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Live-bird markets in the Northeastern United States: a source of avian influenza in commercial poultry

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Abstract

In 1994, an H7N2 subtype avian influenza virus of low pathogenicity was detected in live-bird markets (LBMs) of the Northeast United States. Since that time the H7N2 virus continues to circulate in the LBMs despite efforts to eradicate the virus by market closures followed by extensive cleaning and disinfection. Since 1996, the LBMs have been implicated as the source of virus in five outbreaks of H7N2 avian influenza in commercial poultry. Although the H7N2 virus is of low pathogenicity, several mutations have occurred at, or near, the cleavage site of the haemagglutinin (H) protein, a region of the protein known to influence pathogenicity of H5 and H7 avian influenza viruses. From 1994 to 2002, the amino-acid motif at the H cleavage site has gradually changed from PENP**K**T**R**/GLF to PE**KP**K**KR**/GLF, with the addition of two lysine (K) residues. Also, a 24-nucleotide deletion, believed to be part of the receptor-binding region, was first observed in LBM H7N2 isolates in 1996 and is seen in all isolates tested since 2000. These findings support the need to continue avian influenza virus (AIV) surveillance in the LBMs and to develop new and innovative methods to prevent the introduction of AIV into the LBMs and to find ways to eliminate it when it is detected.

Live-bird markets (LBMs) have been intensely studied in recent years because avian influenza viruses in the markets are closely associated with avian influenza in commercial poultry and the markets may serve as a 'fertile ground' for virus mutations and emergence of new influenza viruses with increased virulence or ability to infect other species, including humans.

In 1997, an H5N1 avian influenza virus (AIV) emerged in Hong Kong LBMs to infect 18 people; 6 of whom died (Claas et al. 1998). The source of human infections was due to direct contact with infected chickens in the LBMs; there was no human-to-human spread. Subsequent studies on the H5N1 virus showed that the virus most likely evolved by the reassortment of virus genes from at least 3 different avian influenza viruses that were circulating within the LBMs in Hong Kong (Guan et al. 1999; Hoffmann et al. 2000).

In the United States, the LBMs were first recognized as a potential source of AIV in 1986 following the re-emergence in Pennsylvania of an H5N2 AIV of low pathogenicity believed to be the precursor virus that caused the outbreak of highly pathogenic H5N2 in 1983-84. The source of the low-pathogenic H5 virus was traced to the LBMs in the Northeast United States. Eradication of the H5N2 virus from the

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LBMs was accomplished by the end of 1987. Since that time, extensive surveillance was conducted to monitor for AIV circulating in the LBMs. In 1994, an H7N2 AIV of low pathogenicity was detected in the LBMs and has persisted since, despite efforts to eliminate the virus. Since 1996, the LBMs have been implicated as a source of H7N2 AIV in at least 5 outbreaks in commercial poultry in the Northeast United States (Akey 2003; Davison et al. 2003; Dunn et al. 2003, D. Senne unpublished observation).

In this paper we: 1. briefly describe the LBM system in the USA; 2. review surveillance activities in the LBMs; 3. review the recent outbreaks of AI for which the LBMs were implicated as a source of virus in commercial poultry; 4. summarize the molecular changes in an H7N2 AIV that has continued to circulate in the markets since 1994; and 5. present past and future plans for the control of AIV in the LBMs.

Keywords: avian influenza; live-bird markets; surveillance; United States

What are live-bird markets?

The LBMs in the Northeast United States are part of a complex marketing system that provide a source of fresh poultry meat preferred by ethnic populations in many of the large cities. The northeastern LBM system is comprised of more than 120 markets in 6 states (New York, New Jersey, Connecticut, Rhode Island, Pennsylvania and Massachusetts) with the majority of the markets being located in New York and New Jersey. Birds entering the LBM system come from a variety of sources including farms that raise birds specifically for the LBMs, backyard flocks and commercial poultry farms. Most of the birds come from adjacent states but some birds are transported several hundred miles from states as far west as Ohio. Birds are collected at the farms by dealers and/or wholesalers and delivered by truck or vans to distribution centres within the city or directly to the LBMs where birds are placed in open holding pens or in cages. Cages are generally stacked 4 to 5 tiers high with separate food and water sources for each tier of cages. Customers can hand-pick birds they wish to purchase and the birds are then individually processed and the carcasses prepared according to the customers' specifications.

The LBMs provide an environment where reservoir species of the AIV, i.e. ducks and geese, are housed closely with chickens, turkeys, guinea fowl and quail etc., which are not natural hosts for the virus. Commingling of different avian species and daily introduction of new birds provides opportunity for the AIV to replicate and adapt to new hosts and the infection to persist within the market system for extended periods. Long-term replication of AIV in unnatural host may facilitate accumulation of point mutations which could lead to increased virulence.

