but can be a hub for the rapid spread of diseases including avian influenza (Al). We assessed the status of biosecurity in 108 LBMs in 37 districts of Uganda. In all LBMs, carcasses were disposed of in the open and birds were introduced in the markets without initial quarantine. A high proportion of markets lacked a dedicated site for unloading of birds (86.1%) and a programme for disinfection (99.1%), had dirty feed/water troughs (93.5%), were accessed by stray animals (97.2%), and had sick and healthy birds (96.3%) or different bird species (86.1%) sold together. Differences in practices occurred among geographical regions and market location. Birds were more likely to be slaughtered in the open in urban compared to rural LBMs (OR=14.6, 95% CI: 1.50 - 142), while selling of un-caged birds was less likely in central compared to western region (OR=0.2, 95% CI: 0.04 - 0.17). Different poultry species confined in the same cage were more likely to be sold in urban (OR=22, 95% CI: 1.14 - 435) compared to rural markets. We conclude that LBMs in Uganda are a potential risk for spread of Al to poultry and humans.

Key words: Biosecurity, live bird markets, avian influenza, environmental hygiene, management practices, segregation measures, traffic control measures.

INTRODUCTION

Poultry has been associated with introduction and spread of avian influenza (AI) viruses in Asia, Europe and Africa (Kung et al., 2007), while the wide genetic diversity and the potential for recombination with human influenza strains continue to pose major animal biosecurity and public health concerns (Li et al., 2010; Fournié et al., 2011).

*Corresponding authors. E-mail: halidkirunda@gmail.com

Live bird markets (LBMs) are essential for marketing poultry in different countries including Uganda, however Al surveillance programmes in several countries in Asia and Africa have demonstrated that Al viruses circulate in LBMs (FAO, 2013). Kirunda et al. (2014) recently detected influenza A viruses in chickens, ducks, turkeys and swine in LBMs in Uganda. These viruses have been detected in waterfowl (Kung et al., 2003; Gaidet et al., 2007) and domestic birds and pigs in several other countries in Africa (Couacy-Hymann et al., 2012; Ma et al., 2008). LBMs have been reported to serve both as key mixing points and sources of disease spread in animals and humans (Kung et al., 2003; Wan et al., 2011). Slaughter and poor disposal of carcasses of birds in markets have particularly been associated with contamination of market environment with influenza viruses (Indriani et al., 2010). In a study by Otim and colleagues (2007), LBMs in Uganda were reported to be positively associated with risk of spread of the closely related Newcastle disease virus. There is also risk of spread of AI to other countries as birds usually sourced from these markets can be moved between Uganda and its neighboring countries (Yongolo et al., 2011).

Implementation of biosecurity measures and husbandry practices to prevent the introduction, replication and spread of infectious agents, has often been recommended to reduce the risk of infection or disease in poultry operations (FAO, 2010; Fox, 2012). However, ineffective measures may create conditions that favour silent spread of disease agents within the poultry sector through such operations (Yupiana et al., 2010). Therefore, it is essential to assess the risk and identify factors that can potentially influence introduction and spread of AI viruses along the production-marketing chain in countries that are at risk of AI (FAO, 2010).

While it is advisable that poultry dealers apply biosecurity measures in LBMs in order to prevent possible outbreaks of AI (FAO, 2010, Wang et al., 2006), information about the application of such measures and practices in LBMs in Uganda is largely unknown. This study was undertaken to assess the existence and application of biosecurity measures in LBMs in Uganda with specific relevance to the risk of outbreaks of AI in the country.

Materials and methods

Study area

The study was conducted in 37 of the 112 districts of Uganda. Based on the national livestock census (UBOS/MAAIF, 2009), 21 of the study districts were among the main poultry keeping districts of Uganda while the rest were in the medium and low categories. A total of 108 LBMs, including seven markets in which positive samples for influenza A viruses in poultry and swine had been reported (Kirunda et al., 2014), were surveyed. Of the 37 study districts, five were located in the central region, while 9, 11, 12 districts were in Eastern, Northern and Western regions, respectively. Most of the study LBMs were from rural areas.

Study Design

This was a cross-sectional survey conducted during September – December 2009. The survey assessed the

status of 24 biosecurity measures and practices (Table 1) grouped into four categories to include: environmental hygiene, segregation, traffic control and management practices. The assessment was made in relation to the potential risk each of the factors could pose in the introduction and/or spread of influenza viruses in LBMs during disease outbreak.

Data Tool and Data Collection

Data was collected using a structured questionnaire and an observational check list. Respondents were poultry trade market leaders in each of the study markets. The independent variables were the market locality (rural or urban), region of operation (central, eastern, northern or western) and the frequency of market operation (daily or weekly). The dependent variables are shown in Table 1. During the study, all surveyed markets were visited only once.

