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Original Article

Pathogenicity Assessment of Seven Variants of Infectious Bronchitis Virus Isolated from Commercial Broiler Chickens during 2013 in Egypt

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ABSTRACT

The aim of this study was to evaluate the pathogenicity of seven variants of IBV isolates (Coded, 11, 15, 21, 13, 19, 22, 24) using commercial broiler chickens. These isolates isolated from 25 commercial broiler flocks raised in El-Behera and Kafr-Elsheikh governorates in 2013, and were identified by the AGPT against reference IBV Beaudette antiserum and RT-PCR and characterized by partial sequence analysis of S1 gene as variant 2 IBV strains. The results at 2 weeks of PI revealed that all isolates were able to induce serological response and respiratory signs in the form of conjunctivitis, mainly of a frothy type, associated with lacrimation, edema and cellulitis of the periorbital tissues, sneezing, coughing, tracheal rales and gasping were observed in all infected groups in a varying degrees and varying times of appearance and disappearance. In addition the birds appeared lethargic, reluctant to move. No mortalities were recorded during the course of experiment in all groups. The main lesions of PI were slight increase of mucin, congestion and presence of nasal exudate in the trachea and small patches of pneumonia in chickens challenged with 4 IBV isolates only (NO. 15, 21, 22, 24). Renal lesions (nephritis and enlarged kidney) which were induced in chickens challenged with 3 isolates (NO. 15, 21 and 24) were not as pronounced as seen in the field. Histopathological finding of PI, exhibited tracheal lesions with inflammatory cells infiltration in the lamina propria and submucosa, edema in the submucosa, desquamation of the lining epithelial of the mucosa and activation of goblet cells. In addition to renal lesions with interstitial nephritis which characterized by infiltration of inflammatory cells (heterophils, lymphocytes and plasma cells) in the interstitial tissue.

Keywords: Pathogenicity, Assessment, Variants, Infectious Bronchitis Virus, Broiler.

INTRODUCTION

Infectious Bronchitis (IB) disease is an acute, highly contagious and infectious disease of poultry in worldwide, possess a major threat to the poultry industry and was first reported in North Dakota, USA, as a novel respiratory disease by Schalk and Hawn (1931). The disease is characterized by respiratory signs including (sneezing, cough, tracheal rales, gasping and nasal discharge), reduction the growth rate of broilers, nephropathogenic strains causing acute nephritis, urolithiasis and may be associated by high mortality (Linda, 2006). Infectious Bronchitis Virus (IBV) belongs to group III of the genus coronavirus of the coronaviridae family (Cavanagh, 1997).

All ages of chickens are susceptible but the severity is great in younger ages (Glahn, *et al*., 1989), as age increases, chickens become more resistant to the nephropathogenic effects and mortality due to infection (Albassam *et al*., 1986). The transmission of IBV is mainly via the respiratory tract from infected chickens. Infection occurs via inhalation of droplets containing the air born virus, which may travel several kilometers. Contaminated feed, drinking water and fomites, including human beings, probably contribute to more local spread. The finding that certain strains have a tropism for the intestine or, at least, the ability to grow well in fecal material suggests that contaminated litter can be a potent method of spread of the virus from site to site. Also, those viruses have the ability to persist on sites if cleaning and disinfection is not effective. (De Fabio, *et al.,* 2000). Natural spread requires about 36 hours or more, while in vitro studies show 18-36 hours depending on dose and route of inoculation. Chickens exposed to an aerosol of undiluted infective egg fluid regularly have tracheal rales within 24 hours (Glahn, *et al*., 1989).

In Egypt, IB was first described by Ahmed (1954), subsequently several reports (Abdel Moneim *et al*., 2002; Sultan *et al*., 2004; Lebdah *et al*., 2004;

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Sediek, 2005 and 2010) emphasized the prevalence of the disease. IBV strains related to the Massachusetts, D3128, D274, D-08880 and 4.91 genotypes have been detected at different poultry farms in Egypt (Abdel-Moneim *et al*., 2006; Sultan *et al.*, 2004; Sediek, 2010). The Egyptian variants which were closely related to the Israeli variant strain were isolated from different poultry farms (Abdel-Moneim *et al*., 2002; Sediek, 2010). The Egyptian variant IBV-CU-2-sp1 and Eg/12120s/2012 were isolated by Afifi *et al.* (2013) and Arafa *et al.* (2013), respectively.

The aim of this study is to evaluate the pathogenicity of seven variants of IBV isolates in commercial broiler chickens.

MATERIAL AND METHODS

IBV virus

The Seven variants IBV isolates (11, 15, 21, 13, 19, 22, 24) with accession numbers in Gene Bank as shown in Table.1 used in the challenges were from infectious allantoic fluid at the level of six –passage, they were isolated from 25 commercial broiler flocks (all flocks were vaccinated against IB disease at the first week of age) of various ages (20-35 days) raised in El-Behera and Kafr-Elsheikh governorates in 2013 and suffering from respiratory symptoms and pathological changes in kidney associated with high mortality rate. These isolates were isolated from the trachea and kidneys of sick and dead broilers in 9 to 11 day-old embryonated specific pathogen free specific pathogen free chicken eggs.

These isolates were identified by the agar gel precipitation test Agar gel precipitation test against reference IBV Beaudette antiserum and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) (Adzhar *et al*., 1997) and characterized by partial sequence analysis of S1 gene as Variant2 IBV strains**.** The seven variants IBV isolates showed 97% to 98.3 % and 96.7% to 98.3% nucleotide sequence identity to IBV-CU-2-SP1 and Eg/12120s/2012 strain (variant 2 like strain), respectively. Nucleotide identity between these seven IBV isolates ranged from 97.7% to 99% and between these isolates and vaccinal strains used in Egypt (M41, H120, Ma5, 4/91, CR88 and D274) ranged from 64.7% to 65.7%, 65.3% to 66.3%, 65.7% to 66.7%, 67.3% to 68.3%, 68.6% to 69.6% and 84.2% to 84.8%, respectively as shown in (Fig.1. and Table. 2.)

