

Live Poultry Markets: A Missing Link in the
Epidemiology of Avian Influenza

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SUMMARY

Surveillance activities for avian influenza virus infection in live poultry markets (LPMs) were instituted in February 1986 when H5N2 virus was first isolated from birds within these markets. Over 19,700 specimens (1,950 submissions) from the LPMs have been tested for presence of virus at the National Veterinary Services Laboratories, Ames, Iowa. Six hundred fifty-three influenza A viruses and 563 Newcastle disease viruses were isolated. Of the 653 isolates of influenza A viruses, 528 (80%) were subtyped as H5N2. None of 61 representative influenza virus isolates were classified as highly pathogenic; however, 11 (18%) killed one or two of eight experimentally inoculated chickens. Of the 218 H5N2 viruses isolated from the LPMs and commercial chickens that were tested for plaquing ability in Madin Darby canine kidney cells, 34 (16%) produced plaques in the absence of trypsin. This suggested that these viruses might be related to the H5N2 highly pathogenic viruses isolated in 1983-84. There has been no isolation of H5N2 virus from the live poultry markets since October 1989.

INTRODUCTION

In 1983 and 1984, an outbreak of highly pathogenic avian influenza (virus subtype H5N2) occurred in Pennsylvania, Virginia, and Maryland. Efforts to control the disease necessitated destruction of over 17,000,000 birds at a cost exceeding \$63,000,000--one of the most expensive animal disease control measures in the history of the United States. Waterfowl were suspected as the source of the virus because they were present on the premise where the first virus isolation from commercial chickens was made.

All available evidence indicated that the disease was successfully eradicated by the end of 1984. Intensive surveillance activities, however, continued through April 1985. In January 1986, additional isolations of H5N2,

genetically related to the 1983-84 virus (1), were made from commercial chickens in Pennsylvania, New Jersey, Massachusetts, and Ohio. The viruses isolated in 1986, however, did not cause high mortality in chickens; the signs observed were those of a respiratory disease and a drop in egg production. The source of the initial infections in 1986 was eventually traced to the live poultry markets (LPMs). Subsequent sampling of the LPMs in New York, New Jersey, Massachusetts, Connecticut, Rhode Island, and Florida revealed that birds in at least 48 markets were infected with H5N2 avian influenza virus.

Live poultry markets or "Botanicas," as they are called in Florida, are popular in metropolitan areas of the United States and cater to many different ethnic patrons. The markets are usually located near residential areas in the inner city. Patrons can select the live bird they wish to purchase and can have the bird defeathered, eviscerated, and in some instances, cut-up and placed in plastic bags to carry home. Occasionally, birds will be taken home live, perhaps to be used in ritualistic rites. This is especially true for the Botanicas located in Florida.

In February 1986, when it was first discovered that birds in the LPMs were infected with H5N2 virus, very little was known about the size and complexity of this segment of the poultry industry. A national survey and census was undertaken in 1986 and 1987. A total of approximately 725,000 birds including chickens, turkeys, ducks, guinea fowl, pigeons, and chuckar partridges, were kept in the live poultry markets at any one time. A majority of these birds (over 685,000) were located in markets in the northeastern United States (5), the largest numbers being in New York and New Jersey. Approximately 93% of birds in the markets were chickens.

Birds find their way into the LPMs through one of several channels. Small-time hucksters and larger dealers or brokers buy poultry directly from the producer. Other birds may find their way to the markets through transfers from dealer to dealer, from dealer to wholesaler, from poultry concentration points, and from auction markets. Some of the larger distributors have the birds delivered to a central facility during the evening or early morning,

then transfer the birds in crates to company-owned vehicles and deliver the birds to individual markets. On many occasions, birds are traded between local markets to meet consumer demands.

Because the LPMs could serve as a source of infection for commercial poultry, intensive efforts were made to rid the markets of the H5N2 virus. As a part of this process, surveillance of the LPMs has continued since 1986. This report summarizes results of the surveillance.

MATERIALS AND METHODS

Virus Isolation and Identification. Pools of tracheal and cloacal swabs, three to five per tube, were collected and placed in tubes containing 2 ml brain-heart-infusion (BHI) broth. Environmental samples from feces, waterers, floors, and floor drains, were also collected and placed in BHI. Specimens were shipped to the National Veterinary Services Laboratories (NVSL), Ames, Iowa, with ice packs by an overnight delivery service. Preparation of inoculum and the procedure for virus isolation have been described (2). Briefly, specimen tubes were vortexed, centrifuged, and the supernatant was treated with antibiotics before inoculation into embryonating chicken eggs. The eggs were candled daily for embryonic mortality. The allantoic-amniotic fluid (AAF) from eggs with dead embryos and from all eggs with surviving embryos after 4 days incubation were tested for hemagglutinating (HA) activity. Allantoic-amniotic fluid with HA activity were tested by the hemagglutination-inhibition (HI) test with Newcastle disease virus (NDV) antiserum. Isolates not inhibited by NDV antiserum were tested for type A influenza subtypes by the avian influenza HI (3) and neuraminidase-inhibition (NI) tests (4). Newcastle disease virus isolates were characterized by mean death time (MDT) in embryonating chicken eggs and/or by monoclonal antibody analysis (hybridomas provided by Dr. David Snyder, College Park, Maryland).

