

A Review of Epidemiological Studies, Pathogenesis and Genetically Ateration in Influenza Viruses Circulating in Iran

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ABSTRACT

Avian influenza is one of the most important and serious virus diseases that has been reported all over the world. So far 16 Subtype HA (H1-16) and 9 NA subtypes (N1-9) have been identified. According to the pathogenicity of these viruses in birds are classified to low pathogenicity to high pathogenicity groups. Adaptation of these viruses in aquatic birds caused these birds as a natural reservoir of the virus. These viruses can break down the barrier between species and transmitted to mammals such as pigs, horses and humans. Using key words such as avian influenza, highly pathogenic types of influenza, influenza epidemiology and genetics of poultry and some other related words, in PubMed, Medline, and SID databases have obtained many documents, mostly for aspects specific for the pathogen or genetic. Finally, of 3760 documents, about 150 related articles were used for this article. The results showed that H9N2 viruses circulating widely in poultry flocks and those who are in contact with poultry. It seems to be in line with variation in the virus infecting humans. Therefore, further investigation and studies ongoing care is essential in order to fight the swine flu. Wild bird's play a very important role in the spread of infection between different subtypes of influenza virus. Therefore, pay particular attention to these birds in the implementation of patient care is recommended.

Keywords: Epidemiological, Influenza Viruses, Pathogenesis, Iran

INTRODUCTION

Influenza virus is the RNA coating type viruses and from family Orthomyxoviridae that causative agent of acute communicable and transferable between man and animal [1]. It has three serotypes C and B, A is the only serotype A is pathogenic in birds [2]. The genome of the virus is eight-piece that encoded several proteins, including nucleoprotein, polymerase internal protein PB1, PB1-F2, PB2 and PA, surface glycoproteins hemagglutinin and neuraminidase, matrix proteins NS1 and NS2 M1 and M2 and non-structural protein. Subtypes based on the two antigens hemagglutinin and neuraminidase are Divided. It is detected 16 subtype hemagglutinin (H1-16) and 9 neuraminidase subtypes (N1-9) for serotypes A [3, 5].

Today, two new subtypes H17N10 and H18N11 isolated from bats and have been reported in China. Avian influenza is highly infectious viral disease in chickens, turkeys and many other birds caused by different subtypes of type A influenza virus [6, 7].

Infection with the virus may have spread to mammals, particularly humans. Current knowledge indicates that the health risks posed by the so-called subtypes have low pathogenicity (LPAI) is lower than the risk of subtypes of highly pathogenic (HPAI) [8].

Epidemiological studies the spread of avian flu in Iran

Flu epidemics spread around the world as seasonal and cause of killing between 250,000 to 500,000 people each year. Each year an average 41,400 people have died by influenza in the United States of America between 1979 and 2001.

Viruses are usually transmit to other species and may cause devastating outbreaks in domestic birds or have such a pandemic in humans. Influenza virus type A patients made three Pandemic in the recent century and killed tens of millions [9]. Additionally the three pandemic viruses emerged a new strain in humans. Most of these new strains occur when the virus is transmitting to humans from other animal species, or when an existing human strain, earn new genes from a virus that usually infects birds or pigs. Influenza A viruses based on the antibody response to this virus can be divided into different serotypes [10, 11].

Serotypes that have been confirmed in humans based on the known human pandemic deaths are:

H1N1, H2N2, H3N2, H5N1, HTN7, H1N2, H9N2, HTN2, H7N3, H10N7.

An avian strain named H5N1 after emerging in Asia in the 1990s, causing a new pandemic of influenza, but still a form of virus that can easily spread among people has not created [12].

In Iran, like many other Asian countries such as Pakistan, China, Saudi Arabia and Korea after the early detection of avian influenza virus H9N2 in 1998, the virus was endemic in the country. Isolates of the virus in poultry farms is common in almost over our country and all viruses H9N2 subtype isolated from vaccinated chickens [13].

H9N2 viruses have been identified as a virus with low pathogenicity in experimental conditions. Although mortality over 60% in some regions on Iran and the Middle East as well as some Asian countries like Pakistan reported [14].