Specific Pathogen Free eggs (SPF)

Fertile, SPF eggs for IBV propagation and titration were obtained from SPF governmental farm (Kom Oshim, Fayoum, Egypt).

Determination of median Embryo Infective Dose (EID50) of IBV isolates

This was carried out after (Villegas and purchase, 1989). Allantoic fluid of 7 IBV isolates were ten fold diluted in Phosphate Buffered Saline (PBS) and inoculated in 10 day old SPF embryonated eggs via allantoic sac (5 eggs/dilution and 0.1ml/egg). The eggs were incubated at 37^0 C and candled daily for mortality up to 6 days Post Inoculation (PI). The dead embryo within 1st 24 hours were discarded as non-specific mortality. Embryos which die later and survivors were opened 6 days PI and the characteristic embryonic changes of IBV infection were recorded.

The EID50 was calculated after using the embryo gross pathological changes (Reed and Muench, 1938) (Table. 3).

Chicks for pathogenicity test

A total of 120 One day old commercial broiler chicks were used for experimental infection. All of the birds were fed ad libitum on commercial starter feed (23 % protein) during their first 2 weeks of life and commercial grower feed (21 % protein) from the third week until the end of the experiment.

Pathogenicity test

120 one day old commercial broiler chicks were kept together under strict hygienic condition in isolated experimental room, previously cleaned and disinfected. Chicks were provided with commercial broiler feed, water and feed were provided ad libitum, and then chicks were divided into 8 equal groups (1-8) at 21 days old age (age of infection). Each group consists of 15 birds and each group was kept in isolate pens in different rooms. 10 random blood samples out of 120 chicks before classification were collected at 21 days old age before infection and the sera tested for IBV and Mycoplasma Gallisepticum antibodies by ELISA. 1-7 groups were infected by IBV field isolates (11, 15, 21, 13, 19, 22, 24) respectively at the 6th embryo passage level, using infective dose 0.1 ml containing 10^5 EID50/bird by intratracheal route at 21 days old. While the other group No. 8 was left as uninfected control and inoculated by 0.1ml PBS. The virus suspensions were prefiltered through 0.22 millipore filter before challenge. The birds were observed daily for clinical signs and mortality for 14 days Post Inoculation (PI). At the 5th day Post Infection (PI), five random birds from each group were autopsied, specimens of trachea and kidney of each bird were collected for histopathological examination.

At 2 weeks PI, six blood samples were collected from each group and the sera tested for IBV antibody by ELISA. The experimental design is summarized in Table 4.

Scoring indexes for clinical and lesions: were recorded according to Avellaneda *et al*. (1994); and Wang and Huang, (2000) as follows:

a) Clinical signs score system of infected chickens

Score 0 = No clinical signs,

Score 1 = lacrimation, slight shaking of head, watery feces,

Score 2 = lacrimation, presence of nasal exudate, depression, watery feces and

Score 3 = strong (lacrimation, presence of nasal exudate, depression, severe watery feces).

b) Gross lesion scores of trachea and kidney systems of infected chicks

Gross lesion scores of trachea

Score 0 = no lesions,
Score 1= slight increase of mucin,
Score 2 = large increase of mucin and
Score 3 = large increase of mucin and mucosal congestion.

Gross lesions scores of kidney

Score 0 = no lesions,
Score 1 = swelling, urate visible only under stereomicroscopy,
Score 2 = swelling with visible urate and
Score 3 = swelling with large amount of urate deposit in kidney.

Histopathological examination

Specimens from tracheas and kidneys, collected from five sacrificed birds at 5th day PI in all groups in pathogenicity test, were preserved in 10% buffered formalin till processed thorough conventional paraffin embedding technique. Sections of 5-10µ in thickness were prepared and stained with Hematoxylin and Eosin stain (Culling, 1983).

ELISA test

ELISA test was carried out on serum samples collected from experimentally infected birds for antibody detection. The test was performed according to the directions of the kit producer company (Synbiotics Corporation, USA).

RESULTS

Virus titrations are shown in Table.3.

Pathogenicity test

The results of studying the pathogencity of seven variants of IBV isolates at 21 days of age in non-vaccinated commercial broiler chickens by intratracheal inoculation are summarized as follows.

Clinical and gross lesion scores of IBV infection at 21 days of age (Table 5 and Table 6)

Respiratory signs in the form of conjunctivitis, mainly of a frothy type, associated with lacrimation, oedema and cellulitis of the periorbital tissues (Fig. 2 and Fig. 3), sneezing, cough, tracheal rales and gasping were observed in all infected groups in a varying degrees and varying times of appearance and disappearance (Table. 5) In addition the birds appeared lethargic, reluctant to move.

The main lesions were slight increase of mucin in trachea, congestion and presence of nasal exudate in the trachea (Fig. 4) and small patches of pneumonia in chickens challenged with 4 IBV isolates only (No. 15, 21, 22, 24), in addition to renal lesions (nephritis and enlarged kidney) in chickens challenged with 3 isolates (No. 15, 21 and 24) (Fig. 5 and Table 5).

Group 1 infected with Isolate (No. 11):

- The first clinical signs appeared after 72 hours PI and disappeared in 6th day to 7th day PI.
- Conjunctivitis associated with lacrimation (4 birds)
- No oedema and cellulitis of the periorbital tissues.
- Sneezing & cough (3 birds)
- Mild tracheal rales (1 bird)
- No gasping
- Depression (2 birds)
- No whitish diarrhea
- Morbidity rate was (4 birds/15 birds).
- The course of the disease was 4 days.
- No mortality was seen.
- No gross lesions in 5 sacrificed birds 5 days PI.
- Clinical score: 0.4
- Tracheal score: 0
- Kidney score: 0

Group 2 infected with Isolate (No.15):

- The first clinical signs appeared after 20 hours PI and disappeared in 6th day to 9th day PI.
- Conjunctivitis associated with lacrimation (15 birds).
- Edema and cellulitis of the periorbital tissues (6 birds).
- Sneezing & cough (13 birds)
- Severe tracheal rales (2 birds)
- Gasping (2 birds)
- Depression (8 birds)
- Whitish diarrhea (2 bird at 6th – 8th day PI)
- Morbidity rate was (15 birds/15 birds).
- The course of the disease was 8 days.
- No mortality was seen.
- Gross lesions in 5 sacrificed birds 5 days PI involving of congestion and presence of nasal exudate in the trachea of 4 birds, small areas of pneumonia in 4 birds and nephritis in 3 birds.
- Clinical score: 1.9.
- Tracheal score: 2.6.
- Kidney score: 0.6.

