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STUDIES ON INFECTIOUS BRONCHITIS IN BROILER CHICKENS IN EL- MENIA GOVERNORATE

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SUMMARY

A total of 986 serum samples were collected from IB non vaccinated 26 broiler farms in El- Menia; A total population of 135500 bird. All tested farms were ELISA positive for IBV antibodies. The percent of positivity were 42.86, 33.04, 48.37, 31.01, 43.09, 34.01 and 33.68 in samples from Abo- Korkas, Bani- Mazar, Dear-Mawas, El-Menia, Malawy, Mtay and Smaloot; respectively. IB virus was successfully isolated by 4 blind passages in SPF chicken embryos, which showed curling and dwarfing and were positive to Dot-ELISA against reference antibodies IBV. Pathogenicity of the obtained virus isolate was studied in both broiler and layer chicken. In broiler chick, pathogenicity was performed in groups aged 1, 7, and 14 days. No mortality was seen in group infected at 14 day of age, while it was 8/25 chicks in both 1 and 7 days. Examined tissue sections of trachea, lung and kidney of birds at 4 and 14 days post infection in different age groups revealed severe histopathological changes especially in birds inoculated at 1 and 7 days old as compared with birds inoculated at 14 day old. In layer chicken the isolate resulted in mild respiratory manifestations (sneezing, coughing), decline of egg quantity and quality (rough shell and shell less eggs) 2 weeks after challenge as well as mortality reached 20%. Postmortem examination of succumbed broiler and layer birds revealed severe renal congestion and mild congestion in trachea and larynx.

INTRODUCTION

Infectious Bronchitis (IB) is an acute highly contagious viral disease of chickens that was firstly

observed in USA in 1930 as a new respiratory disease of baby chicks with mortaility up to 30% (Schalk and Hawn 1931), decline of egg production and quality in layer as well as lesion in kidney, and oviduct (Cavanagh and Naqi 1997a; Lambrechts et al., 1993; Cavanagh 2001). Now days, the disease has a world wide distribution among commercial chickens (Saif et al 2003). IB induces economic losses due to poor weight gain in broilers, as well as decreased both egg production and egg quality in layers and breeders flocks. Different IB serotypes have been isolated in Europe (Davelaar et al., 1984; Capua et al., 1994), Australia (Von B¸low 1967; Sapats et al., 1996) Taiwan (Wang and Tsai 1996), China (Qiang et al., 1998) and Mexico (Escoria et al., 2000) and Egypt (Abdel-Moneim 2003). In Egypt the respiratory form of IBV was firstly detected in 1954 (Ahmed 1954) while the nephrogenic strain of IBV from broiler chicken was firstly isolated by (Amin and Mostageer 1977). Isolates related to Massachusetts, D3128, D274 and the novel genotype Egypt/Beni-Suef/01 were isolated from different poultry farms (Sheble et al., 1986; El-Kady, 1989; Abdel-Moneim 2003). The present study aimed to 1- Serologically monitor the current IBV status in El - Menia Governorate. 2- Isolation of IBV from clinically suspected cases and studying the pathogenicity of the isolated strain (s).

MATERIALS AND METHODS

VIRUS: Egg adapted IBV (M41) vaccinal strain was obtained from (Izo, Italy).

CHICKEN SERA:-

1- **Field sera:-** Blood samples (986) for serum according to Anon (1971) were collected from broiler chicken flocks showing respiratory signs, aged 20 - 40 days and not vaccinated against IB from different localities in El-Menia governorate. The serum were kept at -20°C till serologically examined for IBV antibodies.

2- **Referenc and negative sera:-** Standard and Gray types reference chicken anti-IBV sera were kindly supplied by Virol. Depart., Fac. of Vet. Med., Beni-Seuf Univ. The normal negative chicken serum was supplied by Serum and Vaccine Production Institute, Abbasia, Egypt. These sera were used as negative and positive controls in ELISA.

