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Molecular and Genetic Characterization of Infectious Bronchitis Viruses Isolated from Commercial Chicken Flocks in Egypt between 2014 and 2016

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ABSTRACT

Infectious Bronchitis is one of the major viral diseases affecting poultry causing severe economic losses. The prevalence of Infectious Bronchitis viruses was studied in commercial chicken farms in Egypt between 2014 and 2016. A total of 1722 organ samples (trachea, kidney, caecal tonsils and lungs) were collected from 246 problematic flocks, showing respiratory signs and considerable mortalities, from 13 governorates throughout the examination period and were then subjected to molecular analysis using real-time reverse transcription-polymerase chain reaction. Data from this study have shown a high prevalence (75.6%) of Infectious Bronchitis virus in Egyptian farms. Infections mixed with other respiratory viruses were frequently observed, including very virulent Newcastle disease, Low pathogenic avian influenza, H9N2 and High pathogenic avian influenza H5N1 with 27.9%, 25.7% and 17.1%, respectively with higher detection percentages observed in the winter season. Phylogenetic analysis of 19 selected positive Infectious Bronchitis virus has revealed Infectious Bronchitis virus genotypes closely related to variant II strains Eg/12120S/2012, IS/885, IS/1494, with 4 isolates was clustered in a new group. In conclusion, the present study provides further updates on the circulation and co-circulation of Infectious Bronchitis virus in commercial Egyptian flocks. The continuous existence of field variant Infectious Bronchitis virus in commercial chicken's farms in Egypt emphasizes the need for regular monitoring of Infectious Bronchitis with updating the control and vaccination strategies.

Keywords: Infectious bronchitis, Genetic characterization, Poultry, Prevalence, Epidemiology, Sequencing.

INTRODUCTION

Infectious bronchitis (IB) is an acute, highly contagious disease of chickens caused by coronavirus infection with a major impact to the poultry industry worldwide (Al-Shekaili et al., 2015). While IB virus (IBV) infections in chicks are often manifested with respiratory signs and decrease in feed conversion, infections in mature and layer chickens commonly resulting in urogenital tract affection and sharp decrease in egg production (Cavanagh,

2007; Chen et al., 2010 and Butcher et al., 2011). Chickens and recently pheasants are well-known as main natural hosts for IBV (Ignjatovic and Sapats, 2000).

The morbidity rate in IBV-infected flocks, may reach as high as 100%, while the mortality rate often depends on the presence of secondary infections, flock age, immune status, management and other environmental factors. In young chickens, the mortality rate is typically 25-30% but it can reach 80% relying on the virulence of the IBV infecting strain and the presence of other complicating viral and bacterial agents. Even though all age groups of chicks are susceptible to IBV, young chicks are more susceptible and vulnerable to infections than older ones (Cavanagh and Gelb, 2008).

The coronavirus of domestic chickens is worldwide distributed (Cavanagh, 2007) while in Egypt, IB variants have been firstly reported since 1950s with an identification of variant IBV strain revealed to be closely related to the Dutch strain D3128 (Sheble et al., 1986 and Eid, 1998). The presence of multiple genotypes of IBV has been also reported in Egypt and strains related to the Israeli variants (IS/885 and IS/1494/06) as well as those related to the 793-B genotypes (4/91 and CR 88) have been identified in commercial poultry farms (Abdel-Moneim et al., 2012; El-Mahdy et al., 2010 and Selim et al., 2013). In Egypt, IB and its co-infections with endemic virulent viruses of Newcastle Disease (ND) and Avian Influenza (AI) continue to present a constant threat to the profitability of the poultry industry (ELbayoumi et al., 2013; Selim et al., 2013, Osman et al., 2015 and Kiss et al., 2016). Hence, the present investigation was carried out to provide an update on the epidemiological situation of IB infection in commercial chicken farms in Egypt through molecular analysis of the S1 gene.

MATERIALS AND METHODS

Sampling

In this study, a total of 1722 organ samples were collected from diseased commercial chicken flocks exhibiting respiratory manifestations with variable mortalities during March 2014 to February 2016. Tissue samples from trachea (1107 samples), kidney (178), caecal tonsils (369) and lungs (68) were collected from 246 chicken farms from 13 governorates in Egypt. Samples were transferred on ice to the laboratory and were kept frozen at -80°C until use. From each farm, up to 5 organ samples from each organ type were pooled and subjected to RNA extraction, cDNA synthesis and Real Time Polymerase Chain Reaction (RT-PCR).