Data Management and Analysis

Quantitative data was entered in *EpiData* and analyzed using *SPSS* statistical programs. Analysis was done at univariate level for frequencies/proportions and at bivariate level to determine existence of significant statistical relationships (Chi-square, χ^2) between predictor and outcome variables. At multivariate level of analysis, a binary logistic regression model was run to examine the level of association between the independent variables and exposure to AI viruses (dependent variable) by computing for odds ratios (ORs), significant at p<0.05.

RESULTS

Distribution of Surveyed Live Bird Markets

Markets surveyed were distributed as; 38 in western region, 33 in northern, 24 in central and 13 in eastern. Majority of the surveyed LBMs (70/108, 64.8%) were located in rural areas. Most of the rural LBMs were in northern region (41.1%, 29/70), while central region had the largest proportion of urban markets (44.7%, 17/38). Some of the markets (57.4%, 62/108) operated only once a week, while others (42.6%, 46/108,) operated on a daily basis. Markets operating daily were mainly in the central region, while those operating once a week were mainly in the Western region (Table 2).

Environmental Hygiene in Live Bird Markets

All surveyed LBMs (100%) regardless of region, frequency of operation or location, had two factors pre-

Category	Dependant variables (factor)	Proportion	of	Factors associated with	
		LBMs (%)		Introduction	Spread
Environmental	Slaughter of birds in open sites (N=108)	50.0		+	+
hygiene	Open disposal of carcasses (N=108)	100.0		+	+
	Open disposal of offal (N=108)	100.0		+	+
	Absence of unloading of birds (N=108)	86.1		+	+
	No programmed disinfection of site (N=108)	99.1			+
Segregation	No booth(s) used (N=108)	82.4			+
	No cages for confining of birds (<i>N=108</i>)	69.4			+
	Metallic/plastic cages (<i>N</i> =33)	48.4			+
	Wooden cages (N=33)	51.6			+
	Stacked cages (N=33)	27.3			+
	Birds sold from the ground (N=108)	85.2		+	+
	Birds sold held in hands (N=108)	69.4			+
	Newly introduced birds never quarantined	100.0		+	+
	Sick/healthy birds not separated (N=108)	96.3		+	+
	Different bird species sold together (N=108)	86.1		+	+
Traffic control	Market located in a larger market (N=108)	26.9			+
	Market not fenced (N=108)	86.2			+
	Market uses only one gate (<i>N=108</i>)	96.3			+
	Market is congestion (N=108)	78.7			+
Management	Lack of record keeping (N=108)	95.4			+
practices	Dirty feed and water troughs (N=31)	93.5			+
	Feed stored in open/dirty containers (N=31)	96.8			+
	Stray animals easily access market (N=108)	97.2		+	+
	Other livestock are sold with poultry (N=108)	38.0		+	+

Table 1. Factors affecting biosecurity to avian influenza in live bird markets in Uganda

Key: LBMs=Live bird markets; Introduction (+)=factor has previously been associated with introduction of avian influenza; Spread (+)=factor has previously been associated with spread of avian influenza

Region	Location of mar	ket	Frequency of operation		
	Rural (N=70)	Urban (N=38)	Daily (N=46)	Weekly (N=62)	
Central	10.0%	44.7%	37.0%	11.3%	
Eastern	0.9%	18.5%	15.2%	9.7%	
Northern	41.4%	10.5%	23.9%	35.5%	
Western	40.0%	26.3%	23.9%	43.5%	
Total:	100.0%	100.0%	100.0%	100.0%	

Table 2. Proportions of surveyed markets by region, location and frequency of operation

viously been associated with the risk of introduction and spread of AI, namely; open disposal of carcases and open disposal of offals (Table 1). Other risk practices in majority of markets were non-programmed disinfection of sites (99.1%), and absence of a dedicated area for unloading of birds (86.1%). The largest proportion of markets without dedicated unloading areas were those operated on a daily basis (69.6%, 32/46) and in urban areas (92.1%, 35/38). Although no significant relationship existed between availability of dedicated unloading areas and any of the variables, LBMs in urban areas were three times more likely not to have designated unloading areas (OR=3.0, 95% CI: 0.26 -33.1) compared to markets in the rural setting (data not provided in Table). Slaughter of birds in the open occurred in only 50% of markets. The practice was almost in equal proportions among daily (58.7%, 27/46) and weekly markets (59.7%, 37/62). It was more practiced in urban (78.9%, 30/38) than rural LBMs (34.3%, 24/70). Among regions, slaughter of birds in the open was mainly practiced in eastern (84.6%, 11/13), followed by central (54.2%, 13/24) and western Uganda (50.0%, 19/38). The practice was relatively low (27.3%, 9/33) in markets in northern parts of the country. Binary logistic regression analysis revealed a significant $(\chi^2 = 24.1)$ relationship between location and slaughter/processing of birds in the open (Table 3). Urban LBMs were more likely to have the practice (OR=14.6, 95% CI: 1.50 - 142) compared to rural markets (Table 4).

