SURVEILLANCE IN 2013 OF AVIAN INFLUENZA VIRUS FROM LIVE-BIRD MARKETS IN BANGKOK, THAILAND

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Abstract. Live-bird markets have been implicated in transmission of avian influenza viruses, most recently of influenza A (H7N9) in China. Low pathogenic avian influenza (LPAI) viruses, such as H7N9, cause asymptomatic infections in poultry, and active surveillance is required to detect infection and to prevent transmission to humans. Although limited numbers of live birds for consumption are sold in Bangkok live bird markets (LBM), transmission of H7N9 in nearby China has prompted a program of active surveillance for avian influenza in Bangkok LBM to determine LPAI viruses. In November 2013, Bangkok One Health team organized avian influenza surveillance in all nine districts of Bangkok with LBMs. Oropharyngeal swabs (n = 834), sera (n = 375) and fresh feces (n = 420) were taken from 400 chickens, 20 ducks, 20 geese and 394 pet birds from 75/87 shops. Additionally, drinking water (n = 208) and waste water (n = 26) were collected. Samples were tested for influenza A viruses using RT-PCR. In addition, samples were inoculated in eggs and tested by hemagglutination (HA) and hemagglutination inhibition (HI) assays using H5N1- and H7N9-specific antigens. Sera were tested by HI assay using similar antigens. No sample was found positive for influenza A virus. These data provide evidence that avian influenza viruses, including LPAI viruses such as H7N9, were not circulating in Bangkok LBMs during the period surveyed.

Keywords: avian influenza, H7N9, live-bird market, surveillance, Thailand

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INTRODUCTION

Avian influenza (AI) is caused by avian influenza viruses (AIVs) belonging to genus *Influenza virus* A, family Orthomyxoviridae. There are 18 different known H antigens (H1 - H18) and 11 different known N antigens (N1 - N11) (Tong *et al*, 2013). H5 and H7 viruses can cause highly

pathogenic avian influenza (HPAI), with high morbidity and mortality. Low pathogenic avian influenza (LPAI) can be caused by any HA subtypes (Alexander, 2000). In poultry, AIVs produce symptoms ranging from mild to highly lethal. Poultry infected with LPAI that are asymptomatic or present with mild symptoms, are capable of transmitting avian influenza to humans. For example, H7N9 and H9N2 are LPAI viruses that have been the cause of worldwide outbreaks of AI (Peng *et al*, 2013).

Since March 2013, infections with influenza A (H7N9) virus have caused severe illness in humans in provinces of southeastern China. The China National Health and Family Planning Commission announced that human infections with influenza A (H7N9) virus had occurred in Shanghai and Anhui Provinces (WHO, 2013). In June 2014, 452 human cases of AIV A (H7N9) infection have been confirmed in southeastern China (Feifei *et al*, 2013). Many of the human cases of AIV A H7N9 infection appear to have a link with live poultry markets, places where wild and pet birds and poultries for consumption are sold to household customers (Liu *et al*, 2014). In live poultry markets many different kinds of birds from different sources are densely packed in stacked wire cages, conditions providing an excellent environment for animal to animal and animal to human transmission of influenza viruses, which could lead to an outbreak of HPAI or LPAI in both animals and humans. Thus, live poultry markets are considered a major source of influenza A virus dissemination and a potential opportunity for influenza A virus re-assortment (Wang *et al*, 2006; Chen *et al*, 2014).

Bangkok, the capital of Thailand, is a sprawling area with a population of almost eight million people. In Bangkok there are several large-scale and small-scale live-poultry markets, which have approximately the same type of crowded environment as China's live-poultry markets, leading to close contact between poultry, animals and humans from all over Thailand. People working at these live-poultry markets are at risk of seasonal influenza infection as well as possibly contracting avian influenza viruses (Gilbert *et al*, 2014).

Thus, the aim of this study was to identify AIV A H7N9 and other AIVs in live poultry, environmental samples and live poultry serum in live-bird markets in Bangkok.

MATERIALS AND METHODS

Study sites

Bangkok has nine live-bird markets. Each market has a number of individual shops, which focus on selling live birds for different purposes, *eg*, for consumption, pet and sport. Khlong Toei is the largest market specializing in selling birds for consumption, primarily chickens, ducks and geese; Bang Rak, Samphan Thawong and Rat Burana are smaller-scale markets focused on birds for consumption; and the other five markets focus more on selling birds for pet owners and sport customers (Table 1). For pet owners, birds such as pigeons, doves, love birds, budgerigars, finches, parrots, parakeets, macaws, conures, cockatoo, cockatiels, sparrows, bantams, turkeys, peacocks, swans, and quails are popular. Fighting cocks are raised and sold for sporting competition (Table 2).

