

Short Communication

Molecular Surveillance of Avian Influenza in Live Bird Market of Qom City in Iran

Majidzadeh-A K¹, Soleimanidor M¹, Morovvati A¹, Karimi V², Ghalyanchi-Langeroudi A^{3*}

1. Tasnim Biotechnology Research Center (TBRC), Faculty of Medicine, AJA University of Medical Sciences, Tehran, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Avian influenza (AI) has emerged as a disease with significant potential to disrupt commercial poultry production often resulting in extensive losses (1). Influenza is caused by a zoonotic virus that occurs in lower animals and birds as well as in humans. Influenza viruses belong to the Orthomyxoviridae family of RNA viruses and are divided into five genera: Influenza A, B, C virus, Thogtovirus and Isavirus (2, 3).

A viruses can be divided into subtypes on the basis of the possession of one of 16 antigenically distinct Haemagglutinin (HA) antigens and one of the 9 Neuraminidase (NA) antigens (4). Virtually all HA and NA combinations have been isolated from birds (3).

Live Bird Markets (LBM) have been recognized as a productive source and important man-made reservoir of AI virus (AIV) linked to outbreaks of influenza in commercial poultry farms and humans.

LBMs provide an ideal tool for genetic mixing and spreading of the influenza virus. Because they bring together numerous hosts (e.g. Chickens, Ducks, turkeys and quail) in close contact and high density. So viral reassortment and inter species transmission have accrued. Long term replication of AIV in even unnatural host species can lead to



Fig. 1. Map of Study area (Qom City) in Avian Influenza Molecular Surveillance (10).

accelerated mutation rates for AIV. LBMs are therefore hypothesized to be a missing link in the epidemiology of AIV and it is important that the LBMs be routinely monitored for AIV. Low & High Pathogenic AIV has been isolated repeatedly from LBMs. Presence of this virus in the LBMs poses a significant risk to the commercial poultry in any region (3, 5).

Investigations conducted in Hong Kong following the first H5N1 outbreak in humans in 1997 determined that exposure to poultry in LBM was a key risk factor for human disease (6, 7)

Since 1998, H9N2 AI outbreaks have been one of the major problems in Iranian poultry industry. In 2006, H5N1 report in swans in northern of Iran, but to this time we don't have official report from commercial flocks in Iran

***Corresponding author:** Arash Ghalyanchi Langeoroudi, PhD. Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
Email: ghalyana@ut.ac.ir

Table 1. Primers sequence that was used in Molecular Surveillance of Avian Influenza in Live Bird Market of Qom City In Iran (WHO, 2002).

No	Primer Name	Sequences
1	Uni12	AGCAAAAGCAGG
2	HA-1144	GGAATGATAGATGGNTGGTAYGG
3	HA-Reverse	ATATCGTCTCGTATTAGTAGAAACAAGGGTGTTTT
4	M-WSN-8	GAAGGTAGATATTGAAAGATG
5	M-1023R	GAAACAAGGTAGTTTTTTTACT
6	MF	GGTCTTGTCTTTAGCCAYTCCA
7		AGGTCGAAACGTAYGTTCTCTCTA

(8). Although Fereidoni,S *et al* report detection of other subtypes of avian influenza virus from migratory birds in Iran (9).

Till now, we didn't any molecular surveillance program for tracking of AIV in LBM in Iran. In this study, we do Molecular Surveillance of AI in LBM of Qom City that located in important geographical region.

Qom (also known as Q'um or Ghom) is a city in North-Central of Iran (34°39'N 50°53'E). It lies 156 kilometers (97 mi) by road southwest of Tehran and is the capital of Qom Province. It has an estimated population of 1,042,309 in 2005. The town lies on both banks of the Rud-e Qom and beside a salt desert, the Dasht-e Kavir (10) (Figures 1).

Sample Collection (100 Fecal swabs) was performed according to the standard method from LBM in Qom province. Sampling was from various species such as Pigeons (62), turkeys (13) and Chicken (25). Swabs from similar species within a market were pooled. Specimens were stored at -70 °C until use. Samples were collected in a 2X phosphate buffer solution (PBS, pH 7.4) containing antibiotics (10.000 IU/ml penicillin, 1 mg/ml streptomycin sulphate) and anti antifungal (20 IU/ml Nystatin) (SIGMA, St. Louis, MO, USA).(11-13)

Total RNA was extracted with RNA extraction kit (Bioneer, South Korea) according to the manufacturer's instruction. The extracted total RNA stored at -70°C until use (8, 13, 14).

Reverse transcription was done by using oligonucleotide influenza universal primer, uni12, with "Revert Aid" first strand cDNA synthesis Kit (Fermentas, Canada) (15).

