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Circulating of is/1494-like strains of ibv in vaccinated commercial broiler flocks in Egypt during years 2012 to 2014

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Abstract

In this study, the prevalence of IBV in Egyptian commercial Broiler farms were studied. Samples were collected from flocks in 9 Farms located in 5 governorates (Behira , Giza, Sharkia, Ismailia and Gharbia) between years 2012 -2014. The birds showed severe respiratory signs with moderate number of chalky white droppings, feed consumption and body weight were markedly reduced. Severe rales with difficult respiration and mortalities were high and ranged from 20% to 25% . Flocks included in this study were vaccinated by classic Massachusetts strain similar commercial live (IB -H120 / MA5) vaccine. 8 out of 9 flocks were positive for IBV using Real time RT PCR. The 500-bp of the S1 gene covering the hyper variable region-3 of 5 isolates were amplified and sequenced . Phylogenetic analysis based on that region revealed that these viruses closely related to Variant strains of IBV, The viruses were clustered into two groups, the first group included (port said F118-4/2013, Giza F299/2012) which were resemble to IS 1494 Variant 2 strains. The second group include (Behira F161/2012 , Sharkia F552/2013 and Ismailia F187/2012) were vaccinal strains resemble D274 vaccine.

Monitoring of evolving IBV strains is essential for improving the control of disease in Egypt.

Keywords: Infectious Bronchitis virus (IBV) - S1 Gene -classic and variant -Egypt strains -Classic Vaccines.

Introduction

Avian infectious bronchitis virus (IBV), the prototype species of the family coronaviridae is an acute highly contagious viral respiratory and urogenital tract of chickens, characterized by respiratory signs including gasping, coughing, sneezing, tracheal rales and nasal discharge. In young chicken, severe respiratory distress may occur; while in layer and breeder respiratory distress, decrease in egg production and loss of internal and shell quality of eggs are reported. **(Case et al, 1983)**.

IBV is belong to group 3 corona virus **(Cavanaugh, 2003)**. It is an enveloped , non segmented, positive sense single stranded RNA virus. IBV genome consists of about 27 kb and codes for three structural proteins: the spike (S) glycoprotein, the membrane (M) glycoprotein, and the nucleocapsid (N) phosphoprotein. The S glycoprotein is composed of two glycopolypeptides: S1 and S2 **(Cavanagh, 1983).**

IBV has the ability to mutate or change its genetic makeup readily. As a result, numerous serotypes have been identified and have complicated efforts at control through vaccination. Controlling IB infection is a problem due to wide variations in the serotypes and virulence of strains that have developed from time to time, a highly contagious nature, and the evolution of specific tissue tropism and recombinants due to simultaneous infection of multiple virus types and use of live vaccines **(Bayry et al., 2005) .** Outbreaks can occur in vaccinated flocks due to the lack of cross-protection against antigenically unrelated serotypes and variant strains of the virus **(Gelb et al., 1991; Capua et al., 1994; Jia et al., 1995).** Early study by **Jungherr et al. (1956)** showed that there are sufficient immunological differences among the strains; so that cross-protection would not occur. Previous Studies showed that vaccines can elicit protection against some field challenges **(Davelaar et al., 1984; Parson et al., 1992; Afanador, 1994; Wang et al., 1997).** Other investigations showed that this occurs in function of the genetic variability of originating variant strains **(Wang et al. 1997).** Many authors propose that the inefficiency of the vaccination programs is due to the large diversity of antigenically different strains; because IBV presents the phenomenon of genetic recombination or the virus can suffer a mutation, generating new strains **(Kouwenhoven and Davelaar, 1989).** Variant

strains of IBV have been recovered from vaccinated flocks despite the use of combinations of several strains of live and attenuated IBV vaccines (Gelb et al., 1991).

