Original Article

Molecular characterization of common respiratory viral infections in broilers in Al-Hassa, Eastern Province, Saudi Arabia

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Abstract

Newcastle disease virus (NDV), Infectious bronchitis virus (IBV) and H9N2 avian influenza virus (AIV) are the most significant respiratory pathogens. Several outbreaks have been reported frequently in Saudi Arabia despite of the intensive use of vaccination programs. Ten broiler flocks suffering from complicated respiratory disease were subjected to necropsy and samples were collected for molecular identification of involved viruses and pathogenic bacterial infections. Seven out of the ten flocks were positive for single or combined viral infection. The seven flocks were positive for HA test. All positive samples were subjected for molecular identification, three flocks were positive for single NDV while one flock was positive for both NDV and IBV. Three flocks were positive for H9N2 and IBV. Three flocks were negative for viral infection and subjected for pathogenic bacteria detection. The sequence analysis of F gene of NDV revealed that all isolates exhibited the cleavage site (^{112}RRQKRF^{117}) of virulent NDVs. The neuraminidase-haemagglutinin gene revealed that all AI isolates belonged to H9N2 low pathogenic avian influenza virus (LPAIV) subtype. The S1 gene of IBV showed that one isolate was closely related to the Connecticut IBV strain while another isolate belonged to the H120 IBV strain. In conclusion, combined pathogens causing respiratory infection in broilers is very common, which leads to the increase in disease severity and economic losses. The continuous detection of virulent NDV as well as IBV from broiler flocks emphasize the need to review the vaccination strategies based on further epidemiological and molecular studies.

Keywords: H9N2AIV, IBV, NDV, phylogenetic analysis, sequencing, Saudi Arabia

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Introduction

Respiratory tract infections are considered of greatest importance in poultry industry worldwide, causing high economic losses. The major economic losses due to high morbidity and mortality of diseases in addition to treatment cost and carcass condemnation are frequently high (Ayim Akonor et al., 2013). Many reports mentioned a wide variety of both bacterial and viral pathogens responsible for respiratory diseases in poultry, particularly *Escherichia coli (E. coli)*, *Mycoplasma gallisepticum (MG)*, *Mycoplasma synoviae (MS)*, *Ornithobacterium rhinotracheale (ORT)*, NDV, IBV and AIV, which can cause disease alone or in mixed infections (Roussan et al., 2011; Ayim-Akonor et al., 2013; Mehrabanpour et al., 2014).

NDV is a negative-sense, single-stranded RNA enveloped virus and has only one serotype designed as avian paramyxovirus-1 (APMV-1) belonging to the family *Paramyxoviridae* (Radwan et al., 2013). NDVs are classified according to genetic diversities into two classes, Class I is generally of low virulence while Class II exhibits the majority of virulence and contains eighteen genotypes and subgenotypes (Alexander, 1997; Kim et al., 2013; Wang et al., 2015). According to the Office International des Epizooties (OIE), at least 250 species of birds are susceptible to NDV without fully understanding the route of transmission (Miller et al., 2015; Boroomand et al., 2016). NDV is classified into three pathotypes: lentogenic, mesogenic, and velogenic (Alexander, 1997; Ayim-Akonor et al., 2013).

IBV is an enveloped, positive-sense, ssRNA genome which belongs to the genus *Coronaviridae* of the family *Coronaviridae*. More than 50 serotypes have been reported since the first identification of IBV in 1936 (Roh et al., 2013; Bande et al., 2016). IBV can cause 100% morbidity with 25-80% mortality in young chicks. IBV primarily infects epithelial cells of the upper respiratory tract, resulting in various clinical signs including sneeze, gasp, tracheal rales and nasal discharges. Depending on virus cell tropism, epithelial cells in the kidney or oviduct can be infected, causing nephritis and decreased egg production, respectively (Jahantigh et al., 2013; Roh et al., 2013; Bande et al., 2016; Kiss et al., 2016).

AIV is a negative-sense, segmented (8 segments), single ssRNA, enveloped virus belonging to the Orthomyxoviridae family. Avian influenza virus belongs to type A influenza virus while types B and C might be restricted and cause mild infection to human (Bozorgi et al., 2012). Based on the antigenicity of the two surface proteins, Influenza A viruses have at least 18 subtypes of hemagglutinin (HA) and 11 subtypes of neuraminidase (NA). AIV can be divided into two groups depending on the severity of diseases in poultry: highly pathogenic avian influenza virus (HPAIV), which causes systemic infection with high mortality up to 100%, drop in egg production and occasionally nervous signs; and low pathogenic avian influenza virus (LPAIV), which causes mild infection and usually slight drop in egg production with mortality less than 5% (Seifi et al., 2010; Zhao et al., 2013).

It is important to identify the actual pathogens, particularly because similar clinical symptoms are elicited by different pathogens. However, the conventional virus isolation is time-consuming and laborious. Moreover, the serological methods may face problems of interspecies cross-reaction and non-specific reaction in some pathogens as well as interference with vaccination programs (Rashid et al., 2009; Ayim-Akonor et al., 2013). Therefore, the present study aimed to identify and analyze the evolutionary changes of NDV, IBV and AIV subtypes circulated in the eastern region of Saudi Arabia and involved in the respiratory manifestation of broiler chickens during 2015-2016.

Materials and Methods

Clinical samples: The study was conducted during the period from 2015 to 2016. Ten commercial broiler and backyard chicken flocks aged 14 to 28 days showing cough, gasp, nasal and ocular discharge, conjunctivitis and depression were investigated by clinical veterinarians at the Veterinary Teaching Hospital in King Faisal University. Nasal and oral swabs were collected from live chickens and placed in sterile phosphate buffered saline (PBS) (pH 7.4). Trachea and lungs were collected from freshly dead or scarified chicks, then ground in PBS, followed by three times of freezing and thawing. Supernatants were collected by centrifugation at 5000 rpm for 10 min, then stored at -80ºC until tested.

RNA extraction: Total RNA was extracted from the prepared samples utilizing QIAamp Viral RNA Mini Kit (QIAGEN, USA) according to the manufacturer's instructions. Briefly, 140 µL of the samples were lysed by adding 560 µL of AVL buffer enclosing carrier RNA, then incubated at room temperature for 10 min. After complete lysis 560 µL of absolute ethanol was added and mixed by pulse vortexing for 15 seconds. Aliquots of 630 µL were carried out sequentially to a QIAamp spin column, followed by centrifugation at 8.000 rpm for 1 min after each addition. The binding RNAs were washed by 500 µL of AW1 buffer and centrifuged at 8.000 rpm for 1 min, followed by 500 µL of AW2 buffer, then centrifuged at 14.000 rpm for 3 min. The RNAs were eluted in 50 µL of AVE buffer and stored at -80ºC until used.