Amplification was carried out by PCR as described by using WHO specific primers for All AIV Subtypes .(HA-1144& HA-Reverse) which amplify a 591 bp fragment . To ensure that the RT-PCR is working a reactions for the amplification of the M-gene can be included in parallel for the PCR reaction (M-WSN-8 & M-1023R) which amplify a 1015 bp fragment. Primers sequences are available in Table 1 (14).

The reaction mixture (50 µl) contained 5 µl of cDNA, 15 pmoles of forward and reverse primers (4 µl), and 25 µl Normar PCR master mix.

The PCR reaction is done in 2 minutes at 94°C, 30 cycles including 60 seconds at 94°C, 60 seconds at 50°C, 180 seconds at 72°C, and finally 10 minutes in 72°C as a final extension .After amplification, samples were stored either overnight at 2 to 8°C, or at -20°C for longer-term storage (14).

5 µl of the PCR products were mixed with 1 µl loading buffer and then were electrophoresed on 1.5% agarose gel in Tris-borate EDTA buffer (16).

We didn't detect any AIV RNA in mentioned Collected Samples. Although samples from diverse species of birds were collected and the

RT-PCR test was carried out on the entire specimen.

Live bird markets (LBM) are essential for marketing poultry in many developing countries, and they are a preferred place for many people to purchase poultry for consumption throughout the world. Live bird markets are typically urban and have a permanent structure in which birds can be housed until they are sold. LBMs bring together a mixture of bird species that meet the preferences of their customers and that are commonly produced by multiple suppliers. The mixture of species, the lack of all-in-all-out management, and multiple suppliers are all features that make LBM potential sources of avian influenza viruses (17), especially for their supply flocks (18).

Jadhao *et al* (1) findings suggest that the H5N1 and H5N2 viruses that circulated among geese and ducks in LBMs in Hanoi, Vietnam, during 2001 and 2003 were not the immediate ancestors of the clade-1 viruses associated with fatal human infections in Vietnam.(19)

Surveillance for H5 and H7 subtypes of AIV in the LBM of the northeastern United States has been in effect since 1986 when the markets were first recognized as a potential reservoir for AIV.(20)

Bulaga *et al* examined the suppliers to the LBMs in New York and New Jersey. In 2001, 185 supplier premises in nine states were surveyed for the presence of AIV by virus isolation (17). No H7 or H5 virus was isolated. The survey results suggest that current biosecurity practices at supplier premises could be improved, especially regarding movement of birds (21).

Although ,In another study in 2001by Bulaga *et al*, all 109 retail LBMs in New York and New Jersey were surveyed for the presence of AIV by a real time RT-PCR (RRT/PCR) assay and results compared to virus isolation (VI) in chicken embryo. The RRT/PCR had a 91.9% sensitivity and 97.9% specificity in detecting presence of AIV at the market level. However, the sensitivity at the sample level is 65.87%. The RRT/PCR was a reliable method to identify AIV at the market level (22).

Between 1993 and 2000, gallinaceous birds, waterfowl, and environmental specimens from the LBMs of the northeastern United States and non-LBM premises were tested for the presence of AIV , pathogenic properties of AIV subtypes, especially of hemagglutinin (17) subtypes H5 and H7, and a possible association between LBM and non-LBM infections. Ten H subtypes of AIV were isolated from the LBM specimens: H1, H2, H3, H4, H5, H6, H7, H9, H10, and H11. During this period, the 10 subtypes also were isolated from birds in non-LBM premises. In the LBMs, subtypes H2, H3, H4, H6, H7, and H11 were present for 5-8 yr despite efforts to clean and disinfect the premises.(23)

LBMs have been recognized as a productive source and important man- made reservoir of AIV linked to outbreaks of influenza in commercial poultry farms and humans. LBMs provide an ideal tool for genetic mixing and spreading of the influenza virus. Because they bring together numerous hosts (eg. Chickens. Ducks, turkeys and quail) in close contact and high density.

So in this ideal environment, viral reassortment and inter species transmission. Long term replication of AIV in even unnatural host species can lead to accelerated mutation rates for AIV. LBMs are therefore hypothesized to be a missing link in the epidemiology of AIV and it is important that the LBMs be routinely monitored for AIV. LPAI has been isolated repeatedly from LBMs. Presence of this virus in the LBMs poses a significant risk to the commercial poultry in any region.

We conclude that as LBMs have vital role for outbreak of new Pandemic, more detailed and expansive surveillance program should be done in other regions of Iran for integrate of precise epidemiological map of AI. Continuous surveillance would improve our understanding of the real role of LBMs in ecology of influenza viruses in Iran.

Acknowledgments

We would like to thank A.H.Mohseni , A.Kian and A. Masoumi members of Laboratory of TASNIM Biotechnology Research Center in

AJA University of Medical Science, for their technical supports.