The aim of this study was to investigate the prevalence of IBV in Egypt and their evolutionary relationship to the present work

Material and methods

Sampling

In this study 9 different samples were collected from 9 Broiler flocks showing sever respiratory manifestations , mortalities during surveillance of 3 years from 2012 -2014 to study the prevalence of IBV in 5 Egyptian Governorates . Samples collected included(trachea, kidney, lung , and proventriculus) also other samples collected included tracheal swabs were collected from the suspected flocks (about ten swabs for each flock) under complete hygienic condition for molecular detection of IBV by using real time-PCR.

particularly interested to know whether the recently isolated Egyptian IBV strains which escaped from vaccine elicited immunity were newly introduced in the chicken population or arose by mutations of circulating Egyptian -IBV strains

Table (1) History of examined flocks

Serial No.	Governorate	Chicken type	Breed	Age/d	Vaccination against IB
1	Behira	Broiler	Cobb	28	(L)
2	Port Said	Broiler	Cobb	32	(L)
3	Giza	Broiler	Cobb	24	(L)
4	Sharkia	Broiler	Avian	26	(L)
5	Ismaelia	Broiler	Avian	28	(L)
6	Behira	Broiler	Cobb	29	(L)
7	Giza	Broiler	Avian	39	(L)
8	Behira	Broiler	Cobb	25	(L)
9	Gharbia	Broiler	Hubbard	24	(L)

w=Week d=Day L=Live I=Inactivated

The Vaccination program of IBV for broiler which applied two time only at one day old (H120) and at 14 day (MA5 vaccine).

Real-time RT-PCR (rRT-PCR)

using quantitect probe RT-PCR kit (Qiagen, Inc. Valencia CA), with specific primers and probe named IBV5_GU391 (5-GCT TTTGAGCCT AGC GTT-3) as forward primer, IBV5_GL533 (5-GCC ATG TTG TCA CTG TCTATT G-3) as reverse primer and IBV5-G probe (5-FAM-CAC CAC CAG AAC CTG TCA CCT BHQ1-3) as previously described (Callison et al.,2001).

Virus detection and characterization

QIAamp Viral RNA Mini Kit (Qiagen,Valencia, Calif., USA) as recommended by the supplier was used to extract the RNA from the tracheas of collected samples. One-step reverse transcriptase polymerase chain reaction (RT-PCR) using Qiagen one step RT-PCR (Qiagen, Valencia, Calif., USA) to amplify fragment of the S1 gene (HVR 3) of IBV was conducted using IBV-S1-F as forward primer (CACTGGTAATTTTTCAGATGG) and the IBV-S1- R as reverse primer (CAGATTGCTTACAACCACC) (Adzhar et al., 1997). RT-PCR amplicons were directly sequenced using an ABI Prism Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems). A BLAST analysis was initially performed to compare sequence of isolated local strain with the international strains. ClustalW analysis of partial SP1 gene nucleotide sequences was conducted and deduced amino acid sequences were used for phylogenetic analysis using MEGA 5 (Kumar et al., 2008).

Results

Characteristic of IB outbreaks in poultry farms

The present data represent prospective survey of the presence of IB outbreak in 9 chicken farms. The data collected from 5 governorates include (Giza, Behira. Ismailia , Gharbia and Sharkia) during 3 years from 2012 -2014. They suffered from severe respiratory manifestations with high mortality rate ranged from 20 to 25 % , renal signs in the form of chalky droppings in some flocks , Feed consumption and body weight were markedly reduced.

Real time RT-PCR for screening of samples for ND,AI and IBV.

Pooled of tracheal swabs from each flock (5 swabs/ flock) were tested with real-time RT-PCR (rRT-PCR) for ND,AI and IBV. All samples were negative for ND and AI , where as 8 samples of

the 9 Flocks showed positive results for IBV.
Table 2: summarize the obtained ct values in RT-PCR for the tested samples .

Table (2): The obtained ct values in RT-PCR for the tested samples .