Poultry sample collection

The investigation team visited all live-bird markets in the nine districts of Bangkok (Fig 1). We approached each shop in each market and inquired if they

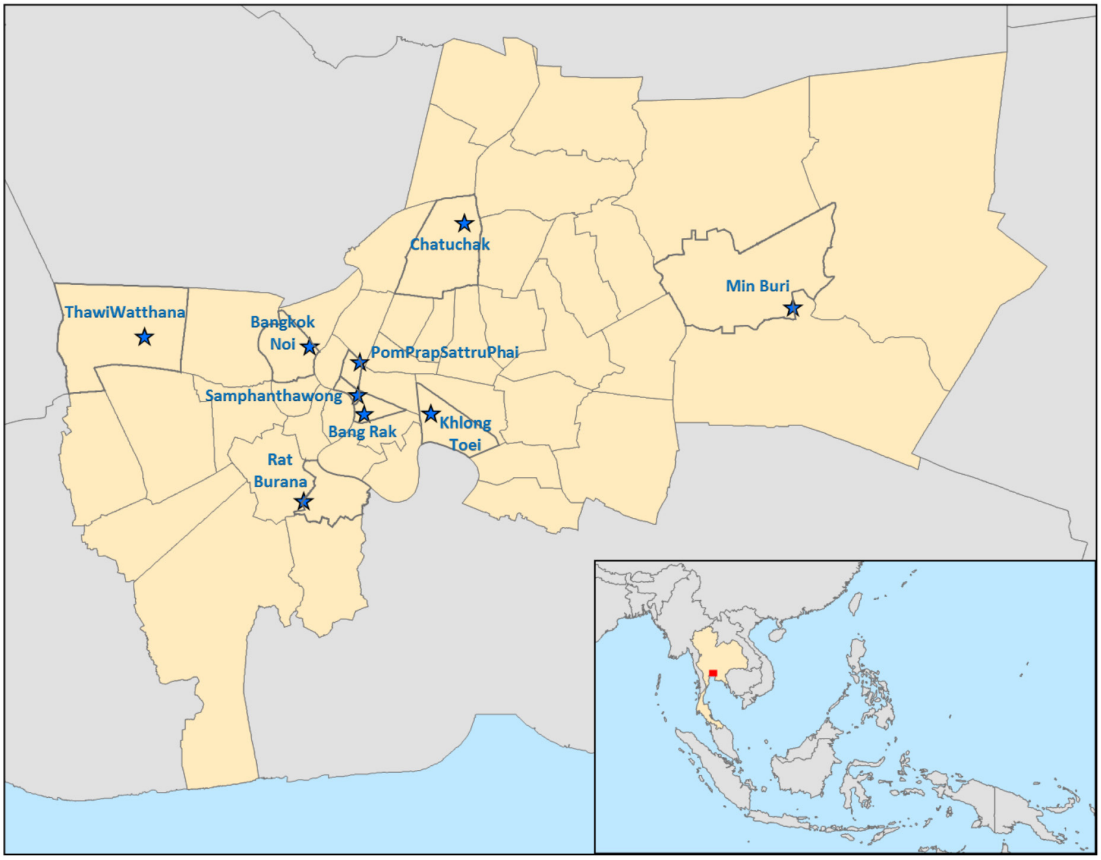

Fig 1–Locations of live-bird markets in Bangkok.

wish to participate in the study. Then team members collected oropharyngeal swab from conveniently selected birds, swabs of fresh feces, and drinking and waste water samples. All swabs samples were placed in viral transport medium (VTM) supplemented with a cocktail of penicillin (2,000 units/ml), streptomycin (2 mg/ml), gentamicin (50 µg/ml), and mycostatin (1,000 units/ml). Water and fresh blood samples were placed in sterile tubes and transported to the National Institute of Animal Health, Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand at 4ºC and analyzed within 24 hours.