References

- Alexander DJ, Brown IH. History of highly pathogenic avian influenza. *Rev Sci Tech*. 2009;28(1):19-38.
- Lamb RA, Kr. Orthomyxoviridae: The viruses and their replication. *Fields' virology*. 5th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2007. p. 1487-531.
- Fields BN, Knipe DM, Howley PM. *Fields virology*. 5th ed. Philadelphia ; London: Wolters Kluwer Health / Lippincott Williams & Wilkins; 2007.
- Alexander DJ. An overview of the epidemiology of avian influenza. *Vaccine*. 2007;25(30):5637-44.
- Shortridge KF, Gao P, Guan Y, Ito T, Kawaoka Y, Markwell D, et al. Interspecies transmission of influenza viruses: H5N1 virus and a Hong Kong SAR perspective. *Vet Microbiol*. 2000;74(1-2):141-7.
- Katz JM, Veguilla V, Belser JA, Maines TR, Van Hoeven N, Pappas C, et al. The public health impact of avian influenza viruses. *Poult Sci*. 2009;88(4):872-9.
- Zhou L, Liao Q, Dong L, Huai Y, Bai T, Xiang N. Risk factors for human illness with avian influenza A (H5N1) virus infection in China. *J Infect Dis*. 2009;199(12):1726-34.
- Nili H, Asasi K. Avian influenza (H9N2) outbreak in Iran. *Avian Dis*. 2003;47(3 Suppl):828-31.
- Fereidouni SR, Aghakhan M, Werner O, Starick E, Bozorghmehrifard MH. Isolation and identification of avian influenza viruses from migratory birds in Iran. *Br Veterinary Assoc*; 2005. p. 526-.
- Wikipedia TFE. Qom.
- Tracking & treating anthrax. Biological terrorism resources for health care providers. *AWHONN Lifelines*. 2001;5(6):31-5.
- Karimi V, Ghalyanchi Langeroudi, A., Fard, M.H.B., Mahboudi, F., Barin, A., Kheiri, M.T Amino Acid Sequence Analysis of Hemagglutinin Protein of H9N2 Isolated from Broilers in Tehran in 2007. . *Iranian Journal of Virology*. 2008;1(3):15-9.
- Ghalyanchi Langeroudi A, Karimi V, Kheiri MT, Fard MHB, Mahboudi F, Barin A. Nucleotide and Amino Acid Sequence Analysis of Hemagglutinin Protein in Cleavage Site Region of H9N2 Isolated from Broilers in Tehran Province during 1998-2007. *Journal of Animal and Veterinary Advances*. 2008;7(5):529-34.
- Concepts and procedures for laboratory-based influenza surveillance. Sect. B-17-B-44 (2002).
- Hoffmann E, Stech, J., Guan, Y., Webster, R.G., Perez, D.R.,. Universal primer set for the full-length amplification of all influenza A viruses. *Arch Virol*. 2001;146:2275-89.
- Guerra HL, Sardinha TM, da Rosa AP, Lima e Costa MF. [Effectiveness of the yellow fever vaccine 17D: an epidemiologic evaluation in health services]. *Rev Panam Salud Publica*. 1997;2(2):115-20.
- Akey BL. Low-pathogenicity H7N2 avian influenza outbreak in Virginia during 2002. *Avian Dis*. 2003;47(3 Suppl):1099-103.
- Cardona C, Yee K, Carpenter T. Are live bird markets reservoirs of avian influenza? *Poult Sci*. 2009;88(4):856-9.
- Jadhao SJ, Nguyen DC, Uyeki TM, Shaw M, Maines T, Rowe T, et al. Genetic analysis of avian influenza A viruses isolated from domestic waterfowl in live-bird markets of Hanoi, Vietnam, preceding fatal H5N1 human infections in 2004. *Arch Virol*. 2009;154(8):1249-61.
- Senne DA, Suarez DL, Pedersen JC, Panigrahy B. Molecular and biological characteristics of H5 and H7 avian influenza viruses in live-bird markets of the northeastern United States, 1994-2001. *Avian Dis*. 2003;47(3 Suppl):898-904.
- Bulaga LL, Garber L, Senne D, Myers TJ, Good R, Wainwright S, et al. Descriptive and surveillance studies of suppliers to New York and New Jersey retail live-bird markets. *Avian Dis*. 2003;47(3 Suppl):1169-76.
- Bulaga LL, Garber L, Senne DA, Myers TJ, Good R, Wainwright S, et al. Epidemiologic and surveillance studies on avian influenza in live-bird markets in New

York and New Jersey, 2001. Avian Dis. 2003;47(3 Suppl):996-1001.

23. Panigrahy B, Senne DA, Pedersen JC. Avian influenza virus subtypes inside and

outside the live bird markets, 1993-2000: a spatial and temporal relationship. Avian Dis. 2002;46(2):298-307.