Surveillance activities

Each year since 1994, between 1,457 and 8,120 tracheal- and cloacal-swab pools were collected from the LBMs in the Northeast United States and tested for presence of AIV by virus isolation in embryonated chicken eggs (Table 1). In 1994, an H7N2 AIV of low pathogenicity was introduced into the LBMs which continued to circulate in the market system. During 1994-2003, between 30 and 808 isolations of H7N2 AIV were made each year (Table 1). In addition to the H7N2 virus, several introductions of H5 and other H7 subtypes were detected but the latter subtypes did not become established. The sporadic detection of the H5 and H7N3 subtype viruses suggests that

these viruses are not well adapted to poultry and disappear when the infected birds are sold and removed from the markets.

Table 1. Number of samples tested and number of isolations of avian influenza virus subtypes H5 and H7 from live-bird markets of the Northeast United States, FY 1994-2003

Fiscal year	Total no. tested	No. isolates (of H5 subtypes*)	No. isolates (of the H7N2 subtype*)	No. isolates (of H7* subtypes)
1994	1,791		30	1 (H7N3)
1995	5,214		170	
1996	1,740		363	
1997	2,060		188	
1998	2,497		372	
1999	3,679	3 (H5N2)	808	10 (H7N3)
2000	1,457		185	
2001	2,756	5 (H5N2)	419	
2002	8,120	3 (H5N2)	745	
2003	5,709	4 (H5N8) 1 (H5N9)	354	

* All H5 and selected H7 avian influenza viruses were characterized as low-pathogenicity viruses

Outbreaks in commercial poultry linked to the LBMs

Since 1996, five outbreaks of low-pathogenic H7N2 in commercial poultry have been linked to the LBMs in Northeastern United States as the source of infection: 1) Pennsylvania, in 1996-97 (18 layer, 2 layer pullet, and 1-meat turkey farms); 2) Pennsylvania, in 2001/2002 (5 broiler, and 2 broiler breeder farms); 3) Virginia/West Virginia/North Carolina in 2002 (210 flocks, 4.7 million birds involving turkey breeders, meat turkeys, broiler breeders, broilers, layers and quail); 4) Connecticut, in 2003 (4 layer farms involving 3.9 million birds); and 6) Rhode Island, in 2003 (32,000 layers). A more detailed account of the outbreaks is given by other authors on this volume (D. Swayne, D. Senne).

A direct epidemiologic connection between the LBMs and the commercial-poultry outbreaks noted above was established in only two outbreaks; the 1996-97 outbreak in Pennsylvania and the outbreak of 2003 in Rhode Island. In both outbreaks, trucks hauling birds to the LBMs had been on the affected premises within a week before the appearance of clinical disease. However, in the remainder of the outbreaks, the LBMs were implicated as the source of viruses because the causative virus was genetically indistinguishable from the H7N2 virus present in the LBMs, the only known source of this strain of AIV. In 2001, more than 185 farms that routinely supplied birds to the LBMs were surveyed for presence of AIV and specific antibodies to AIV in an attempt identify possible sources of the H7N2 virus outside of the LBM system. No H7N2 virus or specific antibodies were detected in the 2,225 swab specimes or 2,450 serums.

Molecular changes in the low-pathogenic H7N2 virus since 1994

The continued circulation of an H7N2 virus of low pathogenicity in the LBM system since 1994 has provided an opportunity to study the genetic changes in the virus following replication in unnatural hosts for an extended period, especially as it

relates to the amino-acid motif at the cleavage site of the H protein. There are four reports where low-pathogenic H5 and H7 viruses, after circulating in poultry for 1 to 9 months, have mutated to highly pathogenic viruses (Banks et al. 2001; Horimoto et al. 1995; Kawaoka, Naeve and Webster 1984; Senne et al. 2002).

Since 1994, several changes in the amino-acid motif have been observed at the cleavage site of the H protein of the H7N2 virus in the LBMs suggesting that the virus is progressing toward becoming highly pathogenic. From 1994 to 2002, the cleavage-site motif gradually changed from PENPKTR/GLF to PEKPKKR/GLF, with the addition of two lysine (K) residues at the -2 and -5 positions of the HA1 (Table 2). In each case, where a virus with a new motif was detected, it eventually replaced the previous virus and became the predominant strain in the markets. For example, in 1994, the amino-acid sequence at the cleavage site of the H protein contained two basic amino acids and was PENPKTR/GLF. In 1996, 1 of 11 isolates tested had a motif containing three basic amino acids (PEKPKPR/GLF), with a K to N (asparagine) substitution at the -5 position. By 1998, the motif with three basic amino acids became predominant and was the only motif detected in isolates tested in 2000 and 2001. In 2002, a fourth basic amino acid was observed in isolates from several markets. This change came about by a P (proline) to K substitution at the -2 position, resulting in a cleavage-site motif of PEKPKKR/GLF. It is possible that the H7N2 virus with four basic amino acids may become highly pathogenic with the acquisition of an additional basic amino acid. The detection of an H7N2 virus with four basic amino acids provided increased urgency to implement a planned 3-day closure of all LMBs in the Northeast United States in an attempt to rid the markets of low pathogenic H7N2 AIV. Details of the market closures are described elsewhere in this chapter.

