Iranian Journal of Virology 2013;7(3): 7-11

Original Article

Phylogenetic Study Based on the Gene of Attachment Protein (G) Avian Metapneumovirus from Broiler Breeder farm in Iran, 2013

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Abstract

Background and Aims: Avian metapneumovirus (aMPV) is RNA virus responsible for upper respiratory disease in poultry which is usually accompanied by secondary bacterial infections. In Iran, some molecular and serological studies indicated that aMPV infection occurred in commercial poultry industry.

Materials and Methods: In this study aMPV was molecularly identified and characterized in an affected broiler breeder flock. The well-vaccinated flock was suffering from a disease with clinical signs and a drop in egg production. After RNA extraction from tracheal and oropharyngeal samples, the presence of viral RNA was demonstrated by reverse transcription-polymerase chain reaction using primers specific to the attachment glycoprotein(G) gene. Positive samples were sequenced and phylogenetic tree based on sequences results was drawn. Based on sequences analysis, field strain of aMPV was recovered from the flock.

Results: Phylogenetic analysis showed that the virus (PCRLAB/HG2013) is located in B subtype cluster and is closely related to the aMPV strain that was previously detected in Iran (PCRLAB/HG2010) and other countries (Nigeria, Japan and Brazil).

Conclusion: The results showed that field strain of aMPV has the potential to cause disease and economic losses in breeder broiler especially during egg production period. It is also concluded that vaccination against aMPV may not provide full protection in field situation and other control measurements including biosecurity should be taken.

Keywords: Avian metapneumovirus; Iran; Phylogenetic analysis; Broiler Breeder

Introduction

Avian metapneumovirus (known as avian pneumovirus or aMPV) cause respiratory diseases and/or egg drops in species such as turkey, chicken, Muscovy or Pekin duck (1). First reported in the late 1970s in South Africa, and subsequently in France and the UK, aMPVs have now been described worldwide (2). The aMPV causes turkey rhinotracheitis (TRT) and swollen head syndrome (SHS) of chickens, which is usually accompanied by secondary bacterial infections that increase mortality (3, 4).

The aMPV (Family Paramyxoviridae, Subfamily: Pneumovirinae, Genus: Metapneumovirus) is a negative-sense,

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[DOI: 10.21859/isv.7.3.7]

nonsegmented single-stranded RNA virus that contains eight genes in the order 3-N-P-MF-M2-SH-G-L-5 (5, 6). Two different subtypes, designated A and B, exist within a single serotype. Also, subtype C and D was reported in the United States and France respectively. (2, 7, 8). One of the major surface glycoproteins of aMPV is the attachment (G) protein which, by analogy with respiratory syncytial virus (RSV), has been proposed to be responsible for virus binding to its cell receptor. The aMPV G gene and its predicted protein have several features in common with their RSV counterparts (9).

Clinical signs are not pathognomonic for a diagnosis of aMPV. A diagnosis may be made by serology, reverse transcription-polymerase chain reaction (RT-PCR) or virus isolation. Virus isolation of aMPV is time consuming and difficult. Current serology tests include enzyme linked immunosorbent assay (ELISA), or virus neutralization (VN) or immunofluorescence (FA). The RT-PCR can be used for detection and differentiation of aMPV. Tracheal swabs and turbinates are considered to be the most appropriate sources of aMPV.

In Iran, some serological studies indicated that aMPV infection is endemic in commercial broiler, layer and breeder farm (10). Also, we reported the first molecular detection of AMPV in broiler farm in Iran, 2010 (11). In this report, we characterized aMPV (PCRLAB/HG2013: detected in broiler breeder farm) type B based on G gene in Iran.

Methods

Case history

In December 2013, an outbreak of avian metapneumovirus occurred in a 32 weeks old broiler breeder farm in Mazandaran Province. Mazandaran province is in the north of Iran and located on the southern coast of the Caspian Sea. Three different vaccines including live and killed aMPV vaccine were administrated before production. Two live vaccines were delivered to the flock at 31 and 70 day -old by drinking water vaccination method and a killed vaccine was intramuscularly injected to the flock at 20 week-old.