Many of the risk factors that may be responsible for the high mortality H9N2 bird flu virus with virulence are low, identify and have been reported to improve the control of this issue is essential [15, 16].

H9N2 avian influenza viruses in birds in Asia have been prevented in Europe. Although viruses usually cause to moderate disease, but be accompanied by infection with other pathogens, these findings suggest that the avian influenza virus in a wide range of hosts, from fish to mammals [17, 19].

H9N2 virus responsible for the outbreak of the disease recently in 1994 and 1999 in birds in other parts of the world including European countries (Germany, Italy and Ireland), Middle East (Iran, Pakistan and Saudi Arabia) have been the United States and South Africa. Therefore, evaluating the H9N2 subtype of avian influenza viruses is essential [20].

Karimnejad and Kind Pour [2012] by testing serum (**ELISA**) and HI and molecular RT-PCR method to determine contamination of cattle for Poultry Meat (with signs breathing) to the avian influenza virus in the province have reported that tested RT PCR, out of 300 serum samples were obtained from 274 and 264 cases respectively in ELISA and HI of H9N2 positive [21]. Positive sera antibody against influenza viruses H7N7 and H5N1 have not seen. The results indicate that the pandemic H9N2 influenza virus plays an important respiratory disease in poultry farms in Fars province is Poultry Meat [22]. Moreover, it was found that despite the presence of high titer antibody influenza virus in infected flocks) with very high casualties, (it has little effect on protecting birds of antibodies against the disease [23].

Total according to research findings sufficient evidence for the claim on the virus subtype H9N2 alone capable of creating havoc and there is a significant decrease in egg production but in field condition its accompaniment with other pathogens (bacterial and viral) it possibility causes very high mortality and sever decreased egg production [24].

Pathogenicity influenza virus subtype H9N2

Clinical diagnosis of avian influenza is very complex because the virus does not cause microscopic lesions clinically Patogenomic. Especially in aquatic birds are the reservoirs of the virus infection without specific symptoms [25]. These birds as carriers, they can be excreted virus in domestic fowl creates lesions in domestic birds in a pathologic subtypes based on the pathotype, the host species and the presence of secondary infections, is different. In order to determine the type of the severity and scope of the waste generated in SPF chickens injected (intravenous) by the influenza virus a / chicken / Iran / 259/1998 (H9N2) [26].

also the organ tropism virus, Hablolvarid et al. [2003] reported a study in which interstitial nephritis tube (Tubolointerstitial nephritis) and swelling of the pancreas (Pancreatitis) the most important waste was found a method of NP virus IHC (immuno histochemistry (**IHC**)) has been tracking the tubules as well as the pancreas [27]. The results showed that the low pathogenic virus in chickens (Low pathogenic) and tend to epithelial tissue (Epitheliotrope) [27]. In another study, the same researchers (Hablolvarid et al. [2004] to study the histopathological

lesions caused by endotracheal inoculation of influenza virus A / Chicken / Iran / 259/1998 (H9N2) and reported that these strains are low pathogenic and the desire to cover tissue and endotracheal insemination tend to tissue trachea, lung (Pneumotropic) and kidney (Nephrotropic) and the injury (ies) [28]. In similar studies Hadipour and colleagues (2010) to detect viral antigen and histopathological lesions in broiler chickens inoculated with influenza virus subtype A / Chicken / Iran / SH-110/99 (H9N2) and A / Chicken / Iran / 772/99 (H9N2) and similar results Hablolvaridand colleagues (2003) reported [29].

Boroujerdi et al. [2010] reported localized pathological lesions (in the lungs and trachea mice, BALB / C inoculated (through the nose) by influenza virus subtypes A / Chicken / Iran / B263 / 106 (H0N2) have been reported [30]. Finding showed that influenza virus H9N2 of them could bring disease in mammals. They concluded that natural infection of humans with avian influenza viruses and the emergence of the phenomenon of recombination could be a new pandemic [30].

In total, according to the findings of the studies seems that the reason for the low pathogenic strain of H9N2 influenza virus. These viruses in natural and experimental infections (endotracheal or nasal) are able to cause infection in the respiratory tract and kidney [31].

