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Original Article

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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 -80^{o}C 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 -80^{o}C 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)
		IBV-S1-R	5/- TTCAGGTTAGCGGCTGGTC -3/				(Bourogaa et al., 2012)
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).

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	+	-	-	-

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).

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).

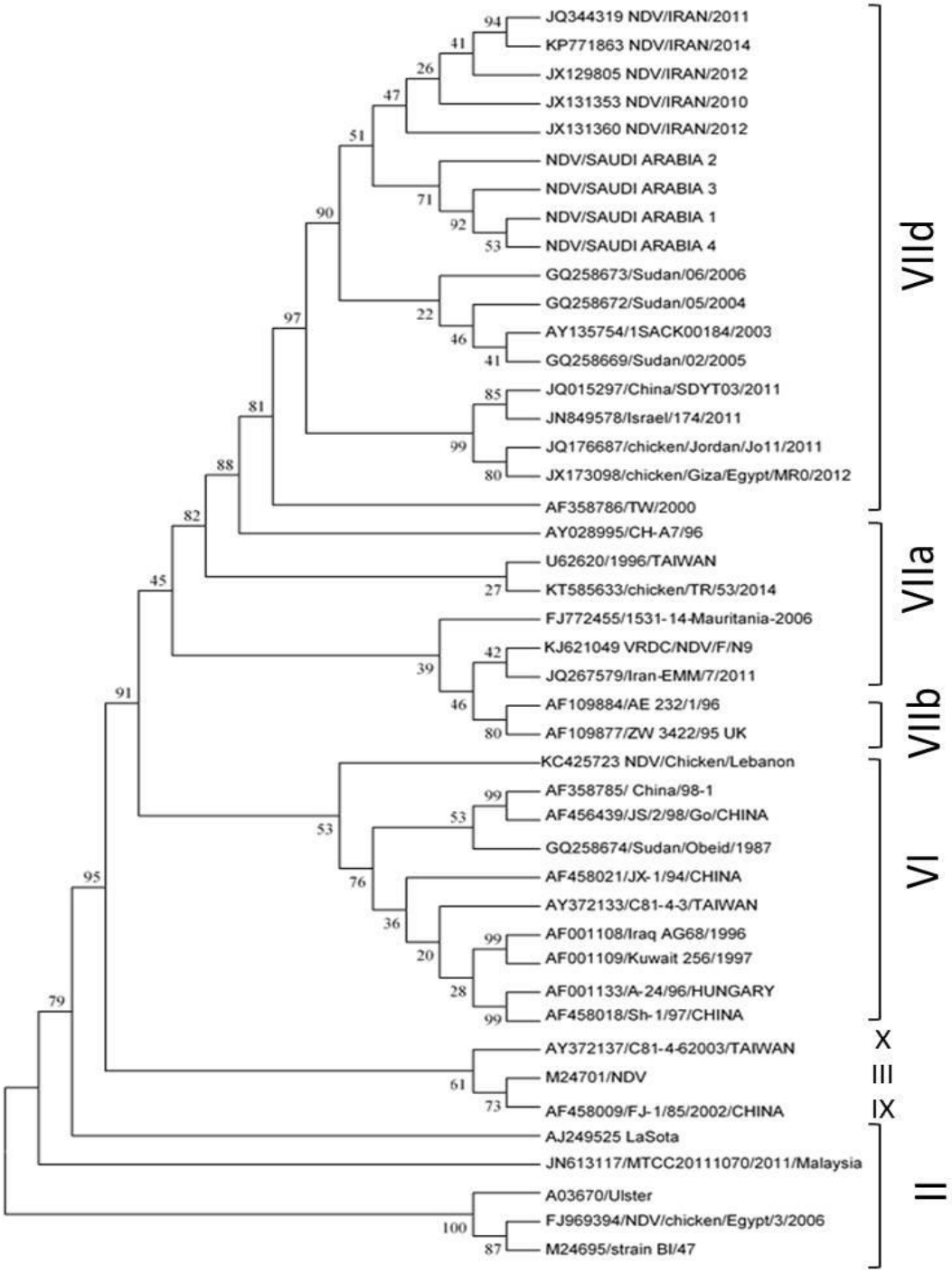

Figure 1 Phylogenetic analysis of the nucleotide sequences of the partial fusion gene of four detected Saudi NDV strains along with other sequences of the reference strains from GenBank by the neighbor-joining method with bootstrap 1000 replicates of MEGA version.