Table 3. Proportions of LBMs with factor	s affecting biosecurity in Uganda by f	requency of operation, location or region
--	--	---

Biosecurity factors	Frequency of operation (%)		Location (%)		Region of operation (%)			
	Daily	Weekly	Rural	Urban	Central	Eastern	Northern	Western
Slaughter of birds	58.7 (27/46	59.7, 37/62	34.3 (24/70)	73.7 (28/38)	54.2 (13/24	84.6 (11/13	27.3 (9/33	50.0 (19/38
Open carcass disposal	100 (46/46)	100 (62/62)	100 (70/70)	100 (38/38)	100 (24/24)	100 (13/13)	100 (33/33)	100 (38/38)
Open disposal of offal	100 (46/46)	100 (62/62)	100 (70/70)	100 (38/38)	100 (24/24)	100 (13/13)	100 (33/33)	100 (38/38)
Lack unloading sites	69.6 (32/46)	98.4 (61/62)	82.3 (58/70)	92.1 (35/38)	65.5 (19/24)	61.5 (8/13)	90.9 (30/33)	94.7 (36/38)
No disinfection	97.8 (45/46)	100 (62/62)	100 (70/70)	97.4 (37/38)	100 (24/24)	100 (13/13)	100 (33/33)	97.4 (37/38)
No booths used	58.7 (27/46)	100 (62/62)	100 (70/70)	71.1 (27/38)	66.7 (16/24)	76.9 (10/13)	90.9 (30/33)	86.8 (33/38)
No cages used	30.4 (14/46)	98.4 (61/62)	82.9 (58/70)	44.7 (17/38)	33.3 (8/24)	38.5 (5/13)	97.0 (32/33)	78.9 (30/38)
Metallic cages	75.0 (24/32)	0.0 (0/0)	0.0 (0/1)	75.0 (24/32)	95.5 (21/22)	50.0 (2/4)	0.0 (0/1)	33.3 (2/6)
Wooden cages	25.0 (8/32)	100 (1/1)	100 (1/1)	25.0 (8/32)	4.5 (1/22)	50.0 (2/4)	100 (1/1)	66.7 (4/6)
Cages stacked	27.3 (9/33)	0.0 (0/0)	0.0 (0/0)	27.3 (9/33)	20.0 (6/30)	0.0 (0/1)	0.0 (0/1)	100 (1/1)
Birds retrained on grounds	43.5 (20/46)	93.5 (58/62)	74.3 (52/70)	55.3 (21/38)	37.5 (9/24)	76.9 (10/13)	81.8 (27/33)	84.2 (32/33)
Birds held in hands, while being sold	17.4 (8/46)	67.7 (42/62)	68.3 (41/70)	6.3 (3/38)	16.7 (4/24)	46.2 (6/13)	69.7 (23/33)	44.7 (17/38)
Lack of quarantine	100 (46/46)	100 (62/62)	100 (70/70)	100 (38/38)	100 (24/24)	100 (13/13)	100 (33/33)	100 (38/38)
Sick/healthy together	91.3 (42/46)	100 (62/62)	98.6 (69/70)	92.1 (35/38)	87.5 (21/24)	100 (13/13)	100 (33/33)	97.4 (37/46)
Different species sold together	73.9 (34/46)	95.2 (59/62)	85.7 (60/70)	86.8 (33/38)	87.5 (21/24)	84.6 (11/13)	78.8 (26/33)	92.1 (35/38)
Located a larger market	60.9 (28/46)	1.6 (1/62)	1.4 (1/70)	73.7 (28/38)	54.2 (13/24)	53.8 (7/13)	12.1 (4/33)	13.2 (5/38)
Market not fenced	91.3 (42/46)	100 (62/62)	98.6 (69/70)	92.1 (35/38)	91.7 (22/24)	92.3 (12/13)	100 33/33)	97.4 (37/38)
Only one gate used	6.5 (3/46)	0.0 (0/62)	0.0 (0/70)	7.9 (3/38)	12.5 (3/24)	0.0 (0/13)	0.0 (0/33)	2.6 (1/38)
Market is congested	65.2 (30/46)	88.7 (55/62)	81.4 (57/70)	73.7 (28/38)	83.3 (20/24)	100 (13/13)	66.7 (22/33)	78.9 (30/38)
No records kept	89.1 (41/46)	100 (62/62)	100 (70/70)	84.2 (32/38)	79.2 (19/24)	100 (13/13)	100 (33/33)	100 (38/38)
Dirty troughs	78.1 (25/32)	100 (9/9)	100 (10/10)	77.4 (24/31)	68.8 (11/16)	100 (7/7)	87.5 (7/8)	90.0 (9/10)
Feed in open containers	97.3 (36/37)	100 (7/7)	100 (9/9)	97.1 (34/35)	100 (17/17)	100 (9/9)	100 (8/8)	90.0 (9/10)
Access by stray animals	100 (46/46)	100 (62/62)	100 (70/70)	100 (38/38)	100 (24/24)	100 (13/13)	100 (33/33)	100 (38/38)
Other livestock sold	6.5 (3/46)	61.3 (38/62)	54.3 (38/70)	7.9 (3/38)	16.7 (4/24)	61.5 (8/13)	39.4 (13/33)	42.1 (16/38)

Key: diff=different; Biosecurity factors=facilities and practices that influence introduction/spread of influenza viruses.