At peak of lay, tracheal rales and drop in egg production and egg shell quality were the first clinical signs which were observed in this flock. In post mortem examination of the dead and cull birds, swollen head syndrome and hyperemic trachea were observed. The egg drop rate was about 13.5%. Also, the flock show decrease about 4% in hatching eggs by changes in egg shell color and thickness. In order identification of causative agent, samples from affected birds have been submitted to PCR Veterinary Diagnostic Laboratory (Tehran, Iran).

RNA extraction and RT-PCR

Tracheal and oropharyngeal swabs from flock were subjected to RNA extraction by RNeasy Mini Kit (QIAGEN, USA) according to the manufacturer's procedure. The aMPV detection and typing primers used in this study were previously evaluated by Bayon-Auboyer *et al* (1999) and Mase *et al* (12). The screening of flocks was performed with the primer pair Nd/Nx and the Ga-G2-, Ga-G12 primers are specific for aMPV subtypes A and B respectively (Table 1) (13). RT-PCR program were performed as described by authors previously (11). The presence of some other endemic and critical avian disease agents such as Newcastle disease virus (NDV), infectious bronchitis virus (IBV) and avian influenza virus (AIV) have been put to test as well (Data are not shown).

Sequence and phylogenetic analysis

RT-PCR (Ga-G12) product was purified using the Qiagen purification kit (Qiangen, USA) and submitted for an automated sequencing in both directions. Nucleotide and predicted amino acid sequences data were aligned with Clustal alignment algorithm by CLC Sequence Viewer. The sequence of PCRLAB/HG2013 was compared with other aMPV genomic sequence data available in GenBank. A phylogenetic tree was created with the program MEGA 5 (Molecular Evolutionary Genetics Analysis, version 3.1) using the Kimura two-

[DOI: 10.21859/isv.7.3.7]

Table 1. Polymerase chain reaction primers used for avian pneumovirus (AMPV) molecular detection and typing (13).

Primer Name	Gene Name	Sequence (5' ------> 3')
Nd	N	AGC AGG ATG GAG AGC CTC TTT G
Nx	N	CAT GGC CCA ACA TTA TGT T
Ga	G	CCG GGA CAA GTA TCT CTA TGG
G2-	G	CCA CAC TTG AAA GAT CTA CCC
G12-	G	CAG TCG CCT GTA ATC TTC TAG GG

parameter model and the neighbor-joining algorithm with 1000 bootstraps. The sequence of G protein gene of PCRLAB/HG2013 has been deposited under accession number KJ933440 in GenBank.

Results

Sequence analysis of the G protein testified that field strain of aMPV and not vaccine strain was circulating in the flock. Other avian pathogens (NDV, IBV, and AIV) have not been found in the flock by molecular investigation. The G protein gene of PCRLAB/HG2013 has high identities to Iranian isolate PCRLAB/HG2010 (99.06%), VCO3/60616 [Japan, 2010] (99.80%), Nigeria/NIR89/2006 [Nigeria, 2006] (99.08%) and turkey rhinotracheitis virus (strain 2119) [99.08%]. Based on phylogenetic analysis (NJ method) PCRLAB/HG2013 has been located in subtype B cluster with previous Iranian aMPV isolate from Broiler farm (PCRLAB/HG2010).

Discussion

At the end of the 1970s in South Africa, a new and acute respiratory, aMPV infection, process appeared, which affected 3-4 week old turkeys, but it has since been isolated in Europe, Israel, Asia and United States. The aMPV is now considered a major disease threat in both turkeys and chickens in many parts of the world (4, 5, 14). Clinical signs of aMPV infection in chickens may include swelling of the periorbital tissue and infraorbital sinuses, torticollis, cerebral disorientation, and opisthotonos. Although widespread respiratory signs are usually observed, mortality caused by aMPV is less than 2%, and less than 4% of the flock will show swelling of the head (15). In broiler breeders and commercial layers, egg production and egg quality are frequently affected (16). In previous studies, serologically has been approved that aMPV infection is endemic in commercial broiler, layer, breeder (10). In order to prevention of disease both types of live and killed vaccines are authorized for use in broiler breeder farms in Iran.