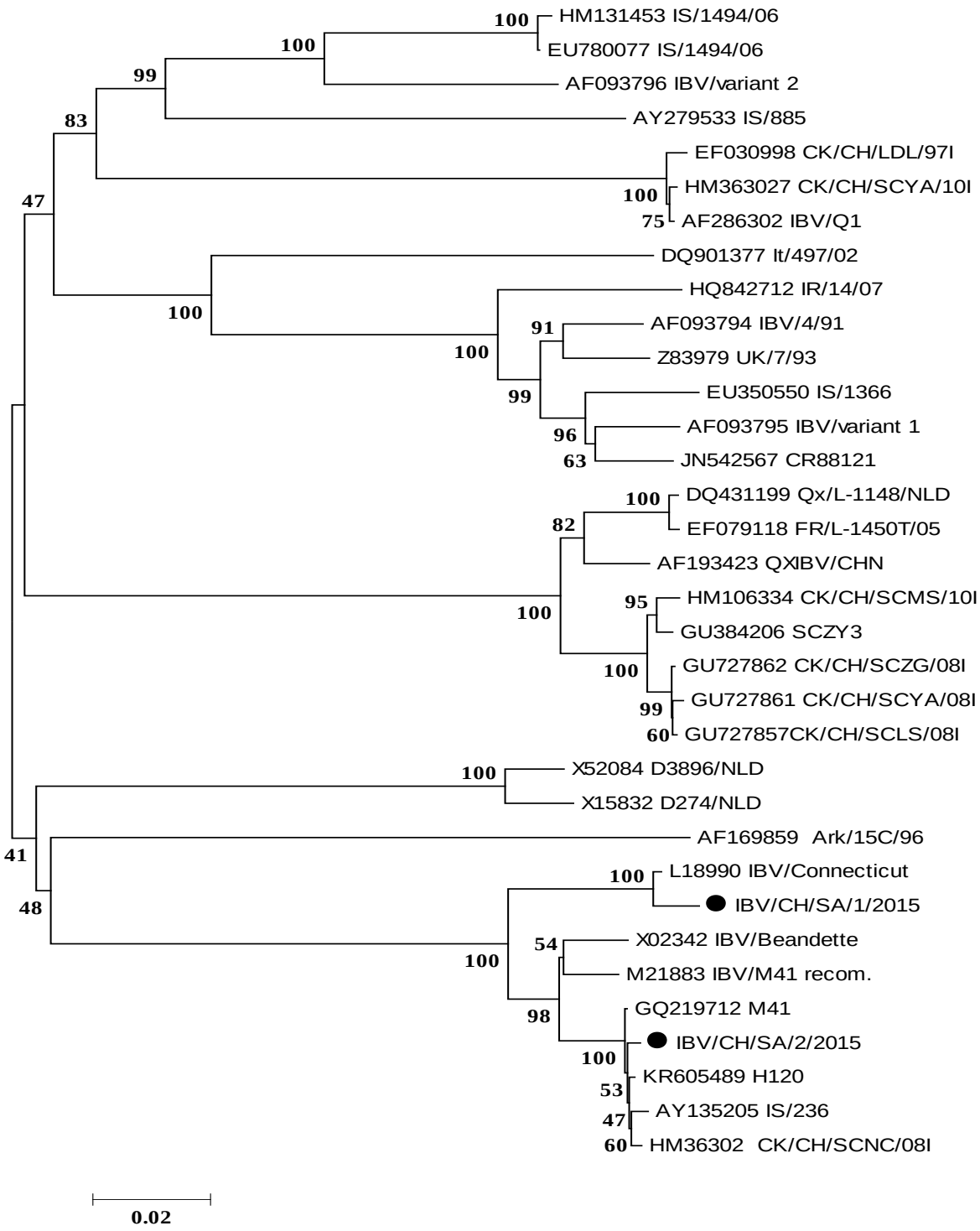

Figure 2 Phylogenetic tree of the nucleotide sequences of the spike (S1) gene of four detected Saudi strains compared with other sequences of the reference strains from GenBank by the neighbor-joining method with bootstrap 1000 replicates of MEGA version. Both Saudi IBV strains were marked with solid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