Oligonucleotide primers: Primers used in this study were selected according to sequence data base, analyzed by OligoAnalyzer 3.1 Integrated DNA Technologies, USA and synthesized by Metabion International AG, Germany. The complete data of primers are listed in Table 1.

Molecular detection of NDV, IBV and AIV in clinical samples: The extracted RNAs were tested for the presence of NDV, IBV and AIV using One-step RT-PCR Kit (QIAGEN, USA). The RT-PCR reaction mixture consisted of 5 µL of the total RNA, 5 µL of 5x Qiagen one-step RT-PCR buffer, 5 µL of Q buffer, 1 µL of dNTPs mix, 1 µL (50 pmol) of each primers, 1 µL of the enzyme mix (containing RT and PCR reaction enzymes), and 6 µL of RNease free water. The RT-PCR

reaction was performed at 50°C for 30 min, then at 95°C for 15 min, followed by 40 cycles starting with denature step at 95°C for 30 s, primers annealing temperature according to Table 1 for 30 s and 72°C for 30 s and final extension step at 72°C for 10 min. The amplified PCR products were electrophoresed in 1.2% agarose gel containing 0.5 µg/mL ethidium bromide and documented using ultraviolet gel documentation system (BIORAD).

Table 1 Details of oligonucleotide primers

Pathogen	Type of primers	Primer's name	Sequence	Target gene	Expected product (bp)	Annealing Temp.	Reference
NDV	Detection & sequencing	APMV1-F-F	5/-ATGGGCYCCAGACYCTTCTAC-3/	F	535bp	60°C	(Radwan et al., 2013)
		APMV1-F-R	5/-CTGCCACTGCTAGTTGTGATAATCC-3/				
IBV	Detection	IBV(N+)	5/- GAAGAAAACCAGTCCCAGATGCTTGG -3/	N	453bp	60°C	(Handberg et al., 1999
		IBV(N-)	5/- GTTGGAATAGTGCGCTTGCAATACCG-3/				
	sequence	IBV-S1-F	5/- TGAAAACTGAACAAAAGACA -3/	S	1700bp	50°C	(Kwon et al., 1993) (Bourogaa et al., 2012)
		IBV-S1-R	5/- TTCAGGTTAGCGGCTGGTC -3/				
AIV	Detection	M52C-M2-F	5/- CTTCTAACCGAGGTCGAAACG -3/	M2	244bp	52°C	(Mady et al., 2010)
		253R-M2-R	5/- AGGGCATTTTGGACAAAGCGTCTA -3/				
	H9 AIV typing	AIVHA-H9-544F	5/- ATTCAAGACGCCCAATACAC -3/	HA	549bp	52°C	(Tang et al., 2012)
		AIVHA-1092-R	5/- TGACCAACCTCCCTCTATGA -3/				
	N2 AIV typing	AIVN2-F	5/- GTAAAAATGAATCCAAATCAAAAG-3/	NA	1420bp	52°C	This study
		AIVN2-R	5/- GCTTATATAGACATGAAATTGATATTC-3/				

Amplification of S1 gene of IBV and H9 and N2 genes of AIV: The supernatants of IBV and AIV positive samples were filtered through monopyrogenic syringe filter (0.2 µM) and inoculated into five specific pathogen-free embryonated chicken eggs of 10 days old. The eggs were inoculated with 200 µl of the sample into the allantoic cavity, then incubated at 37°C with daily candling. Embryos that died within 24 hours were considered as nonspecific death and discarded. Allantoic fluids were harvested and further passaged twice blind successive passages were performed (Alexander and Senne, 2008). The allontoic fluids were harvested and stored at -80°C until used. The IBV and AIV RNAs were extracted from the allontoic fluids utilizing the QIAamp Viral RNA Mini Kit (QIAGEN, USA) as mentioned previously. The RNAs were reverse transcribed using SuperScript™ III Reverse Transcriptase (Invitogen). Ten µl of denatured RNA was mixed with 1 µL Random hexamer and 1 µL dNTPs 10 µM were heated at 65°C for 5 minutes and quickly chilled on ice. Four µL 5X buffer, 2 µL DTT, and 1 µL RNase free water, then incubated at 25°C for 2 min. One µL SuperScript™ III Reverse Transcriptase was added to the mixture. The RT reaction was performed at 25°C for 10 minutes and at 42°C for 50 min, then the reaction was inactivated at 70°C for 15 min. Five µL of each cDNA was used to amplify target gene by specific primers in 20 µL of the final volume of a 2X HotStartTaq Plus Master Mix (QIAGEN, USA) containing 1.5 mM $MgCl_2$, 200 µM of each dNTP, 1 unit HotStartTaq Plus DNA polymerase and 10 µM of each primer. Thermocycling conditions were enzyme activation and initial denaturation at 95°C for 5 min, followed by 35 cycles starting with denature step at 94°C for 30 s, primers annealing temperature according to Table 1 and extension at 72°C for 90 s and a final extension step at 72°C for 10 min.

Sequencing and phylogenetic tree construction: The amplicon bands for F gene of NDV, H9 and N2 for AIV and S1 gene for IBV were excised from agarose gel, then purified using Montage DNA gel extraction kit (Millipore, USA) and sequenced in an automated ABI 3730 DNA sequencer (Applied Biosystems, USA). Obtained sequences were aligned by the Clustal W method. Obtained nucleotide sequences were compared with available sequences in GenBank. A phylogenetic tree was constructed using MECA V5.20 software. Bootstrap values were calculated for 1000 replicates of the alignment.

GenBank accession number: The nucleotide sequences

obtained in this study were submitted to GenBank database with the accession numbers (MG051077, H9SA16; MG051078, H9SA17; MG051079, H9SA18; MG051080, N2 Saudi Arabia 1; MG051081, N2 Saudi Arabia 2; MG022111, F gene NDV1 SA; MG022112, F gene NDV2 SA; MG022113, F gene NDV 3 SA; MG022114, F gene NDV4 SA; MG022115, IBV SA1 and MG022116, IBV SA2).

Sequence analysis and phylogenetic tree construction: The genetic diversity and origin of the detected viruses circulating in broiler farms in Saudi Arabia were determined by sequence analysis and phylogenetic tree construction. The nucleotide sequences of a fusion gene including hypervariable region of the four detected Saudi field strains were compared with 40 reference strains. The Saudi isolates clustered along with the Iranian isolates within the genotype VIId (Fig. 1). The cleavage site of all investigated strains was ^{112}RRQKRF^{117}, which is the characteristic cleavage site of virulent NDVs.