Group 3 infected with Isolate (No. 21):

- The first of clinical signs appeared after 72 hours PI and disappeared in 6th day to 7th day PI.
- Conjunctivitis associated with lacrimation (6 birds).
- Edema and cellulitis of the periorbital tissues (2 birds).
- Sneezing & cough (5 birds)
- Moderate tracheal rales (1 bird)
- No gasping
- Depression (4 birds)
- No whitish diarrhea
- Morbidity rate was (6 birds/15 birds).
- The course of the disease was 4 days.
- No mortality was seen.
- Gross lesions in 5 sacrificed birds 5 days PI involving of slight increase of mucin in the trachea of 4 birds and enlarged kidney in 2 birds.
- Clinical score: 0.7.
- Tracheal score: 0.8.
- Kidney score: 0.4.

Group 4 infected with Isolate (No. 13):

- The first clinical signs appeared after 72 hours PI and disappeared in 5th day to 6th day PI.
- Mild Conjunctivitis associated with lacrimation (2 birds).
- No edema and cellulitis of the periorbital tissues.
- Mild Sneezing & cough (2 birds)
- No tracheal rales
- No gasping
- No depression
- No whitish diarrhea
- Morbidity rate was (2 birds/15 birds).
- The course of the disease was 3 days.
- No mortality
- No gross lesions in 5 sacrificed birds 5 days PI
- Clinical score: 0.1
- Tracheal score: 0
- Kidney score: 0

Group 5 infected with Isolate (No. 19):

- The first clinical signs appeared after 72 hours PI and disappeared in 5th day to 6th day PI.
- Mild Conjunctivitis associated with lacrimation (3 birds)
- No edema and cellulitis of the periorbital tissues.
- Mild Sneezing & cough (2birds)
- No tracheal rales
- No gasping
- No depression
- No whitish diarrhea
- Morbidity rate was (3 birds/15 birds).
- The course of the disease was 3 days.
- No mortality was seen.
- No gross lesions in 5 sacrificed birds 5 days PI.
- Clinical score: 0.2
- Tracheal score: 0
- Kidney score: 0

Group 6 infected with Isolate (No. 22):

- The first clinical signs appeared after 72 hours PI and disappeared in 7th day to 8th day PI.
- Conjunctivitis associated with lacrimation (7 birds).
- Edema and cellulitis of the periorbital tissues (2 birds).
- Sneezing & cough (5 birds)
- Moderate tracheal rales (1 birds)
- No gasping
- Depression (5 birds)
- No whitish diarrhea
- Morbidity rate was (7 birds/15 birds).
- The course of the disease was 5 days.
- No mortality was seen.
- Gross lesions in 5 sacrificed birds 5 days PI involving of slight increase of mucin in the trachea of 4 birds and small areas of pneumonia in 2 birds.
- Clinical score: 0.9
- Tracheal score: 0.8
- Kidney score: 0

Group 7 infected with Isolate (No. 24):

- The first clinical signs appeared after 36 hours PI and disappeared in 6th day to 7th day PI.
- Conjunctivitis associated with lacrimation (12 birds)
- Edema and cellulitis of the periorbital tissues (5 birds)
- Sneezing & cough (11 birds)
- Severe tracheal rales (2 birds)
- No gasping
- Depression (6 birds)
- No whitish diarrhea
- Morbidity rate was (12 birds/15 birds).
- The course of the disease was 6 days.
- No mortality was seen.
- Gross lesions in 5 sacrificed birds 5 days PI involving of congestion and presence of nasal exudates in the trachea of 3 birds, small areas of pneumonia in 3 birds and nephritis in 3 birds.
- Clinical score: 1.4
- Tracheal score: 2.2
- Kidney score: 0.6

Group 8 non infected control:

- No clinical signs and no gross lesions.

Histopathological findings of 5 sacrificed experimentally infected chickens 5 days PI

In non-infected control group, the trachea and kidney showed normal histological structures**.**

In infected groups (No. 1, 4 and 5) by IBV isolates No. 11, 13 and 19 respectively, the trachea revealed only mild inflammatory cells infiltration in the lamina propria and mild degenerative changes of the lining epithelial of the mucosa with the activation of goblet cells**.** And the kidney showed congestion of blood vessel with minor hemorrhage.

In infected groups (No. 3 and 6) by IBV isolates No. 21 and 22 respectively, the trachea exhibited focal desquamation of lining epithelium, activation of goblet cells (Fig. 6) and moderate subepithelial lymphoid infiltration. While the detectable kidney lesions in infected group No. 6 were congestion of blood vessels (Fig.12), and kidney lesions in infected group No. 3 were hemorrhage where the erythrocytes extravasated from the blood vessels mostly in the medulla and mild to moderate interstitial nephritis (Fig. 13) which characterized by infiltration of inflammatory cells (heterophils, lymphocytes and plasma cells) in the interstitial tissue.

In infected groups (No. 2 &7) by IBV isolates No. 15 and 24 respectively, the tracheal lesions were activation of goblet cells, edema which characterized by faint esinophilic albuminous fluid (Fig. 7), diffuse and severe subepithelial lymphoid infiltration (Fig. 8), severe epithelial hyperplasia (Fig. 9). Moreover, lymphoid infiltration in lamina propria and submucosa beside hemorrhages and congestion of blood vessels (Fig. 10) and necrotic lining epithelium with complete

desquamation of lining epithelium (Fig. 11) were noticed. And the common findings in kidney were hemorrhage and congestion of blood vessels and severe interstitial nephritis (Fig. 14) which characterized by infiltration of inflammatory cells (heterophils, lymphocytes and plasma cells) in the interstitial tissue.

