Molecular Surveillance of Avian Influenza in Bird Parks of Tehran, Iran

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Abstract:

BACKGROUND: Avian influenza (AI) viruses have been isolated from a wide diversity of free-living avian species representing several orders. Since 1998, H9N2 AI outbreaks have been one of the major problems in Iranian poultry industry. In 2006, H5N1 was reported in swans in the north of Iran first , but until now there has been no official report from commercial flocks in Iran. **OBJECTIVES:** The aim of this study was Molecular Surveillance of Avian Influenza in Bird Parks of Tehran, Iran. **METHODS:** In this study, 100 fecal samples from different avian species of Public and Bird Parks (The avian species included Pigeon, Duck, Swan, Parrot, Crow and Sparrow) were collected in Tehran, in the central region of Iran during November and December 2009. RNA extraction and RT-PCR have been done according the WHO Instruction for detection of Influenza Type A. **RESULTS:** In 14% of samples genetic materials (RNA) were detected. Species including duck and sparrow were positive. **CONCLUSIONS:** This is the first report of AIV detection in this these species in Iran. Due to emergence of new H1N1 influenza and bird flu throughout the world and in regional countries, surveillance programs for monitoring the spread of these viruses need to be redesigned. Surveillance activities for AI in wild birds should be continued to provide further virological (subtype) and epidemiological (Phylogenic Study) information about circulating viruses.

Introduction

Avian influenza (AI) has emerged as a disease with significant potential to disrupt commercial poultry production often resulting in extensive losses (Alexander, 2000). 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 (Fields et al., 2007; Lamb, 2007). 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 (Alexander). Virtually all HA and NA combinations have been isolated from birds(Fields et al., 2007). Several wild and migratory birds serve as reservoirs and/or mechanical vectors (simply carrying a pathogen or dispersing infected arthropod vectors) for numerous infectious agents. An association with transmission from birds to humans was identified for 10 pathogens.

Wild birds including migratory species may play a significant role in the epidemiology of influenza A virus. The available evidence suggests wild birds play a limited role in human infectious diseases. Direct transmission of an infectious agent from wild birds to humans is rarely identified. Potential factors and mechanisms involved in the transmission of infectious agents from birds to humans need further elucidation (Tsiodras et al., 2008). Annual epidemics and occasional pandemics of influenza in humans depend on the continued evolution of influenza viruses. Although they have numerous potential host populations, most of the available genetic and biologic data are obtained from studies of domestic populations of species such as chickens, turkeys, swine, and horses. Concerning wildlife populations, including wild populations of these domesticated species, much less is known (Webby et al., 2007). Bird Parks are manmade systems in which captive birds are in close contact with free-flying birds, staff and visitors. Bird Parks provide an ideal tool for genetic mixing and spreading of the influenza virus because they bring together numerous hosts (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. Bird Parks are therefore hypothesized to be a missing link in the epidemiology of AIV and it is important that they be routinely monitored for AIV. Since 1998, H9N2 AI outbreaks have been one of the major problems in the Iranian poultry industry. In 2006, H5N1 was first reported in swans in the north of Iran, but to date there has been no official report from commercial flocks in Iran (Nili and Asasi, 2003). Nevertheless, Iranian researchers have reported detection of other subtypes of avian influenza virus from migratory birds in Iran (Fereidouni et al., 2005). In this study, Molecular Surveillance of AI has been done in Bird Parks of Tehran that are located in important geographical regions.

Materials and Methods

Study Area: Tehran is the capital and largest city of Iran, and the administrative center of Tehran Province. Tehran is a sprawling city at the foot of the Tochal mountain range with an immense network of highways unparalleled in Western Asia. The city is famous for its numerous resorts on the Alborz slopes, large museums, art centers, and palace complexes. Tehran is the largest city in the Middle East and is the 16th most populated city in the world with a population of 8,429,807 and is one of Iran's largest urban areas (Wikipedia, 2010).

Sample Collection: Sample Collection (100 Fecal swabs) was performed according to the standard method from Bird Parks in Tehran. Sampling was from various species such as Duck (7), Swan (5), Parrot (18), Crow (27) Pigeons (18) and Chicken (25). Swabs from similar species within a cage were pooled (3 samples together). 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) (2001; Ghalyanchi Langeroudi et al., 2008; Karimi, 2008).