All these biosecurity anomalies occurred despite the observation that all daily LBMs operated throughout the year without a single break.

Segregation Measures in Live Bird Markets

The study observed that in all LBMs, new birds were never quarantined before

being introduced in the market and in almost all markets (104/108, 96.3%), regarless of frequency or region of operation or location, there were no special cages for isolating sick birds from the healthy ones(Tables 1 and 3). Booths were missing in 82.4% (89/108) of the markets (Table 1). They were absent in all (100.0%) weekly and rural markets, and in up to 30/33 (90.9%) of markets in northern region (Table 3). The distance between booths was less than three meters in 94.7% (18/19) of the LBMs (results not shown). Most of

Variable	Odds ratio	95% Confidence Interval (CI)	p-value				
Slaughter of birds from open areas in markets; - 2 Log likelihood – 125.50, χ^2 =24.08, df=5, p=0.000							
Location							
LUCAUON	44.0	4 50 440	0.004*				
Orban Design of energy (inn	14.0	1.50 - 142	0.021				
Region of operation		o 40 o 07	o . .				
Central	0.6	0.19 - 2.07	0.447				
Eastern	4.2	0.78 – 22.7	0.096				
Northern	0.5	0.18 – 1.46	0.208				
Birds sold un-caged - 2 Log likelih	nood – 83.597, χ ² =44.0	03, df=5, p=0.000					
Location							
Urban	2.4	0.39 – 14.5	0.349				
Region of operation							
Central	0.2	0.04 – 0.17	0.015*				
Eastern	1.0	0.17 – 5.92	0.988				
Northern	1.3	0.26 – 6.31	0.769				
Different species of birds sold together: - 2 Log likelihood – 62.455, y^2 =21.43, df=5, p=0.000							
Location			-				
Urban	22	1.14 - 435	0.041*				
Region of operation							
Central	0.9	0.16 - 5.25	0.911				
Eastern	6.6	0.97 - 45.3	0.053				
Northern	3.0	0.39 - 22.5	0.295				
Other livestock sold alongside poultry; - 2 Log likelihood – 94.198, χ^2 =49.20, df=5, p=0.000							
Location							
Urban	0.4	0.02 - 7.74	0.546				
Region of operation							
Central	0.8	0.16 - 3.34	0.783				
Eastern	25	1.77 - 344	0.017*				
Northern	0.93	0.30 - 2.89	0.905				

Table 4. Predictor variables associated with poor biosecurity in live bird markets in Uganda

Note: All Reference category not presented; Key: *=significant at p<0.05

the LBMs (69.4%, 75/108) never used cages to confine birds. Results in Table 3 show that cages were absent in 61/62 (98.4%) of the weekly markets, 58/60 (96.7%) of the rural markets and in 32/33 (97.0%) of markets in northern region. In a few of the markets where cages existed (8/31), the cages were stacked and mostly lined with some material. Cages were made of wooden or metallic/plastic materials.

More than three quarters of the LBMs (85.2%, 92/108) sold birds in the open, un-caged and just restrained on the ground (Table 2). This was practiced in 93.5% (58/62), 74.3% (52/70) and 84.2%,(32/33) in weekly, rural and western region markets, respectively (Table 3). Region of operation was a predictor (χ^2 =44.0) for the practice of selling birds uncaged and in the open. Central region was significantly less likely to practice this vice (OR=0.2, 95% CI: 0.04 - 0.17) compared to western region (Table 4). In 69.4% (75/108) of the study markets, birds were just held in hands while being sold. This was mostly practiced in weekly operated markets, rural areas and in the northern region (Table 3).

A high proportion of markets sold different poultry species confined together. This occurred mostly in weekly operated LBMs (95.2%, 59/62), among urban markets (86.8%, 33/38), and in western region (92.1%, 35/38). This practice was significantly associated with location (χ^2 =21.4), with urban LBMs being 22 times more likely to sell different poultry species confined together compared to rural ones (OR=22,

95% CI: 1.14 – 435). The species sold together comprised chickens, ducks, turkeys and helmeted guinea fowl.

Traffic Control Measures in Live Bird Markets

Majority of LBMs were not fenced (86.2%) and were congested (78.7%) (Table 1). Only 26.9% of the LBMs were located within the general merchandise markets. Table 3 shows the proportions of each of the four biosecuirty factors under the traffic control category among markets of different frequency of operation, region and location. There was a strong relationship (χ^2 =78.1) between location of LBMs and existence of LBM within larger general merchandise market. Urban LBMs were more likely to be situated within larger municipal markets (OR=15.3, 95% CI: 4.10 - 57.6) compared to markets in rural areas.