The obtained 1700 bp PCR products of S1 gene of IBV was sequenced and aligned with the reference strains. The two Saudi field strains showed 94% nucleotide sequence similarities. One of the two Saudi strains (IBV/CH/SA1/2015) showed the highest nucleotide homology of 99% to the IBV Connecticut strain, whereas the second strain (IBV/CH/SA2/2015) showed the highest nucleotide homology of 99.7% to the H120 strain. The identity between IBV/CH/SA1/2015, IBV/CH/SA2/2015 and UK7-91 strains was 78% and 77%, respectively, whereas the identity between IBV/CH/SA1/2015, IBV/CH/SA2/2015 and 793-B strains was 80.9% and 79.8%, respectively (Fig. 2).

The detected AIV was subtyped by amplification of 549 bp of H9 hemagglutinin gene and 1420 bp of neuraminidase N2 gene. Phylogenetic analysis of the three detected Saudi field strains of partial hemagglutinin gene (HA) showed that the Saudi strains of H9N2 were grouped together with the Israeli strains from the G1 lineage (Fig. 3) and the N2 nucleotide sequences of two Saudi field strains showed high homology to the Egyptian strain and another to the Israeli strains of the G1 lineage (Fig. 4).

Results

Molecular detection of AIV, NDV and IBV in clinical samples: The results confirmed that 7 out of the 10 broiler flocks were positive for viral infection. All the positive samples were positive for HA activity. Molecular identification showed that three flocks were positive for single NDV while one flock was positive for both NDV and IBV. Three flocks were positive for H9N2 and IBV. Two out of the three negative flocks for viral infection were positive for pathogenic bacteria detection while one flock was negative for both viral and bacterial infection (Table 2).

Table 2 Details of molecular detection

Flock No.	NDV	H9N2	IBV	Pathogenic Bacteria
1	+	-	-	+
2	-	+	+	-
3	-	-	-	+
4	+	-	-	-
5	-	+	+	-
6	+	-	+	+
7	-	-	-	-
8	-	+	+	-
9	-	-	-	+
10	+	-	-	-

Discussion

Respiratory infection is common and of a major importance in poultry industry all over the world. Respiratory infection in broilers may result from single or mixed infections as many viruses and/or pathogenic bacteria could be involved in the respiratory disease complex. The present study used conserved primers for the detection of different viruses suspected to be involved in the respiratory manifestation according to case history, clinical signs and necropsy. All positive samples were subjected for molecular characterization and subtyping using specific primers.

The detection of H9N2 AIV, IBV and NDV in different combinations in poultry flocks clarifies that multiple viral infection is very common and may have a synergistic role (Roussan et al., 2011). The main indicator of NDV pathogenicity is the formation of active fusion protein from fusion gene (F-gene), which is characterized by the presence of basic amino acid sequences in fusion cleavage site, therefore, molecular pathotyping and phylogenetic analyses of the F gene is a major determinant of NDV virulence instead of conventional methods (Mohamed et al., 2011; Damena et al., 2016). Our results identified the presence of four field NDV strains in broiler chicken flocks. The sequence and phylogenetic tree analysis among the four Saudi strains detected revealed that all Saudi NDV strains were closely related to velogenic types of NDV and classified into genotype VII and subgenotype VIId. Genotype VII is the predominant NDV in Asian domestic poultry (Wang et al., 2015). These Saudi NDV strains clustered with Iranian virulent strain VII and caused outbreaks in 2010 to 2014 in commercial poultry farms as shown in Figure 1 (Langeroudi et al., 2013; Samadi et al., 2014; Boroomand et al., 2016). Wild bird may act as the

reservoir of NDV strains in avirulent form and cause virulent disease in chickens; this could be a worldwide distributor of velogenic pathotypes across regional and international boundaries (Kim et al., 2013; Shabbir et al., 2013; Miller et al., 2015; Damena et al., 2016). Moreover, all commercial NDV vaccines as Hitchner B1-47, Beaudette C-45, Clone30, Komarov-45-LK and LaSota belong to genotype II, which is different from our detected Saudi genotype VII. In addition, a previous study mentioned the challenged vaccinated chicken with LaSota vaccine by variant VII showing symptoms and VII causing disease in vaccinated chickens (Kim et al., 2013; Boroomand et al., 2016). In conclusion, the vaccine used in NDV control strategy in Saudi Arabia should be genotype matched with NDV field strains (genotype VII).