Pathogenicity Testing. Isolates of type A influenza were inoculated into groups of eight chickens derived from a specific pathogen free flock. Isolates prior to 1990 were inoculated in the caudal thoracic air sac (CTAS) whereas isolates made since 1990 were inoculated intravenously. Chickens were observed for 8 days for signs of disease.

Plaque Assay. Confluent monolayers of Madin Darby canine kidney cells, grown in 60 mm tissue-culture-treated Petri dishes, were washed twice with Eagle's minimum essential medium (MEM) to remove residual fetal bovine serum. Plates were inoculated with 0.2 ml of each virus dilution (10-fold dilutions) and incubated for 45 to 60 minutes to allow for virus adsorption. Cells were then overlaid with 5 ml of overlay medium containing 1% Bacto-agar. Duplicate cultures were overlaid with medium with and without trypsin (10 µg/ml). Cultures were incubated at 37°C for 48 hours and overlaid a second time with 3 ml of overlay medium containing 0.004% neutral red and 1% Bacto-agar. Plaques were counted after an additional 24 to 48 hours of incubation at 37°C. Efficiency of plaquing was calculated as the ratio of the average number of plaque forming units (PFUs) observed on plates with trypsin and the average number of PFUs observed without trypsin.

RESULTS AND DISCUSSION

H5N2 Infections in LPMs. Results of the 1986 nationwide survey revealed that at least 48 markets in five states had birds infected with the H5N2 virus: New York (26), New Jersey (12), Rhode Island (1), Connecticut (2), and Florida (7). Annual surveillance was carried out in all states where H5N2-infected markets were identified. Surveillance activities included isolation of the virus from tracheal and cloacal swabs, serology, and the use of sentinel chickens. The level of sampling of the LPMs is shown in Table 1.

Because of surveillance in 1986 and 1987, 528 isolations of H5N2 virus were made (Table 2). The large number of isolations was the result of many markets repeatedly becoming H5N2 virus-positive within 1-2 days following depopulation and cleaning and disinfection (C & D). In order to determine if the virus was reintroduced into the markets via new birds or if the H5N2 virus was persisting after C & D, birds were sampled before they were introduced into the markets. Testing of birds entering the markets did not yield H5N2 virus; therefore, it was assumed that the virus was persisting or being maintained in the markets, or that some infected birds were not being destroyed, moved back into the market after C & D and reinfected new poultry brought into the markets.

Although H5N2 virus was the most common virus isolated from birds in LPMs, many other subtypes of influenza A viruses were found. The subtypes, species of birds from which they were isolated, and the number of isolates are shown in Table 2. Hemagglutinin subtypes in addition to H5 were H1, H2, H3, H4, H6, H9, and H11, most of which were isolated from ducks. Also more than 550 isolations of NDV were made. Those that were characterized belonged to the lentogenic pathotype as determined by MDT or by monoclonal antibody analysis. Conditions within the LPMs are generally conducive to the introduction and adaptation of many viruses to other species. The birds are placed in cages or crates at high densities with many species, including ducks and geese, and are in close proximity to each other.

Cleaning up the LPMs. Eradication of H5N2 virus from the LPMs has been a difficult task. The states were given the responsibility of eradicating the disease from the markets. Three different approaches were used. In New York, the industry chose to depopulate all birds, implement C & D, then leave the market empty for one day before repopulating. This procedure was used for three successive weekends. A random sampling of markets after the first, second, and third C & D revealed the presence of H5N2 virus in four, six, and eight markets, respectively. New Jersey elected to shut down the markets for 5 consecutive days and C & D two times before placing birds. No H5N2 virus was present in samples collected 3 weeks post C & D. Florida elected to use sentinel birds to monitor the Botanicas following depopulation and C & D. The last isolation of H5N2 virus from LPMs in New York, New Jersey, and Florida was in January 1987, June 1987, and October 1989, respectively.

Pathotyping and Plaquing Results. Pathotyping was done as previously described (2). Isolates were inoculated into eight chickens in the CTAS or intravenously and observed for 8 days. Of 61 isolates that were pathotyped, 50 were not pathogenic (did not kill chickens), and 11 (18%) killed one or two of 8 inoculated chickens. Similar results were obtained with the 1986 isolates of H5N2 from commercial poultry. Fourteen of nineteen were not pathogenic, and five (26%) killed one to three chickens. This was to be expected since the source of the virus in commercial poultry was believed to have originated from the markets.