ELISA test

Results of 10 random serum samples collected at 21 days of age (before virus challenge) revealed freedom from specific antibodies against IBV and MG. Sera collected at 14 days PI belonged to 7 IBV field isolates are summarized in Table 7, evidence of seroconversion are 83.3%, 100%, 66.7%, 33.3%, 83.3%, 83.3% and 100% in chickens infected with IBV isolates No. 11, 15, 21, 13, 19, 22 and 24; respectively.

DISCUSSION

In the present study seven Egyptian variants IBV isolates. 11, 15, 21, 13, 19, 22 and 24 were examined to evaluate their pathogenicity in commercial broiler chickens at 21 days of age (susceptible age for IBV infection in field), by intratracheal route because the transmission of IBV is mainly via the respiratory tract (Sediek, 2005 and Sediek, 2010).The criteria for evaluation of pathogenicity based on the clinical symptoms, post-mortem findings and histopathological lesions as well as seroconversion.

All seven IBV isolates (11, 15, 21, 13, 19, 22 and 24) were capable to induce respiratory signs PI, Table 5 with clinical score of 0.4, 1.9, 0.7, 0.1, 0.2, 0.9 and 1.4, respectively (Table 6). Respiratory signs in the form of conjunctivitis, mainly of a frothy type, associated with lacrimation, oedema and cellulitis of the periorbital tissues (Fig. 2 and Fig. 3), sneezing, cough, tracheal rales and gasping were observed in all infected groups in a varying degrees and varying times of appearance and disappearance (Table 5). All of these birds appeared lethargic, reluctant to move. These findings agree with (Calogero *et al.*, 2008).

Chicken infected with isolates (No. 15 and 24) show clinical signs after 20hours and 36hours PI, respectively, and this agree with (Glahn *et al.* 1989), while other isolates (No. 11, 13, 19, 21, 22) show clinical signs after 3 days PI, this agree with (Sediek, 2010), (Table 5).

No mortalities were recorded during the course of experiment in all groups infected with all seven isolates, these result may be due to that the experimental infection in this study was carried out under laboratory conditions (Sediek, 2010), and cannot be compared with commercial situations in which the mortality due to IBV is variable, depending on virulence of infecting serotype, age, status of immunity, stresses such as stocking density and environmental conditions such as high ammonia level, low ventilation rates and cold stress or possibility secondary bacterial infections. Sex, breed and nutrition are additional factors that contribute to the severity of kidney disease (Linda, 2006.). These findings disagree with (Wang *et al*., 1997), who reported that IBV alone in experimental infection could cause death after infection ranged from 10, 20, 50 and 60 percent in experimental infected chicken groups.

Gross lesions of 5 sacrificed birds five days PI showed congestion and presence of nasal exudate in trachea of birds infected with isolates (No. 15 and 24), and slight increase of mucin in trachea of birds infected with isolates (No.21 and 22), these results is supported by (Calogero *et al*., 2008 and Sediek, 2010). In addition to presence of small areas of pneumonia in birds infected with isolates (No. 15, 22 and 24) which was in agreement with some studies (King and Cavangh, 1991; Sediek, 2010), (Table 5).

Renal lesions (nephritis and enlarged kidney) which were induced in chickens challenged with isolates (No. 15, 21 and 24) were not as pronounced as that seen in the field and this can be explained as stated by Albassam *et al*. (1986) and Chandra (1987) who reported that the type of kidney lesions produced by different NIBV strains were similar but their severity varied. Also, McDonald *et al*. (1980) mentioned that the respiratory route of the infection does not lend itself for qualitative studies on kidney susceptibility of IBV strains, the intravenous inoculation proved to be are predicable method to titrate kidney infectivity of IBV strains (Lambrechets *et al.,* 1991). Or these were other factors in naturally infected flocks since Nephritis Nephrosis Syndrome (NNS) which is a disease condition which could be caused specific pathogen or may be attributed to many concurrent factors including viral, nutritional or toxins (mycotoxin and drug toxicity) etc., (Albassam *et al.*, 1986; Bastami *et al.*, 1987; El-Sisi and Eid-Amal 2004).

The three isolates (No. 11, 13 and 19) not induce respiratory or renal lesions. Also isolate (No. 22) induces respiratory lesions but no renal lesions (Table 5). These findings agreed with (Ignjatovic and Sapats 2000; Ignjatovic *et al.,* 2002; Abdel-Moneim *et al.*, 2006), who reported that strains of IBV differ in virulence or pathogenicity for the respiratory tract, kidney or oviduct.

Regarding to histopathological examination of 5 sacrificed infected birds five days PI, tracheal lesions were inflammatory cells infiltration in the lamina propria and submucosa, desquamation of the lining epithelial of the mucosa, activation of goblet cells, edema in the submucosa, subepithelial lymphoid infiltration, epithelial hyperplasia, hemorrhages and congestion of blood vessels, these results agree with (Chen *et al.*, 1996; Cavanagh and Naqi, 2003; Calogero, *et al.,* 2008; sediek, 2010). Furthermore the renal lesions were diagnosed as interstitial nephritis which characterized by infiltration of inflammatory cells (heterophils, lymphocytes and plasma cells) in the interstitial tissue, these findings are supported by similar findings of (Albassam *et al.,* 1986; Chen *et al.*, 1996; Ziegler *et al.,* 2002; Sediek, 2010).

Serum samples taken from chickens14 days post inoculation with Five IBV isolates (No. 11, 13, 19, 21, 22) and subjected to ELISA test showed geometric mean antibody titer lower than 500 which is extremely low, but two IBV isolates (NO. 15 and 24) showed geometric mean antibody titer more than 3000 (Table 7). But generally the occurrence of low seroconversion

after the inoculation of variant IBV isolates is similar to the findings reported by (Gough *et al*., 1992; Mahgoub *et al*., 2010), who reported that variant IBV strains showed slow seroconversion.