RNA extraction

Total RNA for RT-PCR was extracted from 50-100 mg of liquid nitrogen-homogenized samples. The extraction of viral RNA was performed using a QiaAmp viral RNA mini kit (Qiagen, Germany) according to the manufacturer's instructions. RNA was then kept frozen at -80°C until use.

Viral detection by RT-PCR

Real-time RT-PCR for IBV as well as Very Virulent ND (vvND), Highly Pathogenic Avian Influenza Virus (HPAIV) H5N1 and Low Pathogenic AIV (LPAIV) H9N2 was performed using Qiagen one step RT-PCR Kit (Qiagen, GmbH, Hilden, Germany) using strata gene thermal cycler according to the manufacturer's instructions. Target gene amplification, RT-PCR cycling conditions, probe and primer sequences were previously described (Callison et al., 2001; Wise et al., 2004; Slomoka et al., 2007; Ben Shabat et al., 2010).

Genetic characterization of IBV

The genetic analyses of the S1 gene of Egyptian IBV sequences used for comparison in this study were obtained from GenBank and were available for the national center for biotechnology Information Infectious bronchitis viruses resource (https://www.ncbi.nlm.nih.gov/). Sequence identities were calculated using DNA star software (Thompson et al.,1994) and the phylogenetic tree of the nucleotides sequence were constructed using Mega5 (Tamura et al., 2011) and as previously described by Selim et al. (2013).

Ethical approval

This research work did not involve the introduction of any intervention in/on birds, but direct collection of tissues and organs from freshly dead birds was carried out in strict accordance with the recommendations of institutional guidelines for the care and use of laboratory animals. Also, dead chickens were humanly handled.

RESULTS AND DISCUSSION

The present study provided further updates on the molecular characterization of avian infectious bronchitis viruses circulating during 2014-2016 with highlighting the other respiratory viruses which severely complicate infections in poultry farms in Egypt.

Clinicopathological picture of sampled chicken flocks

In this study and throughout the two years survey time, samples were collected from problematic flocks

exhibiting respiratory signs and considerable mortalities reaching in some infected flocks to more than 60%. Clinical manifestations include gasping, rales, respiratory sounds and peeping, ruffled feather, depression and whitish droppings. Post-mortem picture involved tracheal congestion and exudation, tracheal and bronchial casts, rhinitis, serositis (pericarditis, perihepatitis and air sacculitis), splenic and hepatic congestion, salpingitis (in laying chickens) and nephritis with deposition of the ureates in renal tissue and ureters. This is in line with previous literature which reported significant clinical outcomes and gross lesions following respiratory viral infections in chickens (El-Mahdy et al., 2010 and Hassan et al., 2017)

Prevalence of IBVs in commercial poultry farms

Here we report the prevalence of IBVs in commercial poultry farms in Egypt over two years of sampling ranging between 2014 and 2016. Data presented in this study has shown a very high detection rate of IBVs (75.6%) in chicken flocks in Egypt. As shown in Diagram 1, the detection rate of IBVs was highly influenced by sampling season. While a very low detection rate was observed during summer months, the high incidence rate of IBVs was observed during winter (reaching 100%) followed by autumn and spring. Increased detection of IBVs during cold weather could be impacted by many factors, including improper farm ventilation and litter management as well as inadequate biosecurity measures and natural air movements between farms (Abdel-Moneim et al., 2012).

Incidence of IBVs with other avian respiratory viruses

Prevalence of IBVs either alone or in combination with other respiratory viruses commonly complicating poultry farms was also determined (Diagram 2). Whilst the high prevalence of IBVs were seen in problematic Egyptian chicken's flocks, sole IBV infection represented 19.4% and, interestingly, the majority of cases was complicated with one of other avian viruses, including very virulent ND (27.9%), LPAI H9N2 (25.7%) and HPAI H5N1 (17.1%). Involvement of more than two viruses was also detected in a low rate compared double infection cases, although devastating losses were often reported. Previous literature has demonstrated that the prevalence of avian respiratory viruses in Egyptian broiler chicken flocks during 2012-2014, with increased incidence of IBV and H9N2 mixed infections, representing 41.7% (Hassan et al., 2016). In the present study, the incidence of IBV-H9N2 mixed infections was 25.7% during 2014-2016. This reduction in IBV-H9N2 mixed infections could be influenced by the increased coverage of inactivated H9N2 vaccines in different sectors of poultry production. However, clinical observations and mortality rates have significantly increased during the last few years with IBV mixed infections. Indeed, a recent study has shown that experimental co-infection of IBV and LPAI H9N2 resulted in sever clinical outcome and mortality with an increase in the H9N2 shedding from infected chickens (Hassan et al., 2017).