Management Practices in Live Bird Markets

It was observed that most of the variables categorized under management practices varied. In all (100.0%) study LBMs, traders bought birds from unreliable sources, never kept records and LBM lacked a programme for rodent control. Additionally, it was observed that one or more of the other livestock species including goats, cattle and pigs were sold alongside poultry. This occurred in 41/108 (38.0%) of the markets (Table 1). A strong relationship (χ^2 =49.2) existed between region and the practice of selling other livestock species alongside poultry. LBMs in eastern region were more likely to be involved in selling other livestock species alongside poultry (OR=25, 95% CI: 1.18 - 344) compared to those in western region. In most of the markets, water and feed troughs were soiled with faeces, feeds were mostly stored in open containers and stray animals easily accessed the markets (Table 1). The proportions of markets involved in such anomalous biosecurity measures and practices were quite high (68.8% - 100%) regardless of location, frequency of operation or region (Table 3).

DISCUSSION

Study variables either singly or in combination demonstrated poor sanitation in the Ugandan LBMs. This is characteristic of poor biosecurity. Study findings suggest that LBMs in Uganda could be potential sources of infectious disease agents including AI viruses. Slaughter of birds in the open and open disposal of carcasses in the study markets could easily compromise biosecurity as infection can spread to the sale and waste disposal zones during the process of evisceration and disposal of carcasses (Indriani et al., 2010). Poor disposal of carcass is not peculiar to Uganda as it has been reported in other developing countries such as Thailand (Kilpatrick et al., 2006). This is in contrast to other AI outbreak risk prone countries in the Western Pacific Region where proper disposal and other good sanitary practices have become regular phenomena in LBMs (Yee et al., 2008).

Previous studies have particularly demonstrated that AI virus may persist in the LBM environment for weeks (Vong et al., 2008) and LBMs are a known source of these viruses, including source for human infection (Cardona et al., 2009; Santhia et al., 2009; Wan et al., 2011). Un-programmed disinfection in Ugandan markets reported in this study therefore increases the potential for reservoir and spread of AI infections. Although inadequately practiced in Uganda, regular cleaning and disinfection of markets have been reported to be effective at eliminating influenza viruses from environmental surfaces in LBMs (Kilpatrick et al., 2006; Yee et al., 2008) and minimize the reservoir of these viruses in such markets (Kung et al., 2003). This reduces the risk of introduction and spread of these agents (FAO, 2010; Kung et al., 2003).

Majority of study markets sold birds un-caged and confined on the ground. Bird excreta in such markets tend to be littered everywhere and can easily be a source of infection to other birds and humans. Moreover, Songserm and others (2006) demonstrated that influenza viruses including H5N1 subtype survive for 4 to 23 days in wet chicken manure and is easily transmitted through movement of humans and birds (Dorea et al., 2010; FAO, 2007; Nishiguchi et al., 2007). Existence of this practice in markets also contradicts the requirement of prevention of environmental contamination with disease agents (FAO, 2008). In general, ignorance of poultry traders about associated consequences of such actions and inadequate regulation could probably be the reasons for improper environmental hygiene. Poor sanitation in LBMs can hinder national and global efforts aimed at prevention of spread of infectious agents such as influenza viruses (FAO, 2010).

While segregation limits spread of disease pathogens (Fox, 2012), authorities and traders in surveyed LBMs either ignored or were ignorant of most of the recommended measures and practices. This was partly exhibited by the low usage of booths and cages. Lack of cages and booths is regarded a sign of poor biosecurity in LBMs (FAO, 2010) and separating booths and cages by less than one metre increases risk of airborne among birds confined in such facilities transmission (Spekreijse et al., 2011). Continued use of wooden cages, despite the requirement for the easily cleaned plastic or metallic material cages (FAO, 2010) further reduced the level of biosecurity in many of the surveyed markets. Stacked cages missing lining material to prevent cross-contamination encourages spread of disease agents between infected and healthy birds. This irregularity contradicts the World Health Organization recommendation (WHO, 2004) and related studies (Indriani et al., 2010).

In all markets, observation of bio-exclusion measures including quarantining of newly introduced birds and separating of sick from health birds as a requirement for adequate biosecurity in LBMs (FAO, 2008) was disregarded. Poor biosecurity was also exhibited by confining chickens together with other bird species such as ducks, increasing the likelihood of exposing susceptible chickens to ducks that are reported to harbour, yet remain asymptomatic to Al (Nguyen et al., 2005; Gilbert et al., 2006). These biosecurity omissions have also been recently reported in Nigeria (Pagani et al., 2008) and Indonesia (Santhia et al., 2009).