Table 2. Deduced amino-acid sequences of H7N2 avian influenza viruses isolated from live-bird markets of Northeast United States, FY 1994-03

Fiscal year	Amino-acid sequence at HA cleavage site*	No. isolates tested with sequence	Percent isolates with sequence
1994	PENP**K**T**R**/GLF	1	100
1995	PENP**K**T**R**/GLF	6	55
	PENP**K**P**R**/GLF	4	36
	PENP**K**I**R**/GLF	1	9
1996	PENP**K**P**R**/GLF	10	91
	PE**K**P**K**T**R**/GLF	1	9
1997	PENP**K**P**R**/GLF	3	100
1998	PENP**K**P**R**/GLF	12	92
	PE**K**P**K**P**R**/GLF	1	8
1999	PENP**K**P**R**/GLF	4	29
	PE**K**P**K**P**R**/GLF	10	71
2000	PE**K**P**K**P**R**/GLF	20	100
2001	PE**K**P**K**P**R**/GLF	22	100
2002	PE**K**P**KK**R/GLF	9	31
	PE**K**P**K**P**R**/GLF	20	69
	Market Closures (April 2002)		
2003	PE**K**P**K**P**R**/GLF	28	100

*Basic amino acids (lysine[K] and arginine [R]) are shown in bold print

Between 1994 and 2003, other molecular changes were detected in H7N2 viruses circulating in the markets. In 1995, a threonine (T) to P substitution at the -2 position

was first observed. By 1997 this change was observed in most isolates. Proline at the -2 position is unique among H7 subtypes and has been used as a marker to distinguish LBM H7 viruses from other low-pathogenic H7 subtypes isolated outside the LBM system. In addition to the changes at the cleavage site of the H protein, a 24-nucleotide deletion, believed to be part of the receptor-binding region, was first observed in LBM H7N2 isolates in 1996 and is seen in all isolates since 2000. The significance of this deletion is not known but it could be related to the adaptation of the H7N2 virus to poultry.

Past, present and future control activities in the LBMs

In the United States, the authority to control outbreaks of low-pathogenic AIV is given to the individual states, whereas outbreaks of highly pathogenic AIV are managed at the federal level with assistance from the state authorities and the poultry industry. Therefore, control of the low-pathogenic avian influenza (LPAI) in the LBM system has been the responsibility of individual states.

Control of LPAI in the LBMs will differ from state to state; primary focus has been on education, increased surveillance and sanitation, and the adoption of better laws to give states more authority to deal with LPAI. Most states require AIV-positive LBMs to sell off the bird inventory, thoroughly clean and disinfect the premises and leave the market empty of birds from 1 to 3 days before repopulating the market. This approach was successfully used in 1986 and 1993 when low-pathogenic H5N2 AIV was found in LBMs in Northeastern United States, but it has not worked for the H7N2 virus currently present in the LBM system. Surveillance conducted in 2001 showed that up to 60% of the LBMs were positive for the low-pathogenic H7H2 virus that has persisted in the LBMs in Northeast United States since 1994.

The lack of success in reducing the number of H7N2-positive markets prompted the US Department of Agriculture (in March of 1999) to establish a LBM working group. The working group was comprised of representatives from state and federal governments as well as representatives from the LBMs and commercial poultry industry. The charge of the working group was to develop plans to control LPAI in the LBMs. One of the recommendations from the group was to implement a system-wide closure of the retail LBMs in the Northeast United States. The market closure was implemented April 8-10, 2002. Details of the market closures are described elsewhere (Mullaney 2003). In summary, birds in all markets were sold or killed and the markets were thoroughly cleaned and disinfected. Each market was then inspected to ensure that the cleaning and disinfection were properly completed and environmental samples were collected 24 hrs post-cleaning and tested for presence of AIV. The markets were required to stay empty of all birds for a 3-day period. Market owners were paid $3,000 each to cover lost revenue during the closure period. Funding for the closure (>$900,000 USD) was provided by Animal and Plant Health Inspection Service (APHIS) contingency funds.

Following the closure, markets were repopulated with birds only from AIV-monitored (AIV-negative) source flocks. Surveillance showed that the LBMs remained negative for AIV for about 5 weeks before the H7N2 virus was again detected. It is not known if the virus persisted in the markets or was reintroduced into the markets. However, since the closure the H7N2 virus with four basic amino acids has not been detected.

The control of AIV in LBMs has been identified as a critical component of the proposed LPAI control programme in the United States. The LPAI control

programme will most likely be a joint state and federal programme with costs for monitoring and flock indemnities being shared by US Department of Agriculture and participating states. The programme will also consider new approaches to aid in the control of LPAI in LBMs. These may include the licensing of dealers/wholesalers to ensure that birds are obtained only from monitored flocks and that proper cleaning and disinfection of trucks and equipment are performed before going to the farms to pick up birds. Also, vaccination to reduce the susceptibility of the birds in the markets and individual bird identification to trace sources of virus outside the LBM system are being considered.

The LBMs are a reservoir of AIV for commercial poultry and can provide a favourable environment for the emergence of viruses with adaptive changes that can alter host specificity and/or virulence. Therefore, efforts must continue to find ways to prevent the introduction of AIV into the markets and to eliminate AIV when it is introduced

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