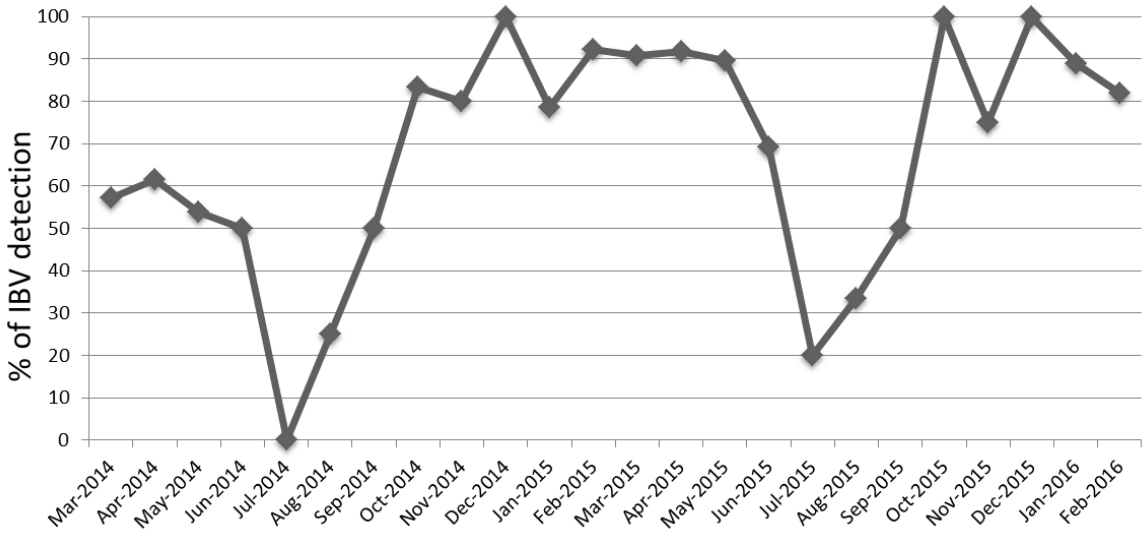

Diagram 1. Prevalence of infectious bronchitis in commercial chicken farms in Egypt during 2014 -2016

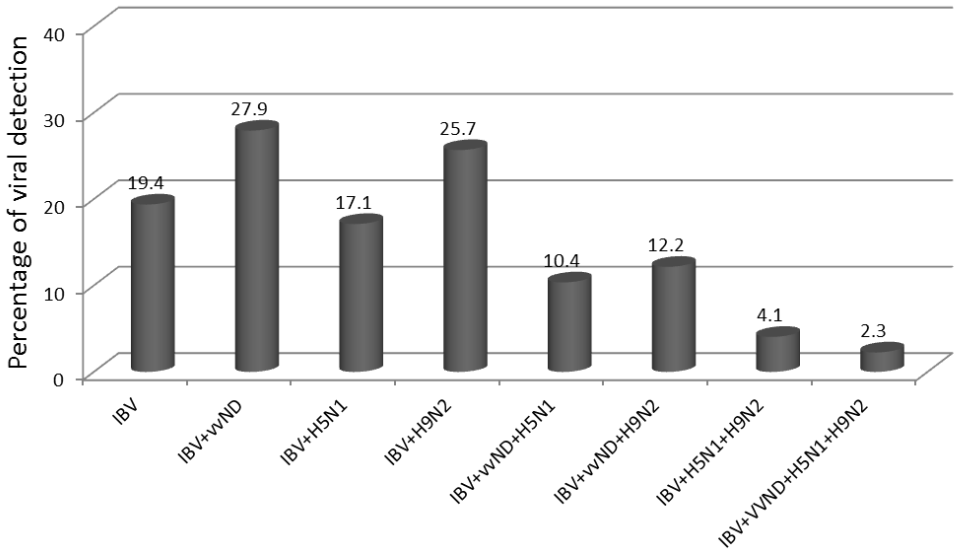

Diagram 2. Incidence of IBV detection with other avian respiratory viruses in commercial chicken flocks during 2014-2016**.** Where IBV is Infectious Bronchitis virus, vvND is very virulent Newcastle Disease, H5N1 is highly pathogenic Avian Influenza H5N1 and H9N2 is low pathogenic Avian Influenza H9N2