In addition to pathotyping isolates in chickens, some isolates from the LPMs and some from commercial poultry were assayed for their abilities to produce plaques in cell culture in the absence of trypsin. Of the 218 viruses tested, 34 (16%) had plaquing efficiencies slightly greater than highly pathogenic isolates (6). Plaquing efficiencies of the 34 viruses were: 1-5 (5), 6-10 (4), 11-35 (5), and 36-200 (20). Most of the 20 viruses with plaquing efficiencies of 36 to 200 lost the ability to plaque following further egg passage. In previous studies, the plaquing efficiencies of highly pathogenic viruses ranged from 0.89 to 3.00, whereas nonpathogenic strains had plaquing efficiencies of >10,000 (6).

Persistence of Virus in the Live Poultry Markets. It would appear the LPMs were the missing link in the epidemiology of the 1986 outbreak of H5N2 in Pennsylvania, New Jersey, Massachusetts, and Ohio. The LPMs represented a previously unrecognized source of influenza A viruses which could infect commercial poultry. The presence of the H5N2 virus in birds in the market system seemed to be subclinical, perhaps because the birds were in the markets for only a few days before being sold. This situation is perhaps most conducive for continued maintenance of the virus--new susceptible birds are continually being introduced to keep the cycle going. Since the H5N2 virus was apparently being maintained within the market system for long periods of time without reintroduction of the virus, it was suggested that the LPMs could have been the source of virus for the epidemic of H5N2 avian influenza in 1983 (7).

The H5N2 virus was not the only subtype of influenza A viruses that was apparently being maintained in the LPMs. In 1989, H2N2 was first isolated from the LPMs and has been recovered from surveillance samples in each of the past 4 years (Table 2). It is interesting to note that in 1988, 22 submissions from turkeys in Minnesota and 1 in North Carolina were diagnosed as being infected with H2N2 virus. In 1989, 127 submissions from turkeys in Minnesota, 9 from Iowa, and 1 from Wisconsin were infected with H2N2 virus.

CONCLUSION

Conclusion 1. No H5N2 virus has been isolated from the LPMs since October 1989; however, monitoring of the markets is continuing.

Conclusion 2. The LPMs provide the opportunity for the adaptation of influenza A viruses to many bird species in the market system. Viruses can be introduced into the markets via ducks, geese, or other species and, in some instances, may persist in the LPMs for long periods of time. The H5N2 virus responsible for the 1983 and 1986 outbreaks has demonstrated a very wide host range, perhaps suggesting it may have been the LPMs for some time.

Conclusion 3. In view of the 1986 outbreak of H5N2 influenza, the LPMs should be considered as a possible source of avian influenza virus infection in domestic poultry.

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Table 1. Sampling of live poultry markets FY 1986 through 1992.

Year	Number of Submissions	Number of Specimens
1986	689	8159
1987	535	7976
1988	226	955
1989	287	1096
1990	100	433
1991	96	839
1992*	21	251

* New Jersey markets only

Table 2. Subtype, source, and number of influenza A viruses isolated from live poultry market samples FY 1986 through 1992.

Year	Subtype	Source*	Number Isolated	Year	Subtype	Source*	Number Isolated
1986	H5N2	CK	293	1987	H3N2	DK	1
		GF	57			EN	1
		TY	36		H3N6 ^c	DK	3
		DK	35		H3N8	DK	3
		EN	20			EN	1
		QU	17		H6N2	DK	1
		PG	2		H6N6	DK	4
		CR	2			EN	2
	H1N1	DK	1		H6N8	DK	4
	H4N6	DK	29			EN	2
		EN	3	1988	H5N2	CK	3
		CK	2	1989	H2N2	EN	1
		GF	1		H6N3	EN	1
	H6N1	DK	3	1990	H5N2	CK	1
	H6N2	DK	14		H2N2	CK	8
		EN	1			DK	2
	H6N6	DK	1			GF	2
	H6N9	DK	4			EN	5
		CK	1			UN	2
	H9N1	DK	2		H3N8	EN	1
	H7N2	CK	1			UN	1
		PG	1		H11N9	UN	1
1987	H5N2	CK	36	1991	H2N2	EN	5
		EN	13			CK	1
		GF	7			GF	1
		DK	5		H6N2	CK	1
		TY	1		H6N8	CK	1
	H1N2	CK	2			EN	1
		DK	1	1992*	H2N2	CK	1
		GF	1				

* CK=Chicken, GF=Guinea Fowl, DK=Duck, TY=Turkey, EN=Environment, QU=Quail, PG=Pigeon, CR=Chuckar Partridge