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Short Communication

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

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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 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).

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

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

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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	GAAACAAGGTAGTTTTTTACT
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 8ºC, or at -20ºC 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

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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.

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