Although key world agriculture and health organizations (FAO/OIE/WHO, 2005) have recommended fencing of markets as a risk reduction measure to protect humans from Al infections, almost all surveyed markets were not fenced off from the rest of the general merchandise markets. The markets also lacked specific entrance and exit doors and only a small proportion had dedicated unloading sites contrary to general biosecurity recommendations (FAO/OIE/WHO, 2005). Isolation anomalies of this category easily expose the general public to risk of infection with AI viruses (Bulaga et al., 2003). It has previously been reported that transmission of these viruses can easily be facilitated by human-mediated transport for a distance of up to 0.8 - 13 Km from site of outbreak (Bataille et al., 2011).

Although LBMs are expected to serve as key active monitoring points for early disease and/or pathogen detection (FAO, 2010), almost all surveyed markets did not keep records. Equally, birds in all surveyed markets were from sources without records. These major lapses in biosecurity provide a missed opportunity of disease control at source (FAO/OIE/WHO, 2005). These omissions have been observed in earlier studies (Santhia et al., 2009; Webster et al., 2005) and are a contradiction to practices in other developing countries (Pagani et al., 2008). Lack of records not only makes traceability of birds along the production-marketing chain impossible, but also makes enforcement of legal requirements difficult. Traceability and compliance with legal disease control measures are critical components of appropriate biosecurity in LBMs (FAO, 2008). Disregarding record keeping also faults the opportunity of using such a measure in reducing the risk of receiving and maintaining infected birds into the market (Senne et al., 2003).

In most of the markets, water and feed troughs were soiled with faeces and feed was stored in open containers. These poor practices could easily facilitate disease transmission in case of faecal contamination from birds infected with influenza viruses. Existing literature indicates that contaminated faeces represent a significant mechanism for exposure of health birds to infection with influenza viruses (EFSA, 2006). Absence of rodent control programmes and easy access to stray animals in all the LBMs equally compromised biosecurity. Rats and mice are known carriers of at least 35 diseases, and constitute major carriers and reservoirs of poultry pathogens, including influenza virus (Adams, 2003). Maas et al. (2007) also reported that dogs may contribute to influenza virus spread. Although pet animals carry virus only over short distance (EFSA, 2006; Hop and Saatkamp, 2010) and that the effect of dryness and solar radiation inactivates influenza viruses, the risk of transmission of these viruses by such animals may remain a possibility.

While literature shows that limiting the number species of livestock sold in a market reduces the persistence of infection in LBMs (FAO, 2010), different species of animals including cattle, goats, sheep and swine were sold alongside poultry in some of the markets. The observation is common in many other African countries (Pagani et al., 2008).

The study results provides evidence that LBMs in Uganda are still far from achieving complete biosecurity, as described by WHO (2004). Whereas no single set of measures has ever completely prevented influenza viruses from occurring and circulating in markets in outbreak countries, there has been a significant decrease in circulation of these viruses in markets where biosecurity measures and practices have been applied (FAO, 2010). Failure to implement proper biosecurity measures in LBMs in Uganda therefore poses a risk of infection and spread of AI viruses to poultry, other livestock species, traders and other humans operating in and around the LBMs. There is need for regular surveillance of these viruses and development of strategies to improve biosecurity in the LBMs in the country. Studies to determine risk factors for spread of AI viruses among poultry handlers in these markets could be beneficial in providing additional information that is essential in development of prevention and control strategies.

ACKNOWLEDGMENTS

This work was supported by the US Department of Defense's Global Emerging Infections Surveillance and Response System (DoD-GEIS) through Contract No. W81XWH-06-C-0414 to Makerere University Walter Reed Project. We thank the district local authorities for assisting during the data collection and the respondents for their time.

REFERENCES

- Adams J (2003). Vector: Rats and Mice. In CAMM Poultry, Chap 10c. pp. 1-5. http://www.clemson.edu/extension/livestock/camm/cam m_files/dairy/dch10c_04.pdf
- Bataille A, van der Meer F, Stegeman A, Koch G (2011). Evolutionary Analysis of Inter-Farm Transmission Dynamics in a Highly Pathogenic Avian Influenza Epidemic. PLoS Pathog 7(6): e1002094.
- Bulaga LL, Garber L, Senne DA, Myers TJ, Good R, Wainwright S, Trock S, Suarez DL (2003). Epidemiologic and surveillance studies on avian influenza in live-bird markets in New York and New Jersey, 2001. Avian Dis 47(Suppl): 996-1001.
- Cardona C, Yee K, Carpenter T (2009). Are live bird markets reservoirs of avian influenza? Poult Sci 88(4): 856-9.
- Couacy-Hymann E, Kouakou VA, Aplogan GL, Awoume F, Kouakou CK, Kakpo L, Sharp BR, McClenaghan L, McKenzie P, Webster RG, Webby RJ, Ducatez MF (2012). Surveillance for influenza viruses in poultry and swine, west Africa, 2006-2008. Emerg. Infect. Dis. 18: 1446-52.
- Dorea FC, Berghaus R, Hofacre C, Cole DJ (2010). Survey of Biosecurity Protocols and Practices Adopted by Growers on Commercial Poultry Farms in Georgia, U.S.A. Avian Dis 54: 1007-1015.
- EFSA (2006). Scientific Report on Migratory Birds and their Possible Role in the Spread of Highly Pathogenic Avian Influenza. p. 19.
- FAO (2007). The importance of biosecurity in reducing HPAI risk on farms and in markets. In International Ministerial Conference on Avian and Pandemic Influenza, 4–6 December. New Delhi.
- FAO (2008). Biosecurity for HPAI: Issues and Options. Rome
- FAO (2010). Good practices for biosecurity in the pig sector Issues and options in developing and transition countries. Rome.
- FAO (2013). Mapping Influenza A (H5N1) virus transmission pathways and critical control points in