However, the Seven IBV isolates in this study (11, 15, 21, 13, 19, 22 and 24), were classified as variant 2 like strain based on partial S1 gene nucleotide sequencing, they displayed a distinct pathotype from each other in experimentally infected birds. Such divergence is not uncommon finding when the sequencing patterns and the pathogenicity of IBV isolates are compared, because the pathotypes of these viruses did not correspond to their genotypes, as based on S1 gene analysis (Wang and Huang 2000). So the genes determining pathogenicity of IBV need further investigation.

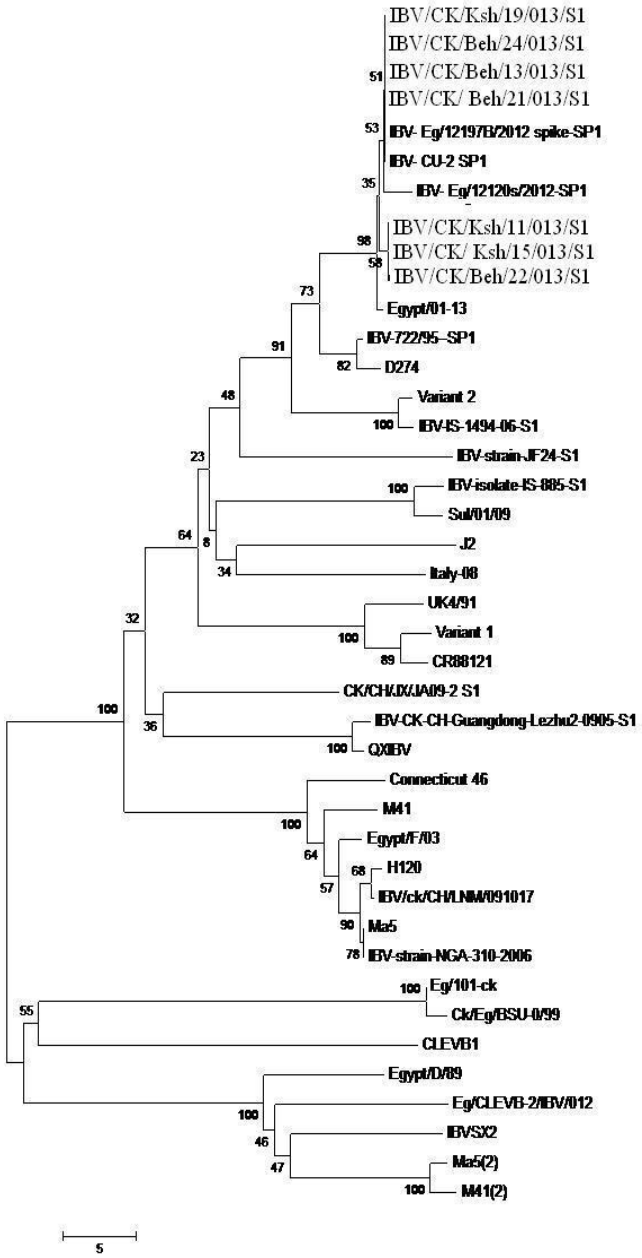

Fig. 1. Phylogenetic tree based on a partial sequence of the S1 gene, showing the relationship between the seven Egyptian IBV isolates in this study, vaccinal strain present in Egypt and other reference IBV world circulated strains.

Fig .2. Serous conjunctivitis with abundan lacrimation, oedema and periorbital cellulitis in bird infected with isolate No. 15.

Fig .3. Periorbital edema and semi-closed eye in bird infected with isolate No. 24.

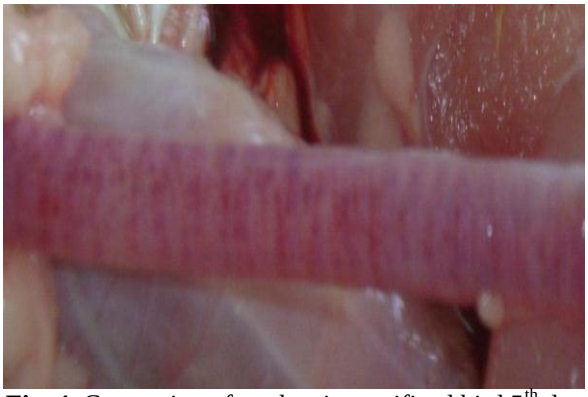

Fig. 4. Congestion of trachea in sacrificed bird 5th day post infection with isolate No. 24.

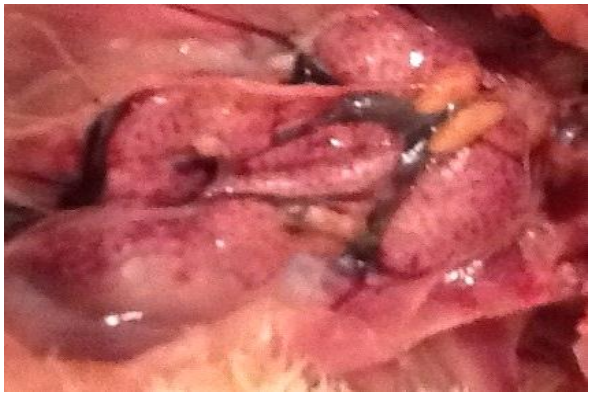

Fig. 5. Nephritis in sacrificed bird 5 days post infection with isolate No.15.

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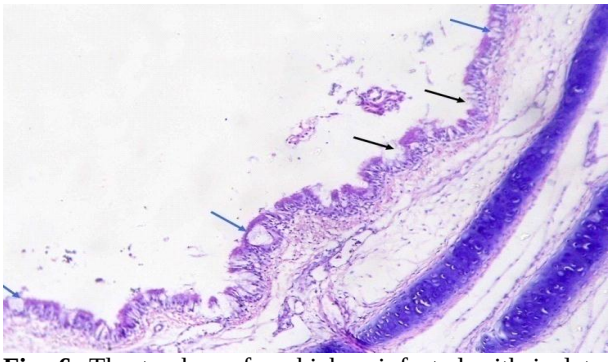

Fig. 6. The trachea of a chicken infected with isolate No. 21 showing focal desquamation of lining epithelium (black arrow) and activation of goblet cells (blue arrow). H&E (X 160).