Genetic variations influenza virus subtype H9N2 gene mutations HA study the phylogenetic tree (Tree Phylogenetic) virus H9N2, indicating that two different branches of the virus, the first branch virus H9N2 American and the second virus H9N2 Eurasian (Category Eurasia) [32]. Viruses branch of Eurasia can be divided into three sub-divided. Sub first isolates from Korea and Japan, which represents virus96 / MS96 / korea / chicken / A sub-second, representing the included A / Quail / Hong Kong / G1 / 97 virus influenza viruses H9N2 isolated from humans in Hong Kong and viruses H9N2 isolated from the Middle East and Pakistan and sub the third includes viruses isolated from China (Like- Beijing), which represents viruses a / Duck / Hong Kong / Y280 / 97 and a / chicken / Beijing / 1/94 [33].

H9N2 subtypes for the first time in 1998 in Iran with the 1998/259 / Iran / chicken / A in the research and diagnosis of poultry diseases Razi Institute and Faculty of Veterinary Medicine, Tehran was isolated [34].

Although during the last decade many reports HA and NA genes on molecular characterization and analysis phylogenic influenza virus (H9N2) (the country has 35 little knowledge about the molecular characterization and analysis of gene phylogenic nucleoprotein (NP: Nucleoprotein) virus bird flu (H9N2) is available for all countries [35].

Homayounimehr et al. [2010] analysis of genes line Nucleotides and phylogenetic HA .Ten strains isolated from influenza virus H9N2 were compared in Gen Bank [36]. All 10 strains studied similar prototype line (-R-S-S-R / G-L-) were hemagglutination break in the local area. Iranian strain HA gene nucleotide line99/1% - 95/2% were matched together.

Methods of diagnosis, prevention and treatment of influenza subtype H9N2

Different methods have been used for detection and identification of influenza subtypes. Method based on hemagglutination inhibition (HI) and neuraminidase (NI) has been recognized as a standard method [37]. But this method has some limitations, such as cross-reactions, personal perceptions and sensitivity and specificity is low (38). RNA sequence and sequencing of the hemagglutinin a sensitive and accurate method for this purpose, but the procedure is time consuming and expensive [39]. PCR-RT as a quick and effective method to detect influenza subtypes and by various researchers has been used [40, 41]. But in some cases loss of sensitivity and false-negative results have been reported

Influenza viruses are RNA viruses and have no bearing on their genome is capable of regenerative properties and therefore an appropriate basis for hemagglutinin and neuraminidase gene mutations and significant changes in nucleic acid provided [42]. It features a complete mismatch primer with target sequence in the hemagglutinin gene and lead to a connection between two nucleotide non-complementary opposite is inconsistent, especially if the connection at the end of 'the primer is the third sensitivity a high level PCR reaction decreased. LEE et al. [2001] was shown that inconvenient connections at the end of '3 hemagglutinin gene-specific primers, PCR-RT is to reduce allergic reactions if perfect adaptation matrix gene target sequence provides early identification of influenza viruses been made. PCR amplification success depends on proper primer design [43].

Vaccination and drug treatment in Subtype H9N2 influenza

Influenza vaccine has been accessible more than 60 years and vast experience during this period has shown a good safety and effectiveness of it. It is known that the effect of vaccination in reducing of hospitalization and death in a population were at risk for severe complications [44].

Two types of vaccines containing killed viruses or passive (in the form of injection) and live attenuated viruses (in nasal spray form is using over the world. vaccine contains killed viruses or passive used in Iran. The injection of killed vaccine is effective (in range of 70 to 90 percent) in preventing the disease. In addition, it can decrease the hospital admissions due to influenza by up to 50 percent.

Studies have shown influenza vaccination of broiler chickens with a killed vaccine, induces poor immune response and lower antibody titration. Therefore it is needed to use of drugs immunostimulant, to increased immune response along with the vaccine [45].

Different studies have been conducted about the effect of immunomodulator drugs and Immune stimulant, to improve the efficiency of bird flu vaccine. Mayahi et al. [2007] were investigated the effect of levamisole on the humeral immune response of broiler chickens against flu A vaccine H9N2 subtype of bird. The results showed that the use of levamisole in simultaneously with vaccination against influenza disease, increased anti-influenza antibody titer [46].