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                                10        20        30        40        50
                    ....|....|....|....|....|....|....|....|....|....|...
variant-2           FVYKQSDFMYGSYHPKCNFRPETINNGLWFNSLSVSLAYGPLQGGCKQSVFNNRATCX
6/82                .I..A..Y.......S.K..L.................G...I.........A......
UK/123/82           .I..E..Y.......S.K..L.......X..P.....G...I.........E......
strain D207         .I..A..Y.......S.K.KL.................G...I.........A......
isolate J2          .R.VE.Y...........S..L.................G.................M....
CK/CH/LDL/98I       .R.VE.............S..L.................G.................M....
Q1                  .R.VE.............S..L.................G.................M....
JF24                ....E.N.................N.............I.....H........KG.....
H120                ....E.N........S....L.................I..............SG.....
IBV-QXIBV           ....A..........S.S.......S...........T..............SGK....
IBV-M41             ....E.N........S....L.................I..............SG.....
IBV-CR88121         ....P..........H.K....N.............T...I.........S......
IB-VAR2-06          ...................D.....................................S......
IS/1494/06          ...................D.....................................S......
Eg/CLEVB-2/IBV/012  ...................D.....................................S......
Israel/720/99       ....A..............D.......................P..............
IS/885              ....A..............D.....................................
Eg/12120s/2012      ....P..........Q.D.....................................
Eg/12197B/2012      ....P..........Q.D.....................................
Eg/1265B/2012       ....P..........Q.D.....................................
CV-28-23            ....P..........Q.D..Q.............F.....................
CV-28-2T            ---------------XSD.S..........KNI....E......DG..CM.SHS....
CV-28-13K           ---------------XRD...........CIKT....HQ.K....SEEHCMNS.S....
CV-28-14            ---------------XSD.S..PL........I.......R.....G.......K....
CV-28-22            ---------------XRD.S..SL......QN...IHQ.R.....GE..MYKHS....
CV-28-35            --------X......Q.D.....................................S......
CV-28-31            ....P..............S.....................T..............S.K....
CV28-28             ....P..........Q.D.....................................S......
CV-28-38            ....P..........Q.D.....................................S......
CV-28-41            ....P..........Q.D.....................................S......
CV-28-26            ....P..........Q.D.....................................S......
CV-28-48            ------------H..QSD.....................................
CV-28-4             ....P..............S.....................T..............S.K....
CV-28-7             ....P..........Q.D..Q..................................S......
CV-28-10            ....P..........Q.D..Q..................................
CV-28-2K            ....P..............S..T.....................T.................K....
CV-28-4K            ....P..............S.....................T..............S.K....
CV-28-17            ....P..........Q.D.....................................
CV-28-18            ....P..........Q.D.....................................
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Diagram 3. Amino acid alignment of partial S1 gene from 19 selected IBVs with other previously reported and reference Infectious Bronchitis Virus