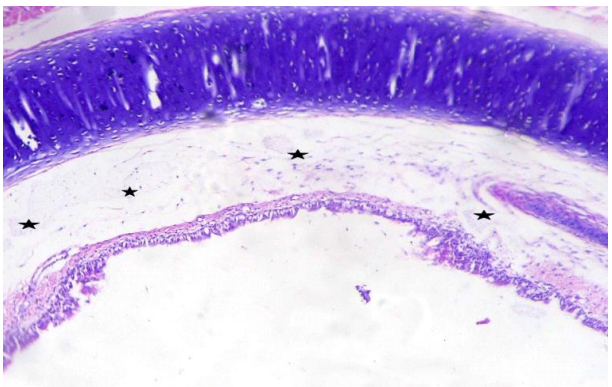

Fig. 7. The trachea of a chicken infected with isolate No. 15 showing faint esinophilic albuminous fluid (stars). H&E (X 160).

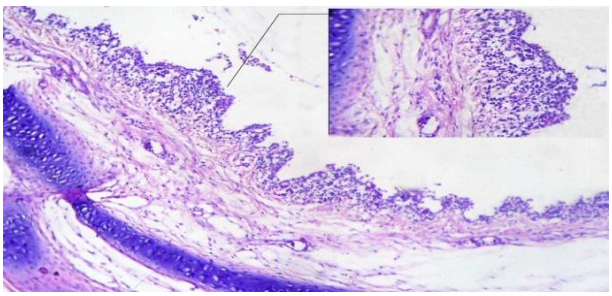

Fig. 8. The trachea of a chicken infected with isolate No. 15 showing diffuse and severe subepithelial lymphoid infiltration. H&E (X 160) (Inset X. 400).

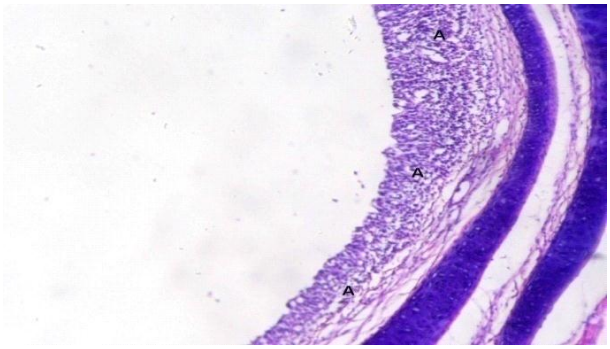

Fig. 9. The trachea of a chicken infected with isolate No. 24 showing severe epithelial hyperplasia (A). H&E (X 160).

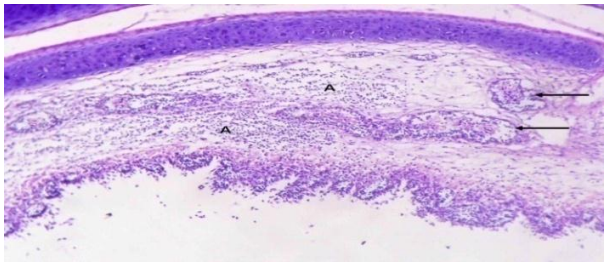

Fig. 10. The trachea of a chicken infected with isolate No.24 showing lymphoid infiltration in lamina propria and submucosa (A) beside congestion of blood vessels (arrows). H&E (X 160).

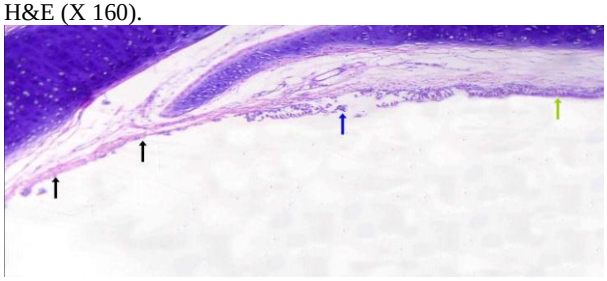

Fig. 11. The trachea of a chicken infected with isolate No.24 showing normal mucosa (green arrow), necrotic lining epithelium (blue arrow) and complete desquamation of lining epithelium (black arrow). H&E (X 160).

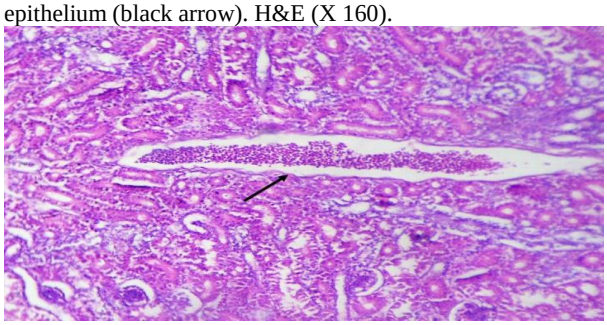

Fig. 12. Kidney of a chicken infected with isolates No. 22 showing congestion of blood vessel (black arrow). H&E (X 160).

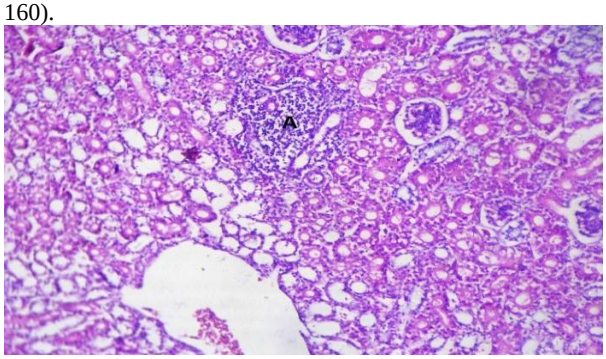

Fig. 13. Kidney of a chicken infected with isolates No. 21 showing mild to moderate interstitial nephritis (A). H&E (X 160).