RNA Extraction: Total RNA was extracted with RNA extraction kit (Bioneer, South Korea) according to the manufacturer's instruction. The extracted total RNA was stored at -70°C until used (Ghalyanchi Langeroudi et al., 2008; Nili and Asasi, 2003; WHO, 2002).

RT-PCR: Reverse transcription was done by using oligonucleotide influenza universal primer, uni12, with "Revert Aid" first strand cDNA synthesis Kit (Fermentas, Canada) (Hoffmann, 2001). Amplification was carried out by PCR as described 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, 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. Also, for a more precise survey, positive samples were checked with other primers (MF, MR) that could detect Influenza A. Primers sequences are available in Table 1 (WHO, 2002). The reaction mixture (50 µL) contained 5 µL of cDNA, 15 pmoles of forward and reverse primers (4 µL), and 25 µL Normal PCR master mix. The PCR reaction is done in 2 minutes at 94ºC, 30 cycles including 60 seconds at 94ºC, 60 seconds at 50ºC, 180 seconds at 72ºC, and finally 10 minutes in 72ºC as a final extension. After

amplification, samples were stored either overnight at 2 to 8ºC, or at -20ºC for longer-term storage (WHO, 2002). 5 µL of the PCR products were mixed with 1 µL loading buffer and were then electrophoresed on 1.5% agarose gel in Tris-borate EDTA buffer (Guerra et al., 2000) .

Results

Genetic materials (RNA) were detected in 14% of our pooled samples. Species including duck and sparrow were positive.

Discussion

Since 1998, H9N2 AI outbreaks have been one of the major problems in the Iranian poultry industry. In 2006, H5N1 was first reported in swans in the north of Iran, but to date, there has been no official report from commercial flocks in Iran (Nili and Asasi, 2003). Nevertheless, Iranian researchers have reported detection of other subtypes of avian influenza virus from migratory birds in Iran (Fereidouni et al., 2005). According the previous surveillance program in 2008, there are no reports on the presence of AI in public Zoos and parks of Tehran (Non-Published Data). Since 1997, multiple avian influenza virus subtypes have been transmitted directly from domestic poultry to humans, causing a spectrum of human disease from asymptomatic to severe and fatal. To assess the pandemic risk that avian influenza viruses pose, multiple strategies have been used to better understand the capacity of avian viruses to infect, cause disease, and transmit among mammals, including humans. Seroepidemiologic studies that evaluate the frequency and risk of human infection with avian influenza viruses in populations with exposure to domestic or wild birds can provide a better understanding of the pandemic potential of avian influenza subtypes. Among poultry workers, butchering and exposure to sick poultry were risk factors for antibody to H5 virus, which provided evidence for infection (Katz et al., 2009). This is the first report of AIV detection in this these species in Iran. Bird Parks have a critical role in emergence of new influenza strains throughout the world and affect public health. Due to the emergence of new H1N1 influenza and bird flu worldwide and in regional countries, surveillance programs for monitoring the spread of these viruses need to be redesigned. Also, continual testing of these birds is justified to ensure that H5 or H7 AIV is not transmitted to human population or commercial poultry farms. Surveillance activities for AI in wild birds should be continued to provide further epidemiological information about circulating viruses. The oldest report of occurrence of Influenza in Zoo was related to the of Hong Kong influenza A (H3N2) virus infection in the Budapest Zoo (Romvary and Tanyi, 1975). In several studies, researchers use form Fecal swabs and RT-PCR method for surveillance of AIV in wild birds and Zoo (Ghersi et al., 2009; Haynes et al., 2009; Karlsson et al., 2007; Pereda et al., 2008). Environmental sampling to monitor AIV in wild bird populations may be a valid alternative to the more-invasive and capture-dependent methods based on cloacae sampling (Pannwitzet al., 2009). In a Serological study, the indirect ELISA was used to detect antibodies to influenza virus A in the sera of wildfowl from the Donana National Park. Infection rates were not high, the wide range of avian species susceptible to AIV A suggests circulation of the virus amongst wildfowl at Donana (Astorga et al., 1994). In an experimental design, sparrows were susceptible to severe infection with H5N1 (Boon et al., 2007). Recent influenza (H5N1) viruses are pathogenic for small terrestrial bird species but the rate of intraspecies transmission in these hosts is very low. Transmission and persistence of AIV among wildlife remains an unresolved issue because it depends on

Table 1. Primers sequence that was used in Avian Influenza Molecular Surveillance in Bird Parks of Tehran (WHO, 2002).