Flock No	RT-PCR	CT value
1	Neg.	32
2	Post.	18.45
3	Post.	20
4	Post.	20.8
5	Post	25
6	Post	29.6
7	Post	32.5
8	Post	30.5
9	Post	34

Post= Positive Neg=Negative CT =cycle thershold

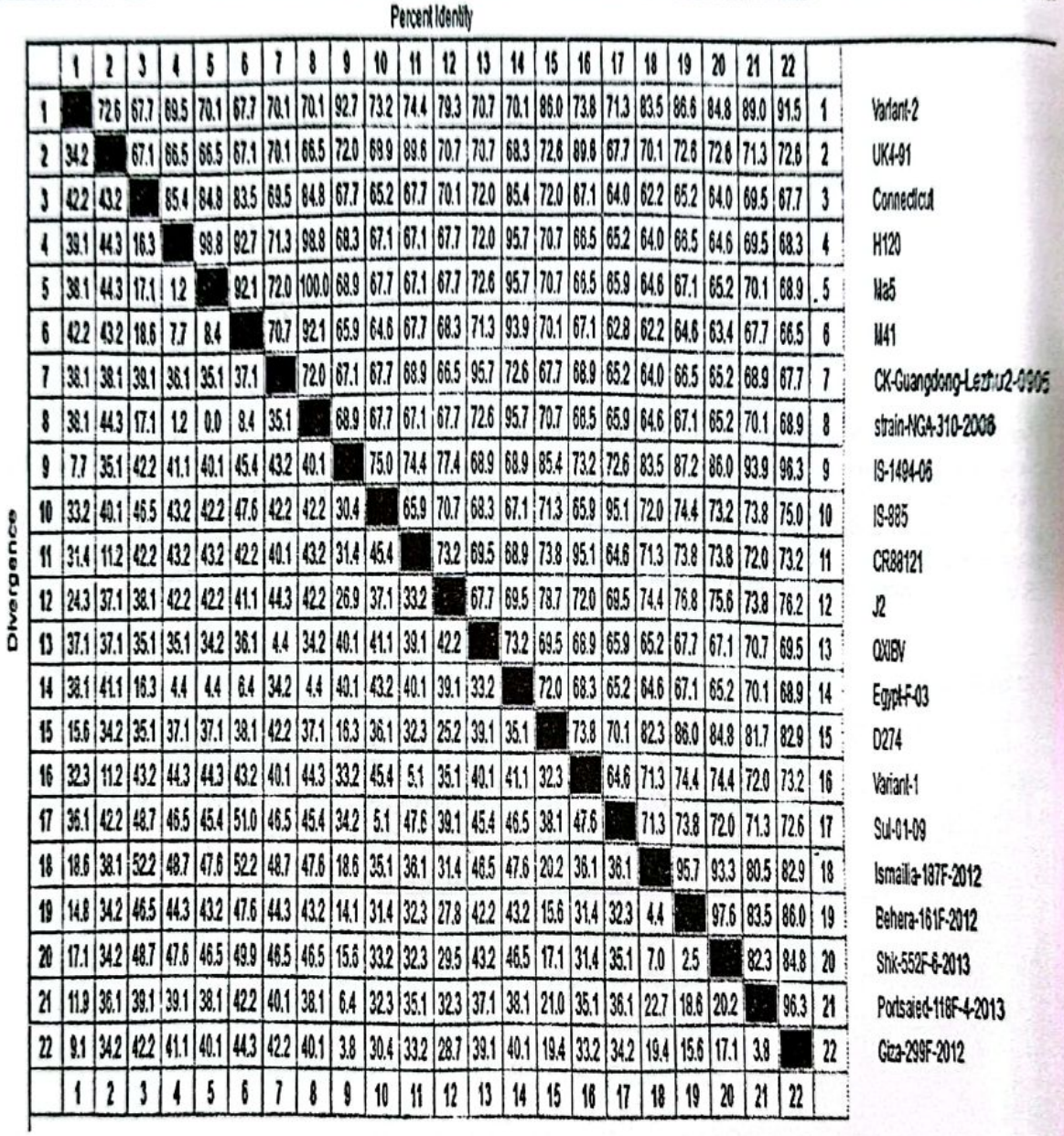
Sequence analysis

Five samples from representative the Five governorates under study with high CT values wereselectedforviruscharacterizationbysequencing .Results revealed that two samples were genotyped as IS / 1494 Like strains (IS / Variant 2) and Three samples were similar to the vaccinal strains D274 (Table 3).The results of RT-PCR showed the expected specific band with corrected size of 500 bp