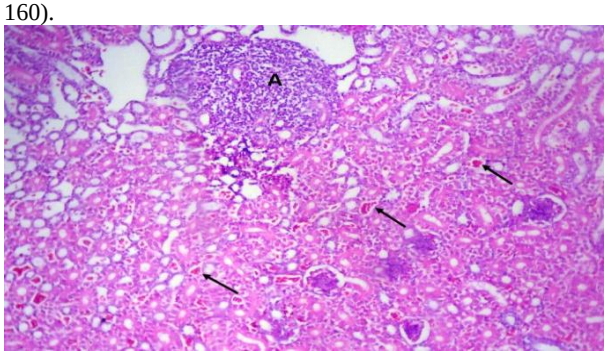

Fig. 14. Kidney of a chicken infected with isolate No. 15 showing moderate to severe interstitial nephritis (A) with congestion of intertubular blood capillaries (black arrows). H&E (X 160).

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Table 1. Isolate codes, nomenclature and accession numbers in Gene Bank of seven IBV isolates from broiler chickens in Egypt investigated in current study.

IBV isolate code	IBV nomenclature	Accession numbers
11	IBV/CK/Ksh/11/013/S1	KM091941
13	IBV/CK/Beh/13/013/S1	KM091942
15	IBV/CK/ Ksh/15/013/S1	KM091943
19	IBV/CK/Ksh/19/013/S1	KM091944
21	IBV/CK/ Beh/21/013/S1	KM091945
22	IBV/CK/Beh/22/013/S1	KM091946
24	IBV/CK/Beh/24/013/S1	KM091947

IBV = Infectious Bronchitis Virus; CK= Chicken; Beh = El Behera Governorate; Ksh= Kafr El Sheikh Governorate

Table 2. Nucleotide identities and divergences of the S1 partial sequence of the seven Egyptian IBV isolates strains in this study with other Egyptian strains, reference strains and vaccinal strains present in Egypt

Percent Identity

Divergence

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
	78.9	77.9	78.2	80.5	95.0	77.1	74.9	75.2	74.9	74.1	76.3	86.2	74.4	77.9	77.6	77.6	78.2	77.6	78.2	77.9	1	Variant-2
21.6		97.7	98.0	89.8	79.9	71.0	68.6	66.0	66.3	65.0	70.0	85.1	67.3	97.4	97.4	97.0	98.3	97.7	97.4	97.7	2	IBV- CU-2 (SP1)
23.0	2.4		98.7	89.8	78.9	70.3	68.0	66.0	66.3	64.4	69.3	84.5	67.0	97.7	96.7	98.0	97.7	97.4	98.3	98.3	3	IBV- Eg/12120s/2012 (SP1)
22.6	2.1	1.4		89.8	79.2	71.0	68.6	66.0	66.3	65.0	70.0	84.8	67.3	97.7	97.4	98.0	98.3	97.4	98.3	99.0	4	IBV- Eg/12197B/2012(SP1)
19.5	11.4	11.4	11.4		80.2	70.3	68.0	68.6	69.0	68.0	70.0	94.4	67.3	90.1	88.8	89.4	89.4	89.1	89.8	89.4	5	IBV-722/95-SP1
5.1	20.3	21.6	21.2	19.9		76.3	74.7	73.8	73.6	72.7	75.8	85.7	73.6	78.9	78.5	78.5	79.2	78.5	79.2	78.9	6	IBV-IS-1494-06-S1
27.4	33.5	34.6	33.5	34.7	28.5		92.8	70.2	70.5	70.0	96.1	75.8	71.1	70.0	69.6	69.6	70.6	70.0	70.3	70.3	7	Variant-1
30.7	37.3	38.4	37.2	38.6	31.0	7.6		71.3	71.6	71.1	93.9	72.7	73.6	67.7	67.3	67.3	68.3	67.7	68.0	68.0	8	4/91
30.3	42.0	42.0	41.9	37.3	32.4	38.4	36.6		99.2	95.9	71.1	72.7	69.4	65.7	65.3	65.3	66.3	65.7	66.0	66.0	9	H120
30.7	41.4	41.4	41.3	36.7	32.9	38.0	36.1	0.8		96.4	71.3	73.0	69.7	66.0	65.7	65.7	66.7	66.0	66.3	66.3	10	Ma5
32.0	43.8	45.0	43.8	38.4	34.2	38.9	37.1	4.3	3.7		70.8	72.7	68.9	65.0	65.0	65.0	65.3	64.7	65.7	65.0	11	M41
28.8	35.3	36.4	35.3	35.3	29.4	4.0	6.3	37.1	36.7	37.6		74.9	70.8	69.0	68.6	68.6	69.6	69.0	69.3	69.3	12	CR88121
15.4	13.4	14.2	13.8	2.8	16.1	29.6	34.4	34.3	33.9	34.3	30.9		71.3	84.8	84.2	84.2	84.8	84.2	84.8	84.5	13	D274
31.8	39.5	40.0	39.5	39.6	33.1	36.8	32.8	39.4	38.9	40.4	37.2	36.6		66.3	66.7	67.0	67.0	67.0	66.7	66.7	14	SUL/01/09
23.1	2.8	2.4	2.4	11.0	21.7	35.2	39.0	42.6	42.0	43.8	37.0	13.8	41.3		98.7	98.7	98.0	98.3	99.0	98.7	15	IBV/CK/Ksh/11/013/S1
23.5	2.8	3.5	2.8	12.6	22.1	35.8	39.6	43.3	42.7	43.9	37.7	14.6	40.7	1.4		98.7	98.3	98.7	98.3	98.3	16	IBV/CK/Beh/13/013/S1
23.5	3.1	2.1	2.1	11.8	22.1	35.8	39.6	43.3	42.7	43.9	37.7	14.6	40.1	1.4	1.4		97.7	98.7	99.0	99.0	17	IBV/CK/ Ksh/15/013/S1
22.6	1.7	2.4	1.7	11.8	21.2	34.1	37.8	41.4	40.8	43.2	35.9	13.8	40.1	2.1	1.7	2.4		99.0	98.0	98.7	18	IBV/CK/Ksh/19/013/S1
23.5	2.4	2.8	2.8	12.2	22.1	35.2	39.0	42.7	42.1	44.6	37.1	14.6	40.1	1.7	1.4	1.4	1.0		97.7	98.3	19	IBV/CK/ Beh/21/013/S1
22.6	2.8	1.7	1.7	11.4	21.2	34.6	38.4	42.0	41.4	42.6	36.5	13.8	40.7	1.0	1.7	1.0	2.1	2.4		98.7	20	IBV/CK/Beh/22/013/S1
23.0	2.4	1.7	1.0	11.8	21.6	34.6	38.4	42.0	41.4	43.9	36.5	14.2	40.7	1.4	1.7	1.0	1.4	1.7	1.4		21	IBV/CK/Beh/24/013/S1
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		