Virus detection and isolation

For surveillance of AIVs, samples were inoculated into allantoic cavity of 9-11-day old embryonated chicken eggs and incubated at 37°C for 4 days with up to two passages in eggs. Allantoic fluids containing dead or dying embryos during the incubation period and all eggs at the end of the incubation period were tested for the presence of hemagglutinating activity (FAO, 2013a). Presence of influenza A virus was confirmed using quantitative reverse transcription polymerase chain reaction (qRT-PCR) (FAO, 2013b). Subtyping of isolates employed hemagglutinin inhibition (HI) assays for specific antigens

Table 1
Number of shops and samples collected from each district of Bangkok during November 2013.

District	Number of shops selling live poultry for consumption	Number of shops	Number of shops providing samples
Sampan Thawong	3	3	3
Klong Toei	24	24	18
Bang Rak	2	2	2
Rat Burana	1	1	1
Pom Prap Sattru Phai		1	1
Bangkok Noi		2	2
Min Buri		11	11
Thawi Watthana		14	13
Chatuchak		28	24
Total	30	86	75

and antisera against H5 and H7 subtypes (FAO, 2013a) and/or qRT-PCR (FAO, 2013b) with confirmation of HA and NA genes by sequencing.

RESULTS

Samples were collected in November 2013 from 75/ 87 (85%) shops where live birds were sold (Table 1). No sample was found positive for influenza A virus among all the types of samples from the various species of fowls and birds (pet and sport) collected from each market (Tables 2 and 3). Markets in five districts sold fowls for consumption, including chicken, duck and geese, and markets in the remaining districts sold pet birds. In markets where fowls for consumption were sold, an average of 155 birds were sold per day, and in markets trading in pet birds, there was an average of 132 birds per shop.

DISCUSSION

We found that none of the samples collected from the nine live poultry markets in Bangkok were positive for AIV. These data provide evidence that AIVs, including LPAI viruses such as H7N9, were not circulating in Bangkok live bird markets during the survey period (November 2013).

Surveillance for H5N1 influenza viruses conducted in live-bird and food markets in central Thailand during July 2006-August 2007 resulted in isolation of 12 subtypes of H5N1 virus (Amonsin *et al*, 2008). In 2010 influenza A virus subtypes H4N6, H4N9 and H10N3 were identified in an active surveillance among avian species in a live-bird market in Bangkok (Wisedchanwet *et al*, 2011). We suggest that our negative finding was due to an absence of H5 HPAI virus circulating in wild birds in Thailand or to limited exposure of our samples to H5N1 carrier(s). Another explanation is that we only used PCR in our surveillance which may have lower sensitivity than egg inoculation in picking up low pathogenic viruses such as H4 and H10.

A cross-sectional virology study in 10 live-bird markets in Hanoi, Vietnam

Table 2
Type and source of birds in each market in Bangkok during November 2013.

District	Type	Source
Bang Rak	Chicken	Nakhon Pathom, Bangkok
Samphan Thawong	Chicken	Nakhon Pathom, Nakhon Nayok, Bangkok
Rat Burana	Chicken	Phetchaburi
Khlong Toei	Chicken, duck, goose	Nakhon Pathom, Phetchaburi, Ratchaburi, Chachoengsao, Ang Thong, Chon Buri
Pom Prap Sattru Phai	Chicken, duck, goose	Samut Sakhon
Bangkok Noi	Pet bird[a]	Bangkok
Min Buri	Pet bird	Bangkok, Samut Prakan, Chachoengsao, Phetchaburi
Thawi Watthana	Pet bird	Bangkok, Ang Thong, Samut Sakhon, Chachoengsao, Nakhon Pathom, Kanchanaburi, Netherlands, Belgium, Australia, Philippines
Chatuchak	Pet bird	Bangkok, Nakhon Nayok, Chachoengsao, Samut Prakan, Phetchaburi, Phra Nakhon Si Ayutthaya, Saraburi, Nakhon Ratchasima, Chon Buri, Pathum Thani, Chiang Mai, Suphan Buri, Buri Ram, Kalasin, Roi Et, Prachin Buri, Songkhla, Yala, Pattani, Narathiwat, Netherlands

[a]Pigeon, dove, love bird, budgerigar, finch, African gray parrot, parakeet, macaw, conure, cockatoo, cockatiel, sparrow, bantam, fighting cock, turkey, peacock, swan, and quail.

in October 2001 collected specimens from 189 birds and 18 environmental samples, which resulted in the isolation of influenza A viruses of H4N6, H5N2 and H9N3 subtypes from healthy ducks and H5N1 viruses were from healthy geese (Nguyen *et al*, 2005). In Indonesia, surveillance for AIV A (H5N1) in 83 live-bird markets in three provinces revealed 47% of the markets were positive for the virus (Risa *et al*, 2010).