TISSUE SAMPLES:- Tracheae and kidneys were collected from suspected broilers chickens field cases aged 38 days and suffering from respiratory distress as well as experimentally infected birds for virus isolation or reisolation; respectively.

EMBRYONATED CHICKEN EGGS:- Specific pathogen free (SPF) chicken eggs were purchased from Koom Oshiem, Fayoum, Egypt. These eggs were used for virus isolation and titration according to Cunningham (1963) and Villegas and Purchase (1989).

CHICKENS:- Hubbard broiler chicks aged 1, 7 and 14 days; 25 per age; as well as 60 commercial balady layers aged 22 weeks old were used for pathogenicity test following methods of Albassam et al., 1986.

BUFFERS AND SOLUTIONS:- Phosphate buffer saline pH 7.2; Carbonate- Bicarbonate coating buffer; Washing and diluent buffer and Blocking buffer were prepared according to (Kok et al., 1996).

ANTIGEN PREPARATION:- IBV vaccine was used directly as described by (Abdel-Moneam 2003) for coating ELISA plates. The optimal dilution of antigen, serum and peroxidase conjugate for indirect ELISA was carried out by Check board titration (Voller and Bidwell 1986).

ELISA TEST PROPER:- Methods of De Wit et al., (1997) was used where antibody titers of standard positive serum, various positive antisera as well as negative serum were determined. Sample /Positive ratio method (S/P ratio): (Snyder and Marquardt 1989): was determined and the standard curve was constructed. The predicted antibody titers were determined directly from the standard curve by solving the regression line equation. The S/P ratio 0.591 (Log titer) - 1.167. Titres more than 500 were considered positive.

VIRUS ISOLATION:- Methods of Cumming (1969) were adopted for preparation of kidney and tracheal samples, while methods of Cunningham (1963) was used for virus isolation in ECE, each sample was passed 3 times before detection of viral antigen in CAM using Dot- ELISA according to Beyer (1984).

VIRUS TITRATON:- Methods of Villegas and Purchase (1989) were used for titration of IBV in the allantoic fluid of the 7th passage. Virus titers were expressed as 50% embryo infectious doses (EID_{50}) and calculated as described by Reed and Muench (1938).

PATHOGENICITY TEST:- Hubbard broiler chicks (1, 7 and 14 day old), 25 per age group and 60 layer balady chickens (22 week old) were used for pathogenicity test to broiler and layer chickens; respectively. Chickens were infected by intraocular instillation of the isolated strain using 105EID50 (Albassam, et al., 1986). Clinical signs, egg quality and gross P. M were recorded.

HISTOPATHOLOGICAL EXAMINATION:- Tissue samples from kidney, trachea and lung were taken in formol saline for histopathology at 4, and 14 days post infection, sectioned, stained and histopathologically examined for lesions.

RESULTS

Predicted ELISA antibody titers by S/P ratio:- A linear relationship existed between the S/P ratio at a single working dilution (1/500) and the corresponding observed serum titers as determined by serial dilution end point method. The titer of the control positive serum was also determined by serial dilution end point method. Regression analysis yielded a regression line (standard curve) with a correlation coefficient of (r=0.9236) (Fig.1). Average absorbance at a single dilutions were corrected by subtraction from an internal control and divided by corrected positive absorbance and predicted antibody titers were directly determined using the regression line equation:

S/P ratio = 0.591(log titer) - 1.167

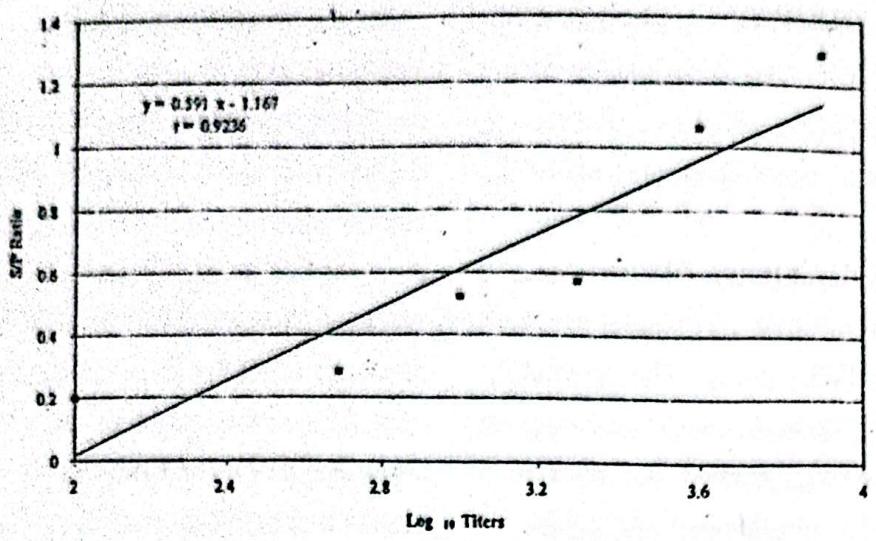

Fig (1): Predicted ELISA antibody titers by S/P ratio

Seroprevalence of IBV ELISA antibody:- Results in table 1 proved that all flocks (26/26) were seropositive with total positive percentage of 37.63%. Positive samples percentage within flocks ranged from 18.42% to 75 %. Most examined samples showed high percentage of positivity (more than 79%) with only one farm showed moderate positivity 59%. IBV ELISA antibody profile in different cities (Table 2) show very high titers profile (more than 10% of the samples are >6000) were noticed in farm 5 (9/40), farm 13 (7/40), farm 14 (7/35), farm 25 (9/41).

Isolation and identification of IBV strains:

Suspected field sample at the 4 th passage were positive for IBV infection as indicated by curling and dwarfing of embryo as well as the presence of virus antigen in CAM as positive Dot - ELISA (Fig. 2).

Pathogenicity of the isolated IBV isolate:

Infected broiler chickens appeared depressed and huddled under heat source with reduced feed intake. Severe conjunctivitis and mild respiratory symptoms including sneezing, cough and rales on 80% of all infected ages at 48 hours from infection. Mortalities appeared only in infected groups aged 1 and 7 days of age but not at 14-days of age. Both groups showed the same mortality pattern with gross lesions including; petecheal haemorrhages in larynx (Fig.3a), congested thymus (Fig.3b) mild airsacculitis, congestion of kidney (Fig 4a), lung (Fig 4c), spleen, as well as on lower border of liver. Kidney lesions and ureates deposit in ureters were detected all over the experiment.

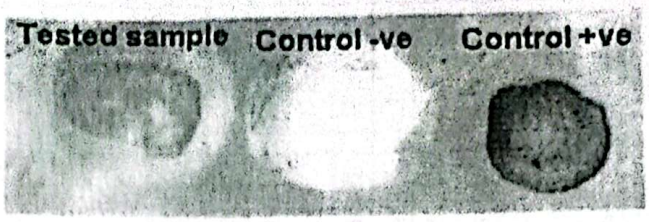

Fig. 2: Nitrocellulose membrane paper. Positive samples showed blue dotes while negative samples showed absence of blue dotes.

Histopathological lesions;

At 4 days P.I.:- Mild tracheal histopathological changes including increased number of goblet cells, mucus, loss of cilia, and focal lymphocytic infiltration and hyperplasia in all examined trachea in all age groups (Fig.5). Kidneys showed lymphocytic infiltration in the outer cortex, hydropic degeneration cloudy swelling and necrosis as well as cellular cast in renal tubules. Hypercellularity of the renal glomeruli was also detected. Congestion and hemorrhages in the renal pelvis submucosa as well as urate crystals in the tubular lumen were detected. Cystic dilatation of many renal tubules in both cortex and medulla were found (Fig.7a) markedly in birds infected at 7 and 14 days of age. Lung lesion was different markedly in different age group, the most sever microscopic finding was detected in 1day old infected birds while moderate and mild lesions were seen in birds infected at 7 and 14 days; respectively (Fig 6). Lesions included atelectasis, emphysema, oedema, intrabroncheal and alveolar haemorrhages with haemosiderosis as well as lymphocytic infiltration.