Table 3. Results of titration of seven IBV isolates used for pathogenicity test in embryonated SPF eggs

Isolate Number	*EID_{50} / ml
11	$10^{6.3}$
13	$10^{6.4}$
15	10^{6}
19	$10^{6.6}$
21	$10^{6.2}$
22	$10^{6.3}$
24	10^{6}

*EID_{50} = Median embryo infective dose

Table 4. The experimental design of commercial broiler chickens infected at 21 days of age with seven IBV isolates

Groups No.	Isolate No.	No. of birds per Group	Infected Dose	Age of infection	Age of histopathology	Age of serum Collection
1	11	15 birds	10^5/0.1ml EID_{50}/bird	21 days	5th day PI	21and 35 days of age
2	13					
3	15					
4	19					
5	21					
6	22					
7	24					
8	NC		-	-		

NC: Negative control; - = not done

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Table 5. Clinical signs and pathological lesions in experimentally infected birds with seven IBV field isolates at 21 days of age

Group No.	1	2	3	4	5	6	7
Isolate No	**11**	**15**	**21**	**13**	**19**	**22**	**24**
I. Clinical signs PI	**No. of birds affected**						
Conjunctivitis & lacrimation	4	15	6	2	3	7	12
edema & cellulitis of the periorbital tissues	-	6	2	-	-	2	5
Sneezing & coughing	3	13	5	2	2	5	11
Tracheal rales	1	2	1	-	-	1	2
Gasping	-	2	-	-	-	-	-
Depression	2	8	4	-	-	5	6
Whitish diarrhea	-	2	-	-	-	-	-
Onset of C.S	72 hrs PI	20 hrs PI	72 hrs PI	72 hrs PI	72 hrs PI	72 hrs PI	36 hrs PI
Disap. of C.S	6-7 d PI	6-9 d PI	6-7 d PI	5-6 d PI	5-6 d PI	7-8 d PI	6-7 d PI
Course of disease	4 days	8 days	4 days	3 days	3 days	5 days	6 days
Morbidity	4 birds	15 birds	6 birds	2 birds	3 birds	7 birds	12 birds
Mortality	0.15	0.15	0.15	0.15	0.15	0.15	0.15
C.S severity	Mild	sever	Moderate	Mild	Mild	Moderate	Sever
II. Gross lesions in 5 sacrificed birds 5 days PI	No gross lesions.	Congestion and presence of nasal exudates in trachea of 4 birds, small areas of pneumonia in 4 birds and nephritis in 3 birds.	Slight increase of mucin in trachea of 4 birds and enlarged kidney in 2 birds.	No gross lesions	No gross lesions	Slight increase of mucin in trachea of 4 birds and small areas of pneumonia in 2 birds.	Congestion and Presence of nasal exudates in trachea of 3 birds, small areas of pneumonia in 3 birds and nephritis in 3 birds.

- = not present; PI = post infection; No. = number; CS = Clinical signs; d= days; Disap= disappearance

Table 6. Clinical and gross lesion scores of broiler chickens infected at 21 days old with seven IBV isolates

Group	IBVs (isolates code)	Observation within 14 days post infection			Clinical score	Gross lesion scores in 5 sacrificed bird 5 day PI	
		Morbidity rate	Survived No.	Mortality		Trachea score	Kidney score
1	11	4.15	15	0	0.4	0	0
2	15	15.15	15	0	1.9	2.6	0.6
3	21	6.15	15	0	0.7	0.8	0.4
4	13	2.15	15	0	0.1	0	0
5	19	3.15	15	0	0.2	0	0
6	22	7.15	15	0	0.9	0.8	0
7	24	12.15	15	0	1.4	2.2	0.6
8	Control	0.15	15	0	0	0	0

PI = Post Infection; No. = Number

Table 7. Results of IBV antibodies monitoring of random serum samples (n=6) by the commercial ELISA at 35 days of age (2 weeks Post Infection) of infected broiler chickens groups

Group numbers	1	2	3	4	5	6	7	8***
Isolate numbers	**11**	**15**	**21**	**13**	**19**	**22**	**24**	**-**
IBV Titer	723	1107	669	0	237	0	2544	0
	3867	4266	4142	11953	4503	588	4729	0
	894	3186	2269	731	2065	1541	8465	0
	0	1937	0	0	3200	247	937	0
	3441	9791	937	0	0	1790	1914	0
	464	4080	0	0	198	170	4173	0
Mean titer	1565	4061	1336	2144	1700	723	3794	0
GMT	398	3244	134	14	334	202	3023	0
Post. No.	5	6	4	2	5	5	6	0
Post. %	83.3	100	66.7	33.3	83.3	83.3	100	0

Infections were carried out at 21 days of age; *Group No.8 is non-infected control. - = not done; Post. No. = Positive Number; Post. %= Positive Percentage; GMT=Geometric Mean Titer

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