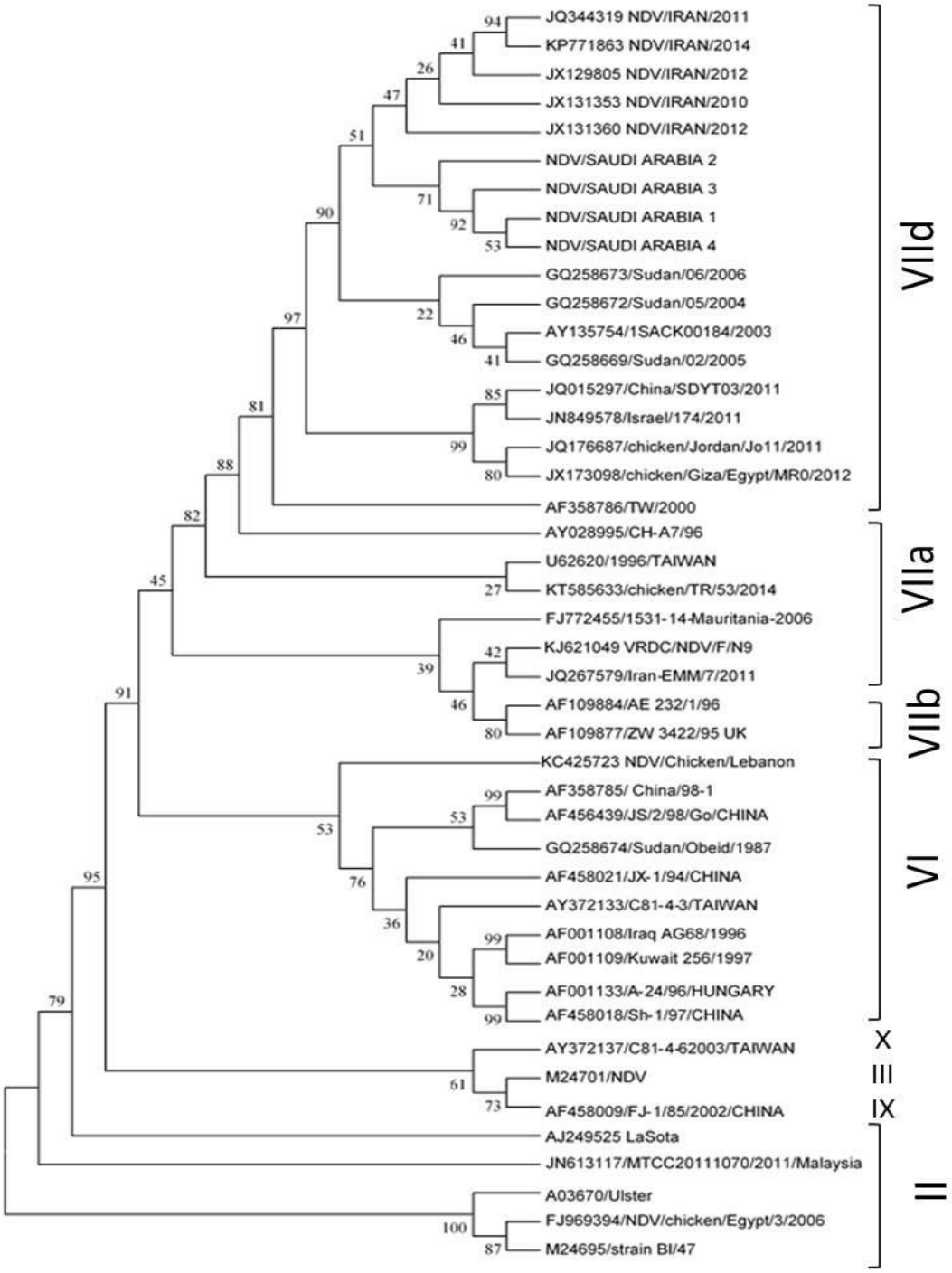

Figure 1 Phylogenetic analysis of the nucleotide sequences of the partial fusion gene of four detected Saudi NDV strains along with other sequences of the reference strains from GenBank by the neighbor-joining method with bootstrap 1000 replicates of MEGA version.

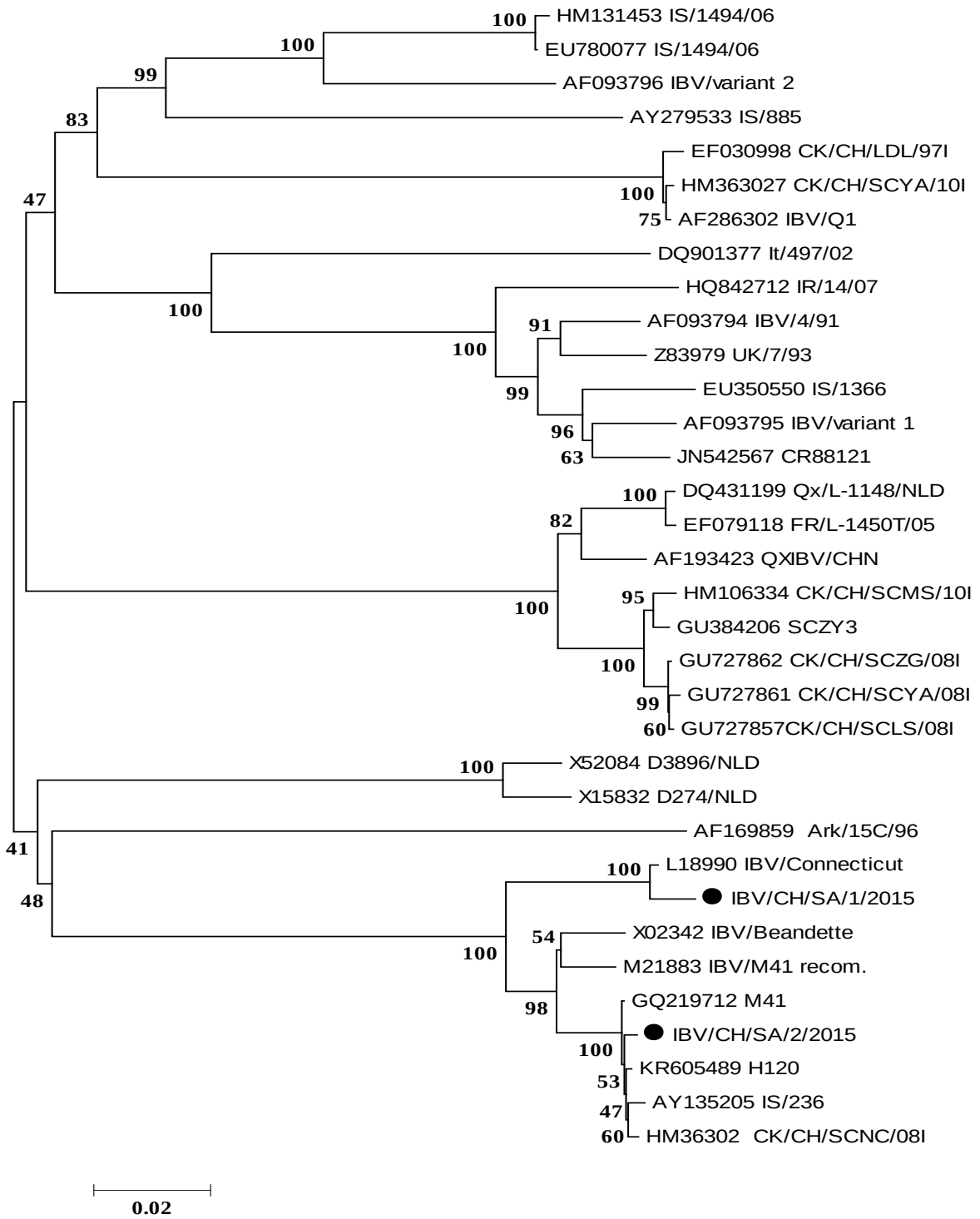

Figure 2 Phylogenetic tree of the nucleotide sequences of the spike (S1) gene of four detected Saudi strains compared with other sequences of the reference strains from GenBank by the neighbor-joining method with bootstrap 1000 replicates of MEGA version. Both Saudi IBV strains were marked with solid circle.

