

Molecular Detection and Characterization of Infectious Bronchitis Virus from Libya

Abdulwahab Kammon, Tan Sheau Wei, Abdul Rahman Omar, Abdunaser Dayhum, Ibrahim Eldghayes, Monier Sharif

Abstract—Infectious bronchitis virus (IBV) is a very dynamic and evolving virus, causing major economic losses to the global poultry industry. Recently, the Libyan poultry industry faced severe outbreak of respiratory distress associated with high mortality and dramatic drop in egg production. Tracheal and cloacal swabs were analyzed for several poultry viruses. IBV was detected using SYBR Green I real-time PCR detection based on the nucleocapsid (N) gene. Sequence analysis of the partial N gene indicated high similarity (~94%) to IBV strain 3382/06 that was isolated from Taiwan. Even though the IBV strain 3382/06 is more similar to that of the Mass type H120, the isolate has been implicated associated with intertypic recombinant of 3 putative parental IBV strains namely H120, Taiwan strain 1171/92 and China strain CK/CH/LDL/97I. Complete sequencing and antigenicity studies of the Libya IBV strains are currently underway to determine the evolution of the virus and its importance in vaccine induced immunity. In this paper we documented for the first time the presence of possibly variant IBV strain from Libya which required dramatic change in vaccination program.

Keywords—Libya, Infectious bronchitis, Molecular characterization.

I. INTRODUCTION

INFECTIONOUS bronchitis virus (IBV) is a highly contagious agent affecting primarily the respiratory tract and also other systems such as urogenital system, including the kidneys and the oviducts depending on the virus strains [1]. The disease caused by this virus was first reported in 1931 and was soon distributed worldwide [2]. IBV is a highly infectious and contagious agent in chickens. Young chickens infected with IBV can acquire permanent damage to their oviducts. Older infected chickens may exhibit poor feed efficiency [1], [2]. IBV is a member of the *Coronaviridae* family, has a 27.6 Kb genome organized as a non-segmented, single-stranded and positive-sense RNA. The virion contains four structural proteins. The glycoprotein S consists of two subunits (S1 and S2) and presents a wide variety of antigenic determinants that may induce the production of specific neutralizing antibodies and also activate cell immunity [3]. In addition to S protein, nucleocapsid protein (N) is another important structural

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protein that may contribute to viral pathogenicity. The N protein is located inside the virion, closely associated with its genome, and interacts with viral and host-cell nuclear proteins [3]. The N protein coding gene is highly conserved among different IBVs, which allows recurrent homolog recombination between different strains [4]. Recent studies have showed the isolation of new variants of IBV in poultry flocks in *neighboring* countries such as Tunisia and Egypt [5], [6]. In other countries, many variant serotypes of IBV were characterized such as Taiwan group I [7], IBV YN strain in China [8] and CK/CH/LDL/97I Chinese strain which recently reemerged in the Middle East [9]. Hence, isolation and characterization of IBV is crucial for the development of effective vaccine in controlling IB in each geographic region or country.

II. MATERIALS AND METHODS

A. History and Collection of the Samples

At the end of last year, Libyan layer and broiler industry faced severe outbreak of respiratory distress associated with high mortality and dramatic drop in egg production. Respiratory signs characterized by coughing, sneezing, excessive lacrimation and swollen head. Tracheal and cloacal swabs were collected from two layer flocks and one broiler flock. Five birds from each flock were randomly chosen and cloacal and tracheal swabs were collected from each bird then necropsy was conducted. The first flock of layers which aged 40 weeks old, showed high mortality, drop in egg production with changes in egg's morphology, and respiratory signs. PM lesions comprised of severe congestion and tracheitis, egg peritonitis, and nephritis. The second flock of layers which aged 15 weeks old appeared healthy. Broilers which aged 30 days showed high mortality and severe respiratory signs. Necropsy showed congested trachea, CRD and nephritis. The broilers were vaccinated against IBV using IB H120 at day old and IB 4/91 vaccine at 14 days old.