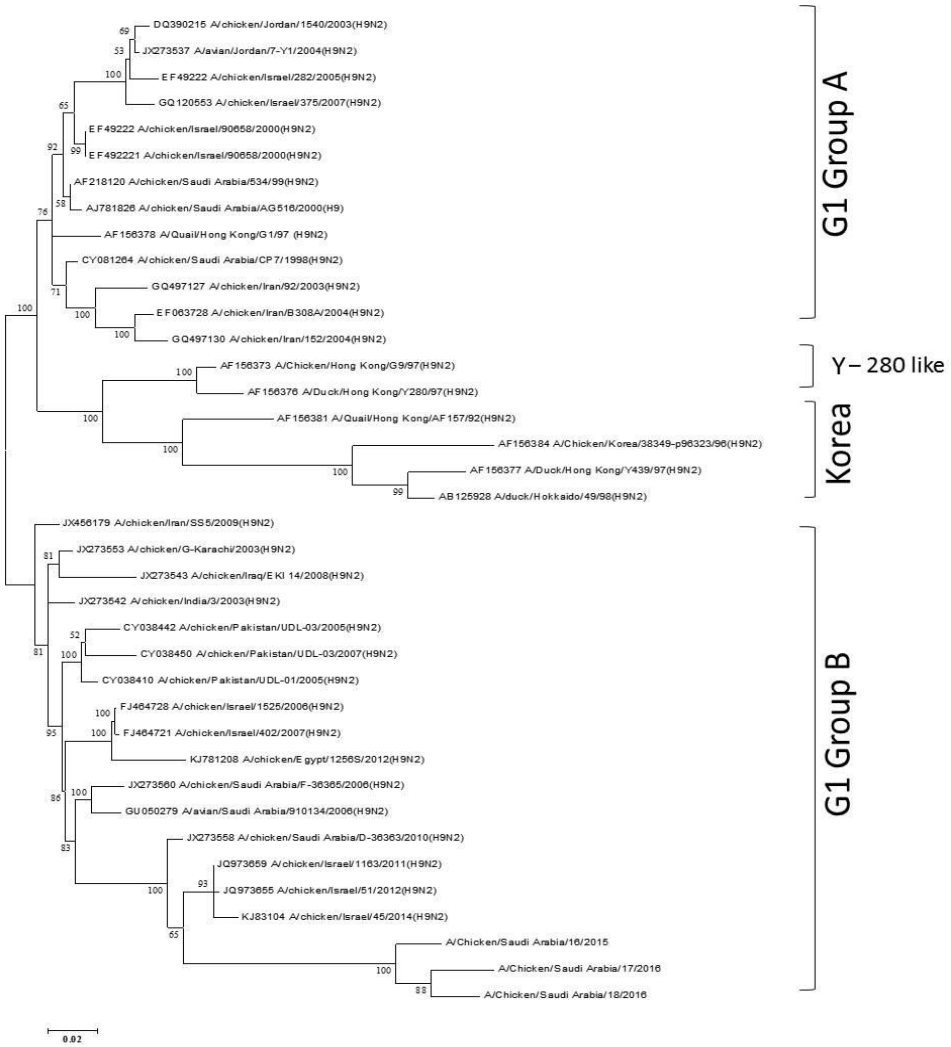

Figure 3 Phylogenetic tree of the nucleotide sequences of the hemagglutinin (HA) H9 gene of the three detected Saudi strains compared with other sequences of the reference strains from GenBank by the neighbor-joining method with bootstrap 1000 replicates of MEGA version.

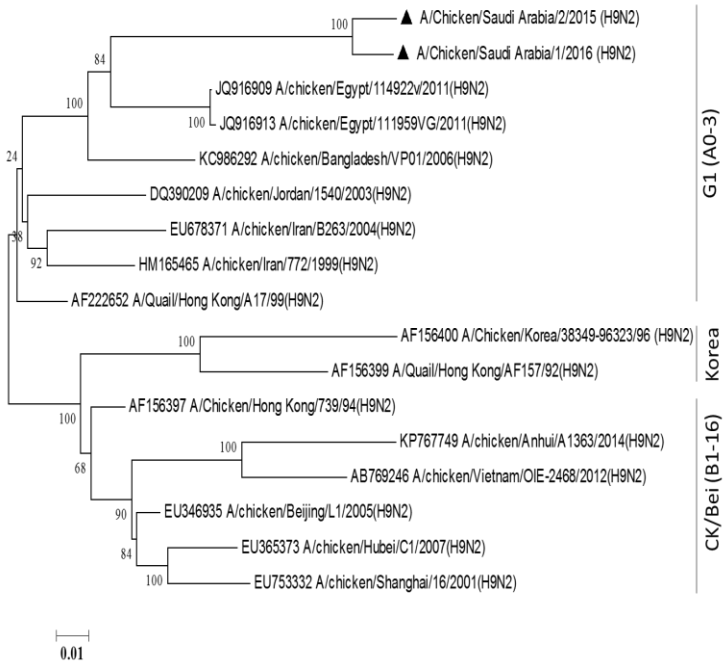

Figure 4 Phylogenetic tree of the nucleotide sequences of the neuraminidase N2 gene of two detected Saudi strains compared with other sequences of the reference strains from GenBank by the neighbor-joining method with bootstrap 1000 replicates of MEGA version. Both Saudi strains were marked with solid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

ลักษณะเฉพาะทางโมเลกุลของเชื้อไวรัสที่ก่อโรคระบบทางเดินหายใจในไก่กระทงของเมืองอัล 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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