No	Primer Name	Sequences
1	Uni12	AGCAAAAGCAGG
2	HA-1144	GGAATGATAGATGGNTGGTAYGG
	HA-Reverse	ATATCGTCTCGTATTAGTAGAAAC AAGGGTGTTTT
3	M-WSN-8	GAAGGTAGATATTGAAAGATG
	M-1023R	GAAACAAGGTAGTTTTTTACT
4	MF	GGTCTTGTCTTTAGCCAYTCCA
	MR	AGGTCGAAACGTAYGTTCTCTCTA

both the ecology of the host (e.g. population density, migration) and on the environment (e.g. AIV persistence in water). For example, in one study, researchers developed a mathematical model that accounts for both AIV epidemics and bird community dynamics. Water-borne transmission is, however, the main determinant of the disease dynamics and observed prevalence level (Roche et al., 2009), so Bird Park managers (especially in suspected case) should regulate instructions for water source of cages. Researchers recently found that feathers could carry the risk for zoonotic infection from infected wild swans by H5N1 (Yamamoto et al., 2009) and this increased the risk of AIV transmission in infected Bird Park. In this regard, the mentioned park managers should use effective methods for disposal of cage waste. As natural hosts for AIV, wild birds, particularly aquatic birds, are the primary reservoir for transmission of AIV to domestic poultry. Therefore, more surveillance programs could continue in order to track new AIV strains in commercial poultry farms in and around Tehran. Vaccination is a useful strategy in Bird Park (Bertelsen et al., 2007; Furger et al., 2008), We recommend the mentioned method for control of AI in high risk parks in Iran. Bird park staff and visitors should be educated about AI. We conclude that as Bird Parks have a vital role for the outbreak of new Pandemics, more detailed and expansive surveillance programs should be done in other regions of Iran for integration of a precise epidemiological map of AI. Continuous surveillance would improve our understanding of the real role of Bird Park in ecology of influenza viruses in Iran and identify any changes in subtype prevalence. Also, our studies on molecular sub typing and phylogenetic study of positive samples in this survey should continue(Boyce et al., 2009; Griot and Hoop, 2007).

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پایش مولکولی آنفلوانزای پرندگان در پارک‌های پرندگان شهر تهران

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چکیده

زمینه مطالعه: ویروس آنفلوانزای پرندگان از طیف وسیعی از پرندگان آزاد زیست از رده‌های گوناگون پرندگان جداشده است. از سال ۱۹۹۸ تاکنون همه‌گیری آنفلوانزای پرندگان H9N2 بعنوان یک معضل در صنعت طیور کشور مطرح گردیده است. در سال ۲۰۰۶ گزارش وقوع H5N1 در قوها در شمال کشور مشاده گردید اما تاکنون گزارش رسمی در گله‌های تجاری وجود ندارد. **هدف:** هدف از این مطالعه بررسی پایش مولکولی انفلوانزای پرندگان در پارک‌های پرندگان استان تهران بوده است. **روش کار:** در این مطالعه ۱۰۰ نمونه سواپ مدفوعی از گونه‌ای مختلف پرندگان از پارک‌های پرندگان در تهران از گونه‌های مختلف (کبوتر، اردک، قو، طوطی، کلاغ، نجشک) در آذر و آبان ماه سال ۲۰۰۹ جمع آوری شد. استخراج RNA و واکنش RT-PCR بر اساس دستورالعمل WHO جهت تشخیص تیپ A ویروس آنفلوانزا صورت پذیرفت. **نتایج:** در ۱۴٪ نمونه‌ها مثبت گردیدند. **نتیجه‌گیری نهایی:** گونه‌های اردک و گنجشک مثبت گردیدند. این اولین گزارش مثبت آنفلوانزای پرندگان در این گونه‌ها در ایران می‌باشد. با توجه به شیوع آنفلوانزای H1 N1 و آنفلونزای پرندگان در منطقه و همچنین اهمیت بهداشت عمومی پارک پرندگان زنده نیاز به بازنگری پایش آنفلوانزای پرندگان ضروری بنظر می‌رسد. پایش بیشتر آنفلوانزای پرندگان در پرندگان وحشی جهت تعیین تحت تیپ و مطالعات شجره‌شناسی ضروری بنظر می‌رسد.

واژه‌های کلیدی: آنفلوانزای پرندگان، ایران، باغ وحش.

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