The putative attachment glycoprotein of aMPV, G, is the most variable protein between aMPV subtypes (5). It has been showed that Iranian field strain can be easily differentiated from available commercial vaccine strains by sequence of G protein gene (11). Based on sequence, the virus was detected in the flock is a field strain. In the absence of other pathogen, detected aMPV can be consider as causative agent of the clinical signs and egg drop production. The former studies had been shown that priming with two or three live vaccines following with inactivated vaccine can induce complete protection. Afterward, some evidences have suggested that field strains may undergo evolution mechanisms to overcome bird's immunity or increase virulence (Jones, and Rautenschlein 2013). This can resulted in disease even in vaccinated flocks.

[DOI: 10.21859/isv.7.3.7]

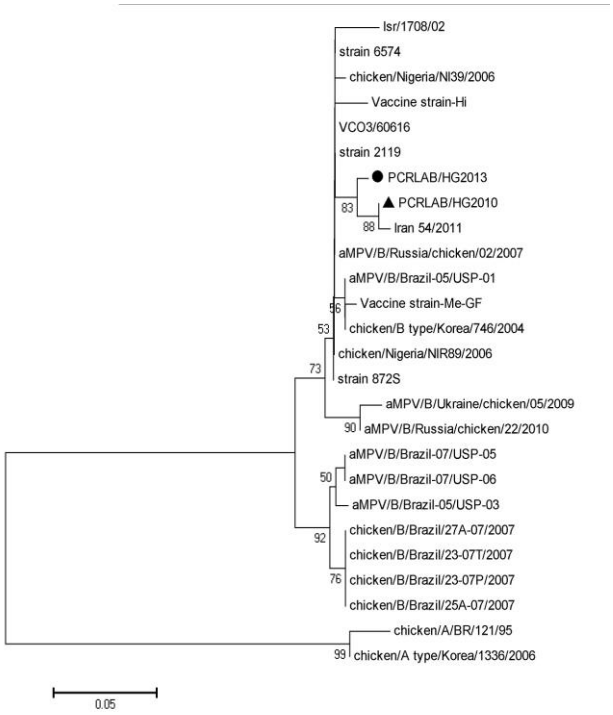

Fig. 1. Nucleic acid Phylogenetic relationships of G glycoprotein gene of avian metapneomovirus genome isolated from Broiler Breeder farm, Iran. Horizontal distances are proportional to the minimum number of Nucleic acid differences required to join nodes. The vertical lines are for spacing branches and labels. The scale bar represents the distance unit between sequence pairs. The virus genome characterized in this report is indicated as Black Circle. Black triangle is indicated for previous isolate from broiler farm in Iran. The sequences obtained from GenBank.

Hitherto, only B subtype of aMPV have been reported from Iran. The probability of circulating of other subtypes cannot be rule out due to few sequences available from Iran. It can be assumed that B subtype is dominant type of aMPV in poultry farm circulating in the country in view of the fact that other subtypes have not been detected yet. Similarly, aMPV subtype B was characterized earlier in Israeli and Jordanian poultry (15). This similarity with Brazil stains which probably reflects the policy of that country to import chicken from most major poultry producing countries in Europe and Asia.

Causing clinical disease by field strain of aMPV shows that vaccination regimen couldn't provide complete protection and for prevention of the disease farmers cannot rely on only vaccines. Understanding mechanism of overcoming bird's immunity by aMPV need further studies. However, development of new generation of aMPV vaccines have been proposed (Jones, and Rautenschlein 2013). By that time, other preventive strategy must be establish in order to control the disease.

Acknowledgment

We would like to thank experts of PCR Veterinary Diagnostic Laboratory for their technical supports.

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[DOI: 10.21859/isv.7.3.7]

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[DOI: 10.21859/isv.7.3.7]