Rajput et al [2007] were studied effect of seed extract *Mudorica* on antibody response to vaccine included virus subtype H5N1 in broiler chickens during the breeding period and concluded that the extract can stimulate the immune system [47].

Zamani Moghaddam and colleagues [2000] in experimental study evaluated the immunogenicity of the some type of killed flu vaccine subtype H9N2 in chickens and reported that of all the groups vaccinated with killed influenza vaccine (subtype H9N2), disposal viral had decreased(48). Vaccine produced in Iran (manufactured by Razi Institute (RI) and the construction of Tehran Faculty of Veterinary Medicine (FVM)) compared with foreign-made vaccine were reduced further virus excretion.

In another study Vasfi Marandi et al. [2002] designed a vaccine with oil emulsion for poultry flu, consists of 4 parts oil Adjuvant ISA-70 and an inactivated antigen of A / Chickne / Iran / ZMT-101 (101) / 98 (H9N2) by formalin to 2 weeks subcutaneously (+ a reminder after ten weeks in laying hens) vaccinated and then after weekly blood tests and HI test serum titre attempted [49].

Half of the chickens at the age of 8 weeks (broilers) and 27 weeks (egg-laying chickens) were infected intratracheally and intravenously by H9N2 strain.

Two weeks after inoculation in both experimental groups and the control group (non-vaccinated) high-titer was observed.

At the same time, the amount of virus isolation from tracheal swabs and cloacae (shedding) was reduced in vaccinated chickens. While non-vaccinated chicks 3 days after challenge had fallen ill and decreased their eggs [52]. The researchers concluded that H9N2 avian influenza inactivated Oil vaccine (emulsion) can reduce virus excretion and egg production in field conditions [50].

In another study, Moghadam Pour et al. [2003] examined the safety level of both domestic and imported poultry flu oily killed vaccine in breeder flocks and declared it was significant difference between the geometric mean antibody titer between the two groups [51].

Hooshmand et al. [2011] in a study to evaluate the efficacy of H9N2 avian influenza oily killed vaccine manufacturing Razi Vaccine and Serum Research Institute against strains in Fars province. They concluded that the vaccine effectively prevents the Replication and virus shedding in broiler chickens [45].

In study by Ebrahimi et al. [2012] reported that the binding C terminal domain of *Mycobacterium tuberculosis* HSP70 to influenza matrixprotein (M2e) and its expression in *Escherichia coli* (E coli) develop a recombinant effective vaccine [31]. Fusion proteins of4xM2e.HSP70c with one dose of intramuscular injection (formulated in buffer F105) to Balb / C mice resulted in complete protection against lethal doses of Iranian isolates of H1N1, H3N2, H9N2 [52].

CONCLUSION

Despite Iranian isolates of influenza virus are categorized in low pathogenicity H9N2 viruses but in conditions along with other pathogens (bacteria and viruses) can cause severe clinical symptoms and the development of large-scale casualties and severe loss of egg production reducers. Research has been done shows that the vaccination against H9N2 influenza virus can be beneficial to reduce virus excretion and decrease in egg production.

Iranian isolates of influenza virus H9N2 undergone genetic variation in some of their genes that made a difference pathogen and changed of effectiveness of vaccines and drugs in the treatment and prevention of it.

However, major changes have not taken place in the hemagglutinin protein breakdown region, in accordance with the characteristic of highly pathogenic viruses [53].

Since the H9N2 virus in Iran based on Qa / HK / G1 / 97 genes with sharing NA and HA, NP protein and circulation of H9N2 viruses in poultry flocks, and those who are in contact with poultry, it seems changes in the virus in order to infect humans.

Therefore, further investigation and ongoing care is needed in order to fight the flu. Wild birds play an important role in the spread of infection with different subtypes of influenza virus. Therefore, it is recommended to special attention to birds in disease surveillance programs.

Consistent implementation of disease surveillance program and avian influenza is essential in the country at different levels (industrial farms, domestic poultry and wild birds) to determine the changes in circulating viruses and preventive and treatment decisions.

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