B. Viral RNA Extraction, RT-PCR and DNA Sequencing

Tracheal and cloacal swabs were streaked gently on FTA® cards (Whatman, USA). These cards were air-dried and sent to Bioscience Institute, University Putra Malaysia for further analysis. Viral RNA was extracted from the FTAcards with RNeasyPlus Mini Kit (Qiagen) according to the manufacturer's instructions. The amplification of N gene of IBV was performed using iScript SYBR Green I one-step real-time PCR Kit (BioRad, USA) using specific primers [10]. After product purification, the RT-PCR products were sent for

sequencing (1st Base SdnBhd, Malaysia). The samples were also tested for Avian Influenza (AI) and Newcastle Disease (ND) by SYBER Green I real time PCR.

C. Sequence Analysis

The sequencing data was first analyzed using Basic Local Alignment Search Tool (BLAST), NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to determine the identities of each sequencing results and their nucleotide similarities as compared to IBV reference strains. All nucleotide sequences were aligned and trimmed using MEGA (ver. 5.2).

III. RESULTS AND DISCUSSION

This work indicated the circulation of a variant strain of IBV in poultry flocks of west region of Libya.

Based on the detection of expected PCR product of 335 bp, 4 tracheal swabs and only one cloacal swab from the IBV positive tracheal swab birds were detected positive for IBV. However, all swabs collected from second layer flock showed negative results. In case of the broilers, all tracheal and cloacal swabs samples showed positive amplification for IBV (Figs. 1, 2). Sequence analysis of the PCR products indicated high similarity (~ 94%) to IBV strain 3382/06 that was isolated from Taiwan. All samples tested were negative for AI and ND. Even though the IBV strain 3382/06 is more similar to that of the Mass type H120, the isolate has been implicated associated with intertypic recombinant of 3 putative parental IBV strains namely H120, Taiwan strain1171/92 and China strain CK/CH/LDL/97I [7]. Isolate 3382/06 possessed the same serotype as its S1 gene-homologous TW-I strains [7]. Therefore, chickens infected with those variants were probably not protected by the current IBV prevention program as shown in the flock vaccination history in current study although IBV serotypes possess some cross protection. The emergence of new infectious bronchitis serotypes creates difficulties in the design of adequate vaccination programs. However, development of vaccines against new IBV variants is not generally an option, owing to the high cost and time required for their final approval. Complete sequencing and antigenicity studies of the IBV strains are currently underway to determine the evolution of the virus and its importance in vaccine induced immunity. Hence, we documented for the first time the presence of possibly variant IBV strain from Libya which closely related to Taiwan group I strain.

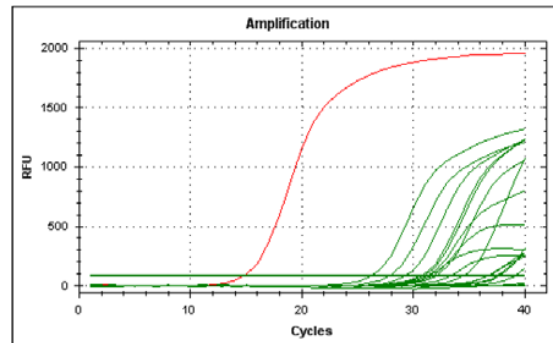


Fig. 1 Amplification curve, positive amplifications were observed for positive control (red color) and tested samples (green color)

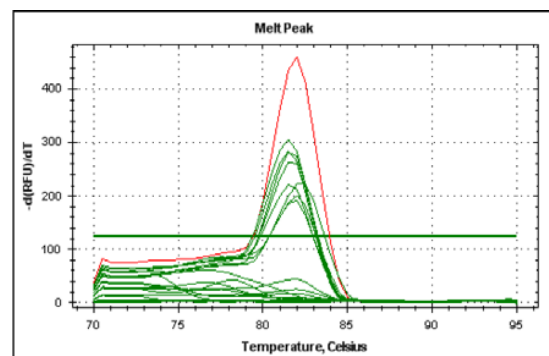


Fig. 2 Melt curve, positive control (red color) and tested samples (green color) showed the specific melting peak

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