Bangkok live-bird markets pose a potential public health risk of avian influenza infection. The humid and warm environment could potentially facilitate the survival, growth and transmission of AIVs. In addition, the environment where traders buy and sell the birds provides ideal conditions for virus transmission among birds kept in the cages stacked one on top of another. Viruses shed in feces and in oral-nasal secretions can easily be transmitted through the stacked wire cages occupied by live fowls and birds. This setting provides an appropriate condition for virus transmission among birds (Yee *et al*, 2009).

The live-poultry markets bring together a number of poultry and poultry species from different sources, providing an ideal environment for re-assortment among AIVs of different subtypes. Influenza A virus found in live-poultry markets can also be a potential risk to humans. Pet birds are potential carriers and/or transmitters of zoonotic diseases, which could have important impact on human health, such as salmonellosis, chlamydophilosis, tuberculosis, cryptococcosis, and avian influenza A H5N1, and

Table 3
Type of samples collected from live-poultry markets in Bangkok tested for avian influenza viruses during November 2013.

District	Number of shops	Poultry type	Sample type				Avian influenza virus[a]
			Oropharyngeal swabs	Fresh feces	Drinking water	Waste water	
Bang Rak	2	Chicken	40	-	4	4	Negative
Samphan Thawong	3	Chicken	35	-	6	6	Negative
Rat Burana	1	Chicken	20	-	2	2	Negative
Khlong Toei	17	Chicken	305	-	50	14	Negative
	1	Duck	20	-	2	-	Negative
Pom Prap Sattru Phai	1	Goose	20	-	4	-	Negative
Bangkok Noi	2	Pet bird[b]	-	40	8	-	Negative
Min Buri	11	Pet bird	105	100	-	-	Negative
Thawi Watthana	13	Pet bird	105	90	39	-	Negative
Chatuchak	24	Pet bird	184	180	93	-	Negative
Total		-	834	410	208	26	

[a]Tested using qRT-PCR and hemagglutination-inhibition assays. [b]Pigeon, dove, love bird, budgerigar, finch, African gray parrot, parakeet, macaw, conure, cockatoo, cockatiel, sparrow, bantam, fighting cock, turkey, peacock, swan, and quail.

H7N9 (De Schrijver, 1998; Van Borm *et al*, 2005; Chomel *et al*, 2007; Vanrompay *et al*, 2007). Bird handlers, professional workers (*eg*, veterinarians, traders and shop owners) have a higher risk for contracting such infections. Studies have shown the important role of migrating birds as pathogen vehicles all over the world, which possibly could contaminate pet birds living in open-air aviaries (Dorrestein, 2009). Viruses could spread from endemic countries to other areas through international trade of exotic birds. Markets where live poultries are sold appear to represent a high risk for zoonotic transmission (Boseret *et al*, 2013).

In Thailand, live-poultry purchasing habits, poultry handling and living conditions increase risk of exposure to AIV-contaminated environment. Although no virus was found in this study, it is critical to maintain a level of biosecurity, which prevents the introduction of AIVs. This could be done by putting into place measures for controlling poultry movement, regular cleaning of markets and shops, establishing quarantine areas at production locations, designating fixed locations for slaughtering, providing health education for shop owners and workers and detection of zoonotic infections, and prohibiting operation of live-poultry markets and culling of poultry in infected areas. Another effective strategy would be to introduce active surveillance programs of poultry, humans and the environment. We also recommend that shop owners and workers receive influenza vaccination annually to reduce the risk of seasonal influenza infection and the probability of re-assortment between viruses in humans and animals. To improve One Health

(CDC, 2015) collaboration, relevant agencies should be invited to join a network and implement necessary activities including surveillance of influenza-like illness together with virology testing of respiratory specimens collected from sick workers and shop owners in live-bird markets. A comprehensive surveillance, prevention and control program for influenza viruses should help to minimize risks of cross-species transmission of the viruses. Future surveillance activities are planned for conducting longitudinal monitoring of live-bird markets and this type of activity might serve as a model for other urban areas of Thailand and for the Southeast Asian region.

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