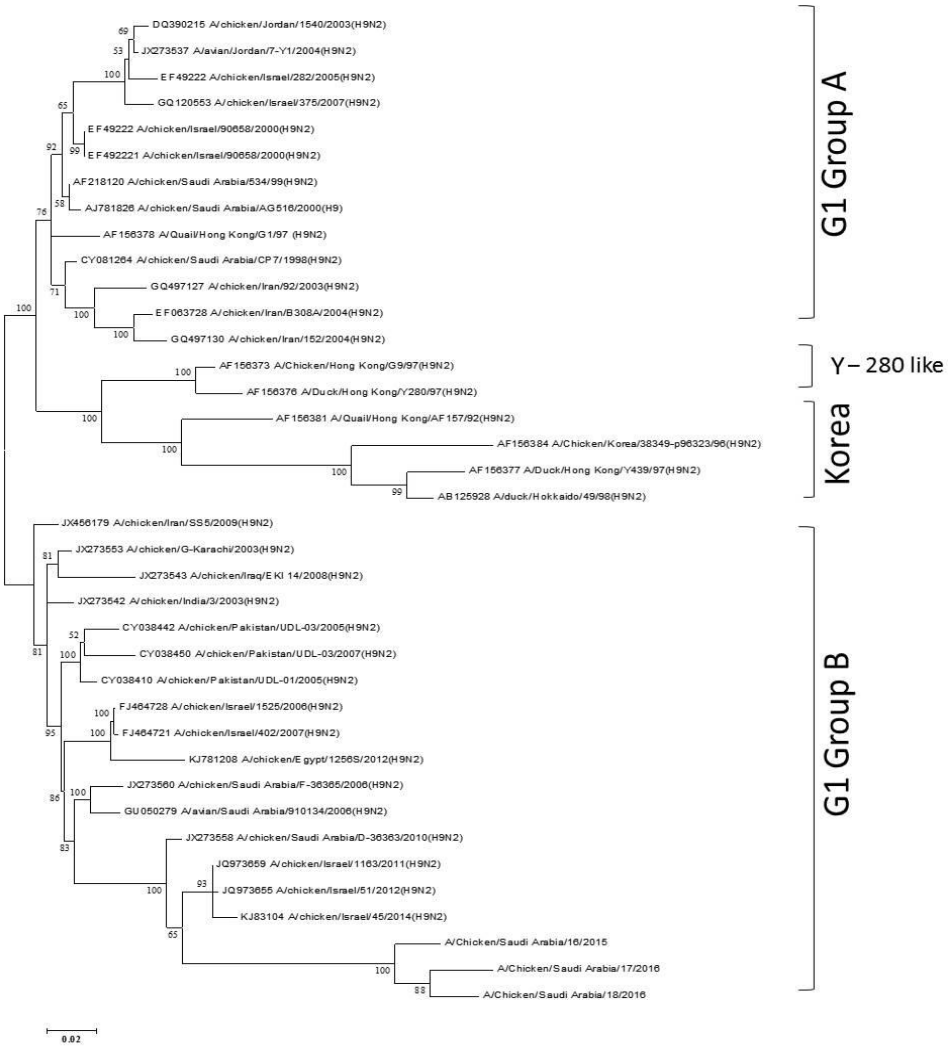

Figure 3 Phylogenetic tree of the nucleotide sequences of the hemagglutinin (HA) H9 gene of the three detected Saudi strains compared with other sequences of the reference strains from GenBank by the neighbor-joining method with bootstrap 1000 replicates of MEGA version.

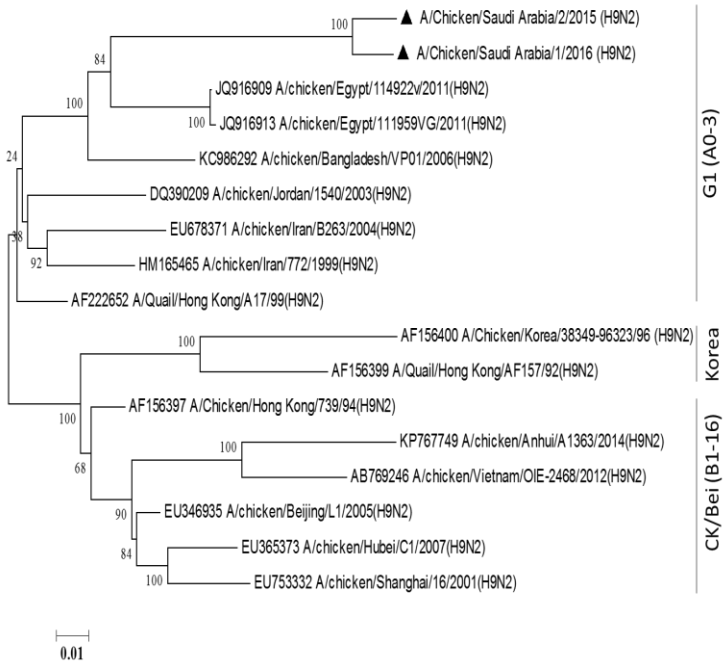

Figure 4 Phylogenetic tree of the nucleotide sequences of the neuraminidase N2 gene of two detected Saudi strains compared with other sequences of the reference strains from GenBank by the neighbor-joining method with bootstrap 1000 replicates of MEGA version. Both Saudi strains were marked with solid triangles.

IBV causes a contagious respiratory disease in chickens and one of the important diseases facing intensive poultry industries worldwide (Zou et al., 2010; Roh et al., 2013; Seger et al., 2016). Many serotypes of IBV have been described and this is due to the nature of coronaviruses, which frequently show point mutation or recombination, leading to the emerge of novel pathogenic variants. This genetic variant may, particularly, be due to changes in nucleotide sequence of the S1 gene, while the IBV genome remains unaltered (Kasem et al., 2015). The sequence and phylogenetic tree analysis of IBV S1 gene among the two detected Saudi strains with other strains from GenBank worldwide revealed that the Saudi 1 IBV strain clustered with the Connecticut IBV strain (L18990) while the Saudi 2 IBV strain clustered with the Massachusetts type vaccines such as M41 (GQ219712) and H120 (KR605489) as shown in figure 2. The nucleotide identities of S1 gene sequences of the Saudi 2 IBV strain showed 99.7% when compared to the Massachusetts types, M41 and H120, which are the most commonly used classical vaccines in Saudi Arabia. Therefore, the Saudi 2 IBV strain could be considered as a vaccinal strain, while the Saudi 1 IBV strain, showing 99% when compared to the Connecticut IBV strain, therefore, could be considered as a field strain. Ganapathy et al. (2015) demonstrated that examined strains that gave <99% homology, when compared with commercial vaccine strains, were considered as field strains and from 99-100% homology as vaccinal strains. Moreover, both Saudi 1 and 2 IBV strains showed 80.9%-79.8% homology, respectively, to the 793B vaccine strain, which supports many studies reporting that the common circulating IBV strain in the Middle East are Massachusetts serotype vaccine strains (H120 serotype vaccine) and in the past few years the vaccination against IBV is performed with 793B and D247 serotype vaccine strains (Awad et al., 2014; Ganapathy et al., 2015). Our result strongly supports the evidence of the spread of live vaccine virus in the field due to the ability of IBV genome for recombination and/or point mutation, particularly in S1 gene causing antigenic drift, leading to decrease in cross-protection and continuous persistence of the vaccine like virus. Therefore, field strain should be considered for the development of vaccine (Farsang et al., 2002; Ganapathy et al., 2015; Kasem et al., 2015).