Egypt. Animal Production and Health Working Paper No. 11. Rome.

FAO/OIE/WHO (2005). Consultation on avian influenza and human health: risk reduction measures in producing, marketing, and living with animals in Asia. In Renaissance Hotel, Kuala Lumpur, Malaysia, 4-6 July 2005.

- Fournié G, de Glanville WA, Pfeiffer DU (2011). Epidemiology of highly pathogenic avian influenza virus strain type H5N1. Health and Animal Agriculture in Developing Countries, eds Zilberman D, Otte J, Roland-Holst D, Pfeiffer D, editors. New York, Springer.
- Fox JQ (2012). Biosecurity in Avian Influenza Control. Retrieved from http://en.engormix.com/MA-poultryindustry/health/articles/biosecurity-avian-influenzacontrol-t2402/165-p0.htm
- Gaidet N, Dodman T, Caron A, Balança G, Desvaux S, Goutard F, Cattoli G, Lamarque F, Hagemeijer W, Monicat F (2007). Avian Influenza Viruses in Water Birds, Africa. Emerg. Infect. Dis. 13: 626-629.
- Gilbert M, Chaitaweesub P, Parakarnawongsa T, Premashthira S, Tiensin T, Kalpravidh W, Wagner H, Slingenbergh J (2006). Free-grazing ducks and highly pathogenic avian influenza, Thailand. Emerg. Infect. Dis. 12: 227-234.

Hop GE, Saatkamp HW (2010). A Pathway diagram for

introduction and prevention of Avian Influenza: Application to the Dutch poultry sector. Prev. Vet. Med. 97: 270-273.

- Indriani R, Samaan G, Gultom A, Loth L, Irianti S, Indryani S, Adjid S, Dharmayanti R, Weaver NLPI, Mumford J, Lokuge E, Kelly K, Darminto MP (2010). Environmental sampling for avian influenza virus A (H5N1) in live-bird markets, Indonesia. Emerg. Infect. Dis. 16(12): 1889-95.
- Kilpatrick AM, Chmura AA, Gibbons DW, Fleischer RC, Marra PP, Daszak P (2006). Predicting the global spread of H5N1 avian influenza. Proceedings of the National Academy of Sciences of the United States of America 103(51): 19368-73.
- Kirunda H, Erima B, Tumushabe A, Kiconco J, Tugume T, Mulei S, Mimbe D, Mworozi E, Bwogi J, Luswa L, Kibuuka H, Millard M, Byaruhanga A, Ducatez MF, Krauss S, Webby RJ, Webster RG, Wurapa K, Byarugaba DK, Wabwire-Mangen F (2014). Prevalence of influenza A viruses in livestock and free-living waterfowl in Uganda. BMC Vet Res 10(1): 50.
- Kung NY, Guan Y, Perkins NR, Bissett L, Ellis T, Sims L, Morris RS, Shortridge KF, Peiris JS (2003). The impact of a monthly rest day on avian influenza virus isolation rates in retail live poultry markets in Hong Kong. Avian Dis 47(Suppl. 3): 1037-1041.
- Kung NY, Morris RS, Perkins NR, Sims LD, Ellis TM, Bissett L, Chow M, Shortridge KF, Guan Y, Peiris MJS (2007). Risk for infection with highly pathogenic influenza virus A (H5N1) in chickens, Hong Kong, 2002. Emerg. Infect. Dis. (13): 412-8.
- Li C, Hattaa M, Nidomb CA, Muramotod Y, Watanabea S, Neumanna G, Kawaokaa Y (2010). Reassortment between avian H5N1 and human H3N2 influenza viruses creates hybrid viruses with substantial

virulence. Proc. Natl. Acad. Sci. USA 107(10): 4687-4692.