At 14 days P.I.:- Kidneys showed, lymphocytic infiltration, degenerative changes, cystic dilatation of few renal tubules in both cortex and medulla. Fibroplasia in the collecting ducts (Fig.7b) and in the ureters (Fig.7c). Plasma cell infiltration as well as urates deposition in the ureters was recorded. No marked differences were found between different age groups. Lung sections revealed necrosis of the alveolar epithelium, atelectasis, emphysema, oedema, intrabronchial as well as alveolar haemorrhages with haemosiderosis and lymphocytic infiltration. Changes in the pulmonary blood vessels included fibroblastic proliferation, hyalinosis of tunica media, swelling of tunica intema as well as perivascular oedema. Fibroplasia was also detected in the bronchi. Different ranges of severity in different age group were also detected as mentioned in lung lesions at 4 days PI.

Table (1): ELISA Antibody Profile of IBV antibodies in different farms allover El-Menia Governorate.

Flock No.	Locality	Type	Age (day)	Density	Sample No.	Titer range					Positive/ sample ratio	Negative/ sample ratio	Infection %
						0-500	500-1000	1000-3000	3000-6000	>6000			
1	Abo-Korkas	Hubbard	23	5000	35	20	3	6	3	3	15/35	20/35	42.86%
2	Bani-Mazar	Hubbard	20	4500	39	29	4	3	0	3	10/39	29/39	25.64%
3		Hubbard	33	4000	39	25	2	6	1	5	14/39	25/39	35.90%
4		Hubbard	21	5000	37	23	6	5	2	1	14/37	23/37	37.84%
5	Dear-Mawas	Ross	35	5500	40	10	7	10	4	9	30/40	10/40	75.00%
6		Ross	21	4500	38	31	2	4	0	1	7/38	31/38	18.42%
7		Ross	27	5000	35	16	5	5	4	5	19/35	16/35	54.29%
8		Ross	27	5000	40	22	6	6	2	4	18/40	22/400	45.00%
9	El-Menia	Hubbard	24	4000	45	31	4	4	4	2	14/45	31/45	31.11%
10		Hubbard	21	4500	40	27	6	4	1	2	13/40	27/40	32.50%
11		Ross	21	6000	33	24	3	3	1	2	9/33	24/33	27.27%
12		Hubbard	33	6000	40	27	0	6	1	6	13/40	27/40	31.50%
13	Malawy	Hubbard	30	5000	34	15	4	5	3	[illegible]	19/34	15/34	55.88%
14		Hubbard	31	5000	35	18	0	4	6	7	17/35	18/35	48.57%
15		Hubbard	25	6000	36	18	4	6	5	3	18/36	18/36	50.00%
16		Hubbard	25	5000	45	30	5	5	1	4	15/45	30/45	33.33%
17		Hubbard	23	8000	38	26	5	3	1	3	12/38	26/38	31.58%
18	Matay	Hubbard	26	4000	38	20	8	5	1	4	18/38	20/38	47.37%
19		Hubbard	21	5000	38	27	4	5	2	0	11/38	27/38	28.95%
20		Hubbard	21	5000	31	19	2	4	4	2	12/31	19/31	38.71%
21		Ross	23	5500	40	31	3	2	2	2	9/40	31/40	22.50%
22	Smaloot	Hubbard	25	5000	36	28	4	2	0	2	8/36	28/36	22.22%
23		Hubbard	28	6500	37	26	5	1	1	4	11/37	26/37	29.73%
24		Hubbard	25	5500	35	24	6	4	0	1	11/35	24/35	31.43%
25		Hubbard	36	6000	41	20	3	6	3	9	21/41	20/41	51.22%
26		Ross	21	5000	41	28	8	4	0	1	13/41	28/41	31.71%
Total				135500	986	615	109	118	52	92	371/986	615/986	37.63%

Table (2): ELISA Antibody Profile of IBV antibodies in different centers allover El-Menia Governorate.