Avian influenza (AI) is a highly contagious disease of poultry caused by avian influenza virus (AIV). Once introduced into domestic poultry, AIV may emerge through genetic shift and drift in two important envelope proteins, hemagglutinin (HA) and neuraminidase (NA) genes, which aid virus to escape, block, and reduce the neutralizing antibody response from host immunity (Lee et al., 2012; Elzahed et al., 2015; Shen et al., 2015). The LPAIV H9N2 virus is the most common virus which occur in domestic poultry populations worldwide with continuous circulation in China and the Middle East (Fusaro et al., 2011; Davidson et al., 2013). H9N2 virus in the Middle East has continuously evolved and has similar genes to those of the Asian H5N1 (Gharaibeh, 2008; Shanmuganatham et al., 2014). The molecular technique assays such as reverse transcription polymerase chain reaction (RT-PCR) are sensitive and rapid than the conventional serology methods for the detection and differentiation of AIV subtypes (Luan et al., 2016; Adel et al., 2017). Globally, H9N2 can be divided into two major lineages: the Eurasian and the North American. The Eurasian lineage can further be categorized into three sublineages: (1) the G1 lineage, A/quail/Hong Kong/G1/97 (G1-like); (2) the Y280 lineage as a member of (Y280-like): A/duck/Hong Kong/Y280/97, (G9-like): A/chicken/Hong Kong/ G9/97, and (BJ94-like): A/chicken/Beijing/1/94; (3) the Korean lineage as a member of (Korean-like): A/chicken/Korea/38349-p96323/96 and (Y439-like): A/duck/Hong Kong/Y439/97 (Langeroudi et al., 2013; Slomka et al., 2013). In this study, the phylogenetic analysis of the detected AIV strains revealed that the Saudi H9N2 viruses clustered within the G1 lineage along with other Middle East countries, suggesting derivation from the single ancestor, A/quail/Hong Kong/G1/97 (Parvin et al., 2014; Chaudhry et al., 2015). The sequence and phylogenetic tree analysis of AIV H9 gene among the three Saudi strains detected with other strains from GenBank worldwide revealed that the Saudi H9 AIV strain clustered together with the Israeli, Iranian, Egyptian, Pakistani, Iraqi, Indian and previous Saudi isolates in 2006-2010 as shown in Figure 3. Our results are in agreement with those of many studies reporting that these viruses have originated from a common source, mainly the A/quail/Hong Kong/G1/97 lineage (Davidson et al., 2013; Bahari et al., 2015; Peacock et al., 2016). The sequence and phylogenetic tree analysis of the AIV N2 gene among the detected Saudi strains also clustered to the A/quail/Hong Kong/A17/99 lineage as from Egypt, Jordan, Iran and Bangladesh as shown in Figure 4, in agreement with previous studies (Bahari et al., 2015). The Arabian Peninsula has shown a rapid increase in the imports of live poultry in the world and this may play an important role in the H9N2 virus transmission from Hong Kong and viral interspecies transfer (Fusaro et al., 2011; Shanmuganatham et al., 2014). The detected Saudi H9N2 virus was co-infected with infectious bronchitis virus (Roussan et al., 2009; Seifi et al., 2010).

In conclusion, our results clarify that the investigation into combined infection is very important and should be conducted regularly. Continuous investigation into the currently circulating pathogens and evaluation of vaccination strategies are necessary for improvement in the disease prevention and control.

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References

Adel A, Arafa A, Hussein HA and Elsanousi AA 2017. Molecular and antigenic traits on hemagglutinin gene of avian influenza H9N2 viruses: Evidence of a new escape mutant in Egypt adapted in quails. Res Vet Sci 112: 132-140.

Alexander DJ 1997. Newcastle disease and other avian Paramyxoviridae infections. In B.W. Calneck (10th ed.) Dis Poultry. p. 541-569.

Alexander DJ and Senne DA 2008. Newcastle disease virus and other avian paramyxoviryses. In: Dufour-Zavala L, Swayne DE, Glisson JR, Pearson JE, Reed WM, Jackwood MW, Woolcock PR, editors. A laboratory manual for the isolation, identification, and characterization of avian pathogens. 5th ed. Georgia: p. 135-141.

Awad F, Baylis M and Ganapathy K 2014. Detection of variant infectious bronchitis viruses in broiler flocks in Libya. IJVSM. 2(1): 78-82.

Ayim-Akonor M, Baryeh K and Asante IA 2013. Molecular Based Survey of Pathogens Associated with Respiratory Disease Outbreaks in Broiler Chickens in Accra. J Nat Sci Res. 3(10): 25-31.

Bahari P, Pourbakhsh SA, Shoushtari H and Bahmaninejad MA 2015. Molecular characterization of H9N2 avian influenza viruses isolated from vaccinated broiler chickens in northeast Iran. Trop Anim Health Prod. 47(6): 1195-1201.

Bande F, Arshad SS, Omar A, Bejo MH, Abubakar MS and Abba Y 2016. Pathogenesis and Diagnostic Approaches of Avian Infectious Bronchitis. Adv Virol. 11: 1-11.

Boroomand Z, Jafari RA and Mayahi M 2016. Molecular characterization and phylogenetic study of the fusion genes of Newcastle disease virus from the recent outbreaks in Ahvaz, Iran. Virus Dis J. 27(1): 102-105.

Bourogaa H, Hellal I, Hassen J, Fathallah I and Ghram A 2012. S1 gene sequence analysis of new variant isolates of avian infectious bronchitis virus in Tunisia. Veterinary Medicine: Res Reports. 3: 41-48.

Bozorgi A, Keyvanfar H, Shushtari H, Bahmaninejad M and Eshratabadi F 2012. Molecular characterization and phylogenetic analysis of hemagglutinin and neuraminidase genes of H9N2 avian influenza viruses isolated in Iran in 1999 and 2009. Afr J Microbiol Res. 6 (21): 4550-4556.

Chaudhry M, Angot A, Rashid HB, Cattoli G, Hussain M, Trovo G, Drago A, Valastro V, Thrusfield M, Welburn S, Eisler MC and Capua I 2015. Reassortant Avian Influenza A (H9N2) Viruses in Chickens in Retail Poultry Shops, Pakistan, 2009–2010. Emerg Infect Dis. 21(4): 673-676.

Damena D, Fusaro A, Sombo M, Belaineh R, Heidari A, Kebede A, Kidane M and Chaka H 2016. Characterization of Newcastle disease virus isolates obtained from outbreak cases in commercial chickens and wild pigeons in Ethiopia. Springerplus. 5: 476-453.