- Ma W,Kahn RE, Richt JA (2008). The pig as a mixing vessel for influenza viruses: Human and veterinary implications. J. Mol. Genet. Med., 3(1): 158-166.
- Maas R, Tacken M, Ruuls L, Koch G, van Rooij E, Stockhofe-Zurwieden N (2007). Avian Influenza (H5N1) Susceptibility and Receptors in Dogs. Emerg. Infect. Dis. 13(8): 1219-1221.
- Nguyen DĆ, Uyeki TM, Jadhao S, Maines T, Shaw M, Matsuoka Y, Smith C, Rowe T, Lu X, Hall H, Xu X, Balish A, Klimov A, Tumpey TM, Swayne DE, Huynh LP, Nghiem HK, Nguyen HH, Hoang LT, Cox NJ, Katz JM (2005).Isolation and characterization of avian influenza viruses, including highly pathogenic H5N1, from poultry in live bird markets in Hanoi, Vietnam, in 2001. J. Virol. 79(7): 4201–4212.
- Nishiguchi A, Kobayashi S, Yamamoto T, Ouchi Y, Sugizaki T, Tsutsui T (2007). Risk factors for the introduction of avian influenza virus into commercial layer chicken farms during the outbreaks caused by a low-pathogenic H5N2 virus in Japan in 2005. Zoonoses Public Health 54(9-10): 337-43.
- Otim MO, Kabagambe EK, Mukiibi GM, Christensen H, Bisgaard M (2007). A study of risk factors associated with Newcastle disease epidemics in village free-range chickens in Uganda. Trop. Anim. Health Pro 39(1): 27-35.
- Pagani P, Abimiku JEY, Emeka-Okolie W(2008). Assessment of the Nigerian poultry market chain to improve biosecurity. Rome.
- Santhia K, Ramy A, Jayaningsih P, Samaan G, Putra AAG, Dibia N, Sulaimin C, Joni G, Leung CYH, Peiris JSM, Wandra T, Kandun N (2009). Avian influenza A
- H5N1 infections in Bali province, Indonesia: a behavioral, virological and seroepidemiological study. Influenza Other Respir. Viruses 3: 81–89.
- Senne DA, Suarez DL, Pedersen JC, Panigrahy B (2003). Molecular and biological characteristics of H5 and H7 avian influenza viruses in live-bird markets of the northeastern United States, 1994-2001. Avian Dis 47(3 Suppl): 898–904.
- Songserm T, Jam-on R, Sae-Heng N, Meemak N (2006). Survival and stability of HPAI H5N1 in different environments and susceptibility to disinfectants. Dev. Biol. (Basel) 124: 254.
- Spekreijse D, Bouma A, Koch G, Stegeman JA (2011). Airborne transmission of a highly pathogenic avian influenza virus strain H5N1 between groups of chickens quantified in an experimental setting. Vet Microbiol 152(1-2): 88-95.
- UBOS/MAAIF (2009). Livestock numbers, National Data Base. Uganda Buraeu of Statistics and Ministry of Agriculture, Animal Industry and Fisheries, Kampala.
- Vong S, Ly S, Mardy S, Holl D, Buchy P (2008). Environmental contamination during influenza A virus (H5N1) outbreaks, Cambodia, 2006. Emerg Infect Dis 14(8): 1303-1305.
- Wan X, Dong L, Lan Y, Long L, Xu C, Zou S, Li Z, Wen L,

Cai Z, Wang W, Li X, Yuan F, Sui H, Zhang Y, Dong J, Sun S, Gao Y, Wang M, Bai T, Yang L, Li D, Yang W, Yu H, Wang S, Feng Z, Wang Y, Guo Y, Webby RJ, Shu Y (2011). Indications that Live Poultry Markets Are a Major Source of Human H5N1 Influenza Virus Infection in China. J. Virol. 85(24): 13432-13438.

- Wang M, Di B, Zhou D-H, Zheng B-J, Jing H, Lin Y-P, Liu Y-F, Wu X-W, Qin P-Z, Wang Y-L, Jian L-Y, Li X-Z, Xu J-X, Lu E-J, Li T-G, Xu J (2006). Food markets with live birds as source of avian influenza. Emerg. Infect. Dis. 12: 1773-1775.
- Webster RG, Guan Y, Poon L, Krauss S, Webby R, Govorkovai E, Peiris M (2005). The spread of the H5N1 bird flu epidemic in Asia in 2004. Arch Virol 19(Suppl): 117-129.

- WHO (2004). Healthy marketplaces in the Western Pacific: guiding future action. Geneva: The Organization; 2004. Geneva.
- Yee KS, Carpenter ET, Mize S, Cardona CJ (2008). The Live Bird Market System and Low-Pathogenic Avian Influenza Prevention in Southern California, Case Report. Avian Dis., 52: 348-352.
- Yongolo MG, Christensen H, Handberg K, Minga U, Olsen JE (2011). On the origin and diversity of Newcastle disease virus in Tanzania. Ondertepoort J. Vet. Res., 78(1): E1-8.
- Yupiana Y, de Vlas SJ, Adnan NM, Richardus JH (2010).Risk factors of poultry outbreaks and human cases of H5N1 avian influenza virus infection in West Java Province,Indonesia. Int. J. Infect. Dis. 14(9): e800-e805.