Table (3):-

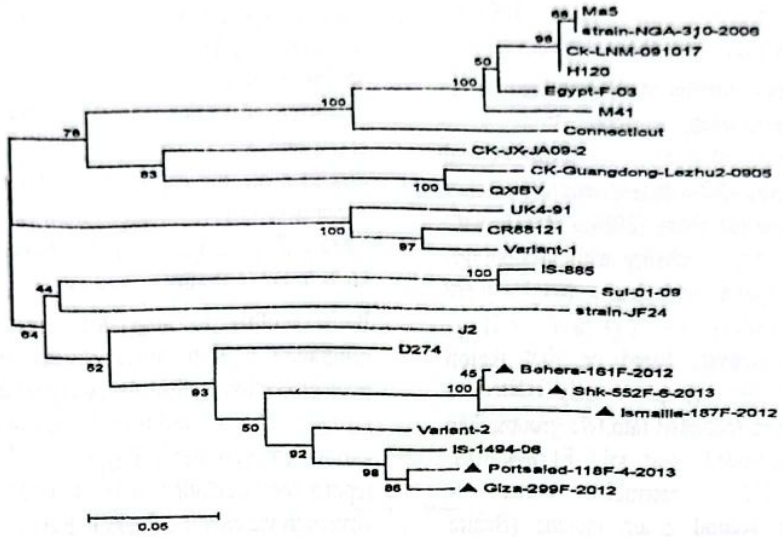
Sample code	Governorate	Phylogenetic origin	Year
F118-4	Port said	(IS/ 1494) - Like	2013
F299	Giza	(IS/ 1494) - Like	2012
F552	Sharkia	(D274) - Like	2012
F187	Ismelia	(D274) - Like	2012
F161	Behira	(D274) - Like	2012

Figure 1 :
Amino acid identities of the characterized IBV strains in relation to some reference strains of IBV.



Amino acid identities of the characterized strains ranged from 70.1-72.6 % to 4/91 vaccinal strains of IBV,
Amino acid identities of the characterized strains ranged from 64-70.1 % to H120 and MA5 strains of IBV,
Amino acid identities of the characterized strains to other variants strains were ranged from 72-75 % to IS / 885 , 71.3-73.8 % to CR 88 strains and 81.7-86 % to D274 strains.

Figure 2:Phylogenetic tree for the characterized IBV strains based on the partial sequence of S1 amino acids. sequences



Phylogenetic tree of the charactrized strains was constructed based on sequence of HVR 3 of S1 gene in which the viruses were divided into two Variant groups.

The first group includes port said F118-4 / 2013 and Giza F299 / 2012 were resemble of the IS /1494 variant 2 viruses . The second group includes Behira F161/2012 , Sharkia F552/2013 and Ismailia F187/2012 Resembled Variant 2 (D274 vaccine).

Discussion

Concerning the characteristics of IB outbreaks in the present investigations, examination of 9 Broiler chicken farms distributed in 5 governorates, revealed that the IBV is prevalent since the initial description and isolation of the virus **(Ahmed, 1954; Eissa et al., 1963; Ahmed, 1964 ; Amin and Mustagger, 1977).**

One of the major problems with IBV is the frequent emergence of new variants. The detection and identification of these new variants is crucial to disease control.

Many tests are used for the detection and identification of IBV such as :- serological examination (Agar gel precipitation test ; Serum neutralization Test (SNT) ; Fluorescent antibody techniques "FAT; Haem agglutmation (HI) ; Enzyme Linked immuno sorbent assay (EliSA)),Molecular based diagnosis Conventional and RT-PCR- Sequencing., Isolation and propagation of infectious bronchitis in cell Culture or embryonated chiken egg.

In this study, all samples which collected from 9 Broiler chicken farms from 5different governorates are tested by molecular based diagnosis Conventional and RT-PCR-Sequencing Direct automated cycle sequencing (DACS) of a reverse transcription-polymerase chain reaction product of the S-1 subunit of the spike peplomer gene was used to identify IBV serotypes. Degenerate primers CK4 and CK2 used according to **Kingham et al., (2000)** were selected because they successfully amplify a wide range of serotypes represented by various reference strains and field isolates , and the resulting PCR product contains diagnostically relevant S1 sequence that can be used to identify the serotype of IBV.