Davidson I, Shkoda I, Golender N, Perk S, Lapin K, Khinich Y and Panshin A 2013. Genetic characterization of HA gene of low pathogenic H9N2 influenza viruses isolated in Israel during 2006–2012 periods. Virus Genes. 46(2): 255-263.

Elzahed ME, Arafa A, Elsanousi AA and Shalaby MA 2015. Isolation and characterization of avian influenza from different species of ducks in delta region. Egypt J Virol. 12: 1-9.

Farsang A, Ros C, Renstrom LHM, Baule C, Soos T and Belak S 2002. Molecular epizootiology of infectious bronchitis virus in Sweden indicating the involvement of a vaccine strain. Avian Patho. 31(3): 229-236.

Fusaro A, Monne I, SalviatoA, Valastro V, Schivo A, Amarin NM, Gonzalez C, Ismail MM, Alankari A, Alblowi MH, Khan OA, Ali ASM, Hedayat A, Garcia JG, Ziay GM, Shoushtari A, Alqahtani KN, Capua I, Holmes EC and Cattoli G 2011. Phylogeography and Evolutionary History of Reassortant H9N2 Viruses with Potential Human Health Implications. J Virol. 85(16): 8413-8421.

Ganapathy K, Ball C and Forrester A 2015. Genotypes of infectious bronchitis viruses circulating in the Middle East between 2009 and 2014. Virus Res. 210: 198-204.

Gharaibeh S 2008. Pathogenicity of an Avian Influenza Virus Serotype H9N2 in Chickens. Avian Dis. 52(1): 106-110.

Handberg KJ, Nielsen OL, Pedersen MW and Jurgensen PH 1999. Detection and strain differentiation of infectious bronchitis virus in tracheal tissues from experimentally infected chickens by reverse transcription-polymerase chain reaction. Comparison with an immunohistochemical technique. Avian Patho. 28(4): 327-335.

Jahantigh M, Salari S and Hedayati M 2013. Detection of infectious bronchitis virus serotypes by reverse transcription polymerase chain reaction in broiler chickens. Springerplus. 2(1): 36-39.

Kasem S, Abdelkader A, Tahoon A and Shaban H 2015. Phylogentic analysis of recent infectious bronchitis virus isolates from broiler chicken farms in Kafrelsheikh, Egypt. Benha Vet Med J. 29(1): 189-195.

Kim SH, Wanasen N, Paldurai A, Xiao S, Collins PL and Samal SK 2013. Newcastle Disease Virus Fusion Protein Is the Major Contributor to Protective Immunity of Genotype-Matched Vaccine. PLoS ONE. 8(8): e74022.

Kiss I, Mato T, Homonnay Z, Kis TT and Palya V 2016. Successive occurrence of recombinant infectious bronchitis virus strains in restricted area of Middle East. Virus Evol. 2(2): 1-8.

Kwon HM, Jackwood AMW and Gelb JJr 1993. Differentiation of Infectious Bronchitis Virus Serotypes Using Polymerase Chain Reaction and Restriction Fragment Length Polymorphism Analysis. Avian Dis. 37(1): 194-202.

Langeroudi AG, Karimi V, Kheiri MT and Barin A 2013. Full-length characterization and phylogenetic analysis of hemagglutinin gene of H9N2 virus isolated from broilers in Iran during 1998–2007. Comp Clin Pathol. 22: 321–330.

Lee CH, Byun SH, Lee YJ and Mo IP 2012. Genetic evolution of the H9N2 avian influenza virus in Korean poultry farms. Virus Genes. 45: 38-47.

Luan L, Sun Z, Kaltenboeck B, Huang K, Li M, Peng D, Xu X, Ye J, Li J, Guo W and Wang C 2016. Detection of influenza A virus from live-bird market poultry swab samples in China by a pan-IAV, one-step reverse-transcription FRET-PCR. Sci Rep. 22(6): 30015.

Mady WH, El-Sanousi AA, Amer HM and El-Sabagh IM 2010. First year of the highly pathogenic avian influenza H5N1 Outbreak in Egypt: Rapid Antigenic/Molecular diagnosis and Virus isolation. Int J Virol. 6(2): 73-81.

Mehrabanpour MJ, Khoobyar S, Rahimian A, Nazari MB, and Keshtkar MR 2014. Phylogenetic characterization of the fusion genes of the Newcastle disease viruses isolated in Fars province poultry farms during 2009-2011. Vet Res Forum. 5(3): 187-191.

Miller PJ, Haddas R, Simanov L, Lublin A, Rehmani SF, Wajid A, Bibi T, Khan TA, Yaqub T, Setiyaningsih S and Afonso CL 2015. Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features. Infect Genet Evol. 29: 216-229.

Mohamed, MHA, Kumar S, Paldurai A and Samal SK 2011. Sequence analysis of fusion protein gene of Newcastle disease virus isolated from outbreaks in Egypt during 2006. Virol J. 8: 237.

Parvin R, Heenemann K, Halami MY, Chowdhury EH, Islam MR and Vahlenkamp TW 2014. Full-genome analysis of avian influenza virus H9N2 from Bangladesh reveals internal gene reassortments with two distinct highly pathogenic avian influenza viruses. Arch Virol. 159(7): 1651-1661.

Peacock T, Reddy K, James J, Adamiak B, Barclay W, Shelton H and Iqbal M 2016. Antigenic mapping of an H9N2 avian influenza virus reveals two discrete antigenic sites and a novel mechanism of immune escape. Sci Rep. 6: 18745.

Radwan MM, Darwish SF, El-Sabagh IM, El-Sanousi AA and Shalaby MA 2013. Isolation and molecular characterization of Newcastle disease virus genotypes II and VIId in Egypt between 2011 and 2012. Virus Genes. 47(2): 311-316.

Rashid S, Naeem K, Ahmed Z, Saddique N, Abbas MA and Malik SA 2009. Multiplex polymerase chain reaction for the detection and differentiation of avian influenza viruses and other poultry respiratory pathogens. Poultry Sci. 88(12): 2526-2531.

Roh HJ, Hilt DA, Williams SM and Jackwood MW 2013. Evaluation of Infectious Bronchitis Virus Arkansas-Type Vaccine Failure in Commercial Broilers. Avian Dis. 57(2): 248-259.

Roussan DA, Alrifai RH, Khawaldeh GY, Totanji WS, and Shaheen I 2011. Ornithobacterium rhinotracheale and Mycoplasma synoviae in broiler chickens in Jordan. Rev Sci Tech Off Int Epiz. 30(3): 931-937.

Roussan DA, Khawaldeh GY, Alrifai RH, Totanji WS and Shaheen IA 2009. Avian influenza virus H9 subtype in poultry flocks in Jordan. Prev Vet Med. 88(1): 77-81.