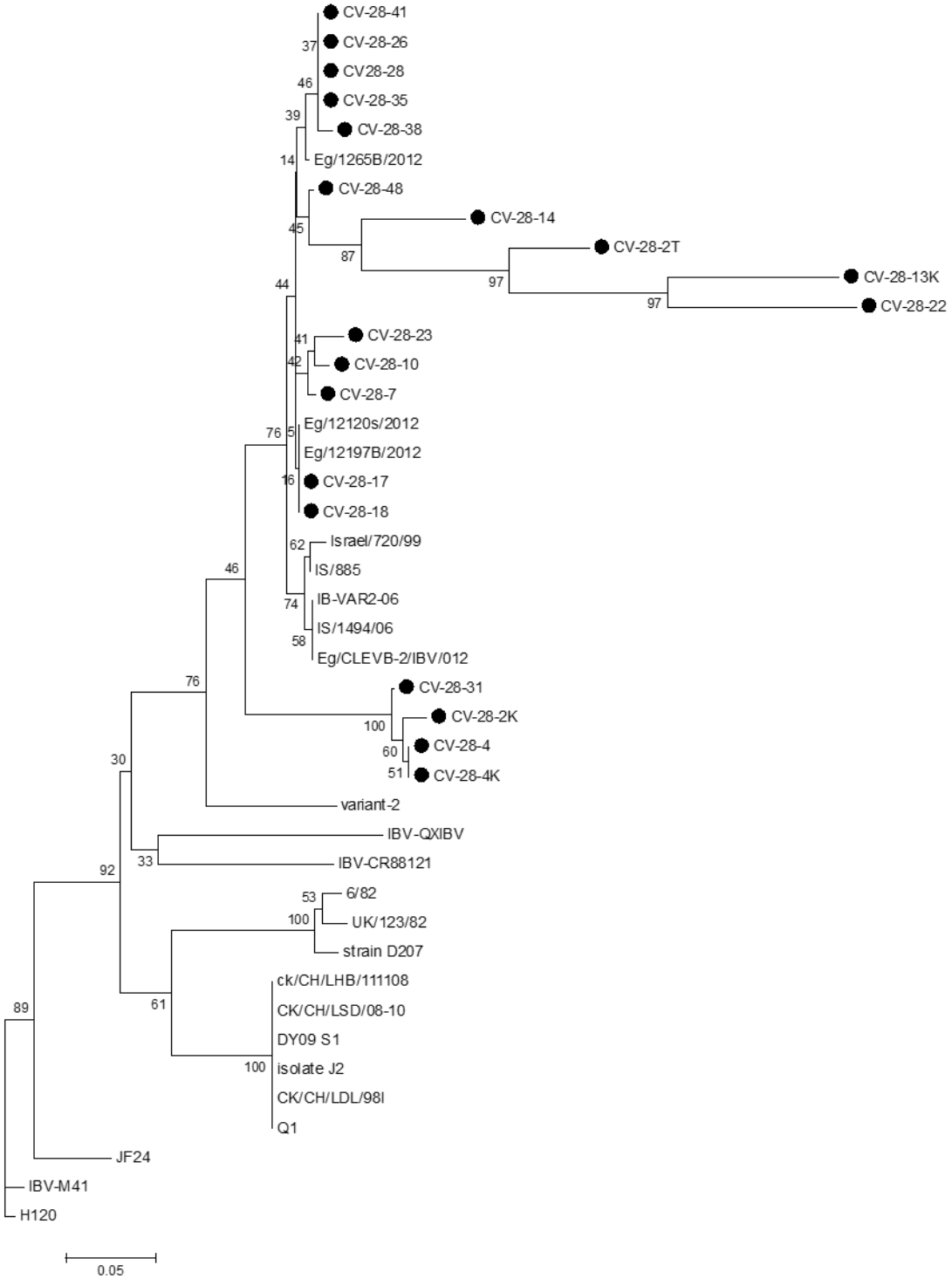

Diagram 4. Phylogenetic tree of 19 selected Infectious Bronchitis Virus generated from S1 genes of Egyptian IBVs detected during 2014-2016, the previously reported IBVs and other selected reference IBVs

S1 sequencing and phylogenetic analysis

Selected IBV positive samples were further examined for genetic identification. Amino acid alignment of partial S1 gene from 19 samples with other previously detected and reference IBV viruses For IBV is seen in Diagram 3. The obtained results have shown that all the 19

IBVs were genotyped as a variant type closely related to variant II-like strain. These viruses were then subdivided into two main groups; 15 viruses closely related to Eg/12120S/2012, IS/885 and IS/1494 like strains, while 4 viruses (CV-2K, CV-4, CV-4K and CV-31) were clustered separately in another group as seen in Diagram 4. Previous investigations have also shown the prevalence of variant IBVs closely related to Israeli variants (IS/1494/06 and IS/885/00) and original Egyptian variant strain (Egypt/Beni-Suif/01) (Abdel-Moneim et al., 2012; Selim et al., 2013; Zanaty et al., 2016). The uncontrolled field circulation of various IBV variants in chickens is highly relevant from the epidemiological point of view since possibility of emergence of new variants is highly expected. Indeed, recent study of genome sequencing of IBV has shown the emergence of a new QX-like strain in Sudan from ancestor ITA/90254/2005 genotype (Naguib et al., 2016). In this study, no evidence of detection of other IBV variants in the tested positive samples although previous investigators have recorded the presence of Q1-like IBV strain in Egyptian poultry during 2012-2013 (Abdel-Sabour et al., 2017).

CONCLUSION

The present paper provides an update about the molecular epidemiology of IBV circulating, and challenging, commercial poultry flocks in Egypt. The presented data have shown genetic makeup of field IBV is closely related to variant II strains. These variants have been detected from problematic flocks with severely impacted performance parameters, pointing out the significant role of complicating viral pathogens, mainly vvND and LPAIV H9N2. With no doubt, enhancing biosecurity measures, regular IBV monitoring and updating the vaccination schemes are crucial components to control IBV infection in commercial chickens in Egypt, and perhaps elsewhere in the region.

DECLARATIONS

Acknowledgments

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Competing interests

The authors declare that there is no conflict of interest.

Consent to publish

All persons gave their informed consent prior to their inclusion in the study.

Author`s contributions

All authors participated in making the design, support with sampling and surveillance work, interpretation of results and writing the paper.

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