The DACS procedure provided high-quality and reproducible S1 sequence for all IBV serotypes tested before including variant serotypes and reference strains **(Kingham et al., 2000).**

Regarding to the method used for identification of these IBV isolates, DACS included two steps: the first step was the amplification of the genome using PCR method. These methods depend on the degenerate primers CK4 and CK2. These primers could amplify all serotypes of IBV due to producing a product of about 500 bp (base pair).This product included two highly conserved regions which were conserved in all serotypes of IBV. In between the two highly conserved regions there were the two highly variable regions in which the variation between serotypes occur. This work agree with the previously published work by **Keeler et al., (1998) ; Kingham, et al. (2000) and Bayry, et al. (2005).**

The Surveying of IBV for 9 chicken Broiler farms were collected from 5 different governorates include (Giza, Ismailia , Behira , Gharbia and Sharkia) are showed in (table 1).

The clinical examination of all examined Farms in this investigation, suffered from sever respiratory manifestations , the birds showed severe rales with difficult respiration, mortality was higher ranged from (20% - 25%), with moderate number of chalky white droppings, Feed consumption and body weight were markedly reduced.

Phylogenetic analysis based on that region revealed that these viruses closely related to Variant 2, were separated into two groups, The first group included (port said F118-4/2013, Giza F299/2012), resembled Variant 2-IS/1494. The second group include (Behira F161/2012 , Sharkia F552/2013 and Ismailia F187/2012) resembled D274 vaccine 2 of strain. Amino acid identities of the characterized strains ranged from 70.1-72.6 % to 4/91 vaccinal strains of IBV, Amino acid identities of the characterized strains ranged from 64-70.1 % to H120 and MA5 strains of IBV, Amino acid identities of the characterized strains to other variants strains were ranged from 72-75 % to IS / 885 , 71.3-73.8 % to CR 88 strains and 81.7-86 % to D274 strains.

It is worthly to note that the vaccination programm used in these flocks may be induced poor protection against the newly circulating IB serotypes charactrized from disease outbreaks in various governorates in Egypt. Indeed, the study reports the circulation of IS/1494-like variant 2 viruses in vaccinating flocks in Egypt.

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الملخص العربى

فى هذه الدراسة تم دراسة حدوث وإنتشار مرض إلتهاب الشعب الهوائية فى دجاج التسمين فى مصر حيث تم جمع عينات من 9 قطعان تقع فى خمسة محافظات مختلفة هى (البحيرة- الجيزة -الشرقية - الإسماعلية - الغربية) فى الفترة من 2012- 2014 . ومن أهم الأعراض المرضية للطيور محل الدراسة أعراض تنفسية حادة وأعداد محدودة من الزرق الطباشيرى وإنخفاض ملحوظ فى إستهلاك العلف و الزيادة الوزنية للطيور وأيضا"صعوبة بالتنفس ونفوق مرتفع يتراوح بين 20-25 % . وكانت الطيور محل الدراسة محصنة بلقاح مرض إلتهاب الشعب الهوائية العترة الكلاسيكية ماسوشوستس (إتش 120 - إم أى 5). وأسفرت النتائج عن إيجابية عدد 8 من 9 قطعان أعطت نتيجة إيجابية لوجود فيروس مرض الإلتهاب الشعبى بإستخدام إختبار البلمرة المتسلسل المسبوق بنسخ وعمل تتابع نيوكلوتيدى للجين إس 1 عند المنطقة شديدة التغير المنطقة (3) لعدد 5 معزولات . أوضحت النتائج أن هذه الفيروسات متشابهة مع العترات المغايرة لمرض الإلتهاب الشعبى وهذه الفيروسات متجمعة فى مجموعتين المجموعة الأولى (بورسعيد إف 118-4 / 2013 - الجيزة إف 299 /2012) والتى تشبه العترة الإسرائيلية 1494 النوع2 والمجموعة الثانية تشمل (بحيرة إف 161 /2012- شرقية إف 552 /2013- الإسماعلية إف 187/ 2012) والتى تشبه العترة المغايرة نوع دى274.