Samadi S, Kianizadeh M, Najafi MF, Mousavi Nasab SD, Davatgar AMH, Royaee A and Pilvar P 2014. Molecular characterization and phylogenetic study of velogenic Newcastle disease virus isolates in Iran. Virus Genes. 48(2): 290-295.

Seger W, Langeroudi AG, Karimi V, Madadgar O, Marandi MV and Hashemzadeh M 2016. Genotyping of infectious bronchitis viruses from broiler farms in Iraq during 2014-2015. Arch Virol. 161(5): 1229-1237.

Seifi S, Asasi K and Mohammdi A 2010. Natural co-infection caused by avian influenza H9 subtypes and infectious bronchitis viruses in broiler chicken farms. Veterinarski Arhiv. 80(2): 269-281.

Shabbir MZ, Zohari S, Yaqub T, Nazir J, Shabbir MA, Mukhtar N, Shafee M, Sajid M, Anees M, Abbas M, Khan MT, Ali AA, Ghafoor A, Ahad A, Channa AA, Anjum AA, Hussain N, Ahmad A, Goraya MU, Iqbal Z, Khan SA, Aslam HB, Zehra K, Sohail MU, Yaqub W, Ahmad N, Berg M and Munir M 2013. Genetic diversity of Newcastle disease virus in Pakistan: a countrywide perspective. Virol J. 10: 170.

Shanmuganatham K, Feeroz MM, Engel LJ, Walker D, Alam SMR, Hasan MK, Mckenzie P, Krauss S, Webby RJ and Webster RG 2014. Genesis of avian influenza H9N2 in Bangladesh. Emerg Microbes Infect. 3(e88): doi:10.1038/emi.2014.84.

Shen HQ, Yan ZQ, Zeng FG, Liao CT, Zhou QF, Qin JP, Xie QM, Bi YZ and Chen F 2015. Isolation and phylogenetic analysis of hemagglutinin gene of H9N2 influenza viruses from chickens in South China from 2012 to 2013. J Vet Sci. 16(3): 317-324.

Slomka MJ, Hanna, A, Mahmood S, Govil J, Krill D, Manvell RJ, Shell W, Arnold ME, Banks, J and Brown IH 2013. Phylogenetic and molecular characteristics of Eurasian H9 avian influenza viruses and their detection by two different H9-specific Real Time reverse transcriptase polymerase chain reaction tests. Vet Microbiol. 162(2-4): 530-542.

Tang Q, Wang J, Bao J, Sun H, Sun Y, Liu J and Pu J 2012. A multiplex RT-PCR assay for detection and differentiation of avian H3, H5, and H9 subtype influenza viruses and Newcastle disease viruses. J Virol Methods. 181(2): 164-169.

Wang JY, Liu WH, Ren JJ, Tang P, Wu N, Wu HY, Ching CD and Liu HJ 2015. Characterization of emerging Newcastle disease virus isolates in China. Virol J. 12: 119.

Zhao M, Liu Q, Sun Q, Zhang W, Zhao G, Gu M, Wang X, Hu S, Liu X and Liua X 2013. Full Genome Sequence of a Natural Reassortant H9N2 Avian Influenza Virus Isolated from Domestic Ducks in Jiangsu Province, China. Genome Announc. 1(4): e00463-13.

Zou NL, Zhao FF, Wang YP, Liu P, Cao SJ, Wen XT and Huang Y 2010. Genetic analysis revealed LX4 genotype strains of avian infectious bronchitis virus became predominant in recent years in Sichuan area, China. Virus Genes. 41(2): 202-209.

บทคัดย่อ

ลักษณะเฉพาะทางโมเลกุลของเชื้อไวรัสที่ก่อโรคระบบทางเดินหายใจในไก่กระทง ของเมืองอัล ฮาซา ภาคตะวันออกของประเทศซาอุดิอารเบีย

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เชื้อไวรัสที่ก่อโรคทางระบบทางเดินหายใจสำคัญ คือ เชื้อไวรัสนิวคาสเซิล (NDV) เชื้อไวรัสหลอดลมอักเสบ (IBV) และเชื้อไวรัสไข้หวัดนก (AIV) มีรายงานการระบาดของโรคเหล่านี้อย่างต่อเนื่องถึงแม้ว่าจะมีการใช้โปรแกรมวัคซีนที่เข้มงวด ฝูงไก่กระทงจำนวน 10 ฝูงที่ป่วยด้วยโรคระบบทางเดินหายใจ ถูกส่งมาชันสูตรซากและทำการเก็บตัวอย่างเนื้อเยื่อเพื่อศึกษาเชื้อไวรัสและแบคทีเรียที่เกี่ยวข้อง ฝูงไก่จำนวน 7 ฝูงให้ผลบวกต่อการติดเชื้อไวรัสชนิดเดียวและหลายชนิดร่วมกันและให้ผลบวกต่อ HA test ฝูงไก่จำนวน 3 ฝูงให้ผลบวกเฉพาะเชื้อ NDV ในขณะที่ฝูงไก่จำนวน 1 ฝูง ให้ผลบวกต่อเชื้อ NDV และ IBV ฝูงไก่จำนวน 3 ฝูง ให้ผลบวกต่อเชื้อ H9N2 และ IBV ฝูงไก่ จำนวน 3 ฝูงที่ให้ผลลบต่อเชื้อไวรัส ถูกนำส่งตรวจหาเชื้อแบคทีเรียต่อไป การศึกษาลำดับเบสของเชื้อ NDV ส่วนยีนเอฟ พบว่ามีตำแหน่งตัดที่ส่วน (^{112}RRQKRF^{117}) ยีน neuraminidase-haemagglutinin ของเชื้อ AIV พบว่าเป็นกลุ่มเชื้อที่มีความรุนแรงต่ำ (LPAIV) ยีน S1ของเชื้อ IBV จำนวน 1 เชื้อมีความใกล้เคียงกับสายพันธุ์Connecticut ในขณะที่เชื้ออื่นๆ จัดอยู่ในกลุ่ม H120 IBV สรุปโรคระบบทางเดินหายใจในไก่เป็นการติดเชื้อร่วม ซึ่งนำไปสู่ความรุนแรงและการสูญเสียทางเศรษฐกิจ การตรวจพบเชื้อ NDV และ IBV ในฝูงไก่กระทงควรนำมาพิจารณาในการทำโปรแกรมวัคซีนเพื่อการศึกษาทางระบาดวิทยาและชีวโมเลกุลของเชื้อไวรัสต่อไป

คำสำคัญ: H9N2AIV IBV NDV วิเคราะห์สายวิวัฒนาการ ตรวจสอบลำดับเบส ซาอุดิอารเบีย

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