Flock No.	Locality	Density	Sample No.	Titer range					Positive/ sample ratio	Negative/ sample ratio	Infection %
				0-500	500-1000	1000-3000	3000-6000	>6000			
1	Abo-Korkas	5000	35	20	3	6	3	3	15/35	20/35	42.86%
2	Bani-Mazar	13500	115	77	12	14	3	9	38/115	77/115	33.04%
3	Dear-Mawas	20000	153	79	20	25	10	19	74/153	79/153	48.37%
4	El-Menia	20500	158	109	13	17	7	12	49/158	109/158	31.01%
5	Malawy	29000	188	107	18	23	16	24	81/188	107/188	43.09%
6	Matay	19500	147	97	17	16	9	8	50/147	97/147	34.01%
7	Smaloot	28000	190	126	26	17	4	17	64/190	126/190	33.68%
Total		135500	986	615	109	118	52	92	371/986	615/986	37.63%

Fig.3: Tracheal and Thymus lesions associated with IB infection.
a . Petechial hemorrhage in larynx. b. Congested thymus. c. Control thymus.

Fig.4: Kidney and lungs of chickens infected with IBV:
a. Congested kidneys with tubules and ureters distended with urates.
d. Control kidney. c. lung congestion d. Control lung.

Fig. 5: Trachea 4 days PI showing focal lymphocytic infiltration, oedema and cell proliferation (x:40).

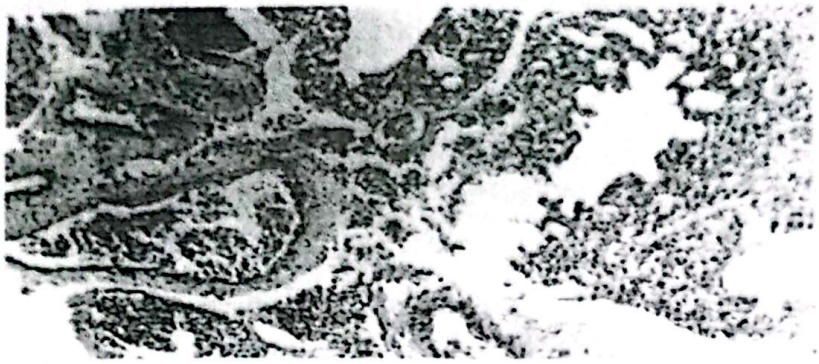

Fig.6:- Lung sections of chickens show excessive haemorrage in the bronchial lumen and alveoli (x:4).

Fig. (7): Kidney sections of chickens infected at:

(a) 1 day showing multiple cystic dilatation and focal lymphcytic infiltration at 4 days PI (x:10);

(b) 1 day-old showing fibroplasia in the renal collecting tubules at 14 day PI (x:10);

(c) 14 days showing fibroplasia and lymphocytic infiltration in the ureter at the 14 th day PI (x:10).

Pathogenicity of the isolated IBV strain in layer chicken showed a mild sneezing, cough and rales at the 2nd day PI. Singes of lower egg production and deterioration of egg quality (rough shell and shell less egg) (Fig.8) appeared after 2 weeks PI. A total of 12 birds were succumbed after infection with sever kidney congestion, urates deposition (Fig.9b) and excessive mucous in the tracheal lumen. Mortality pattern appeared at 16t day PI (2 chickens), 20th day PI (8 chickens) ar 21st day PI (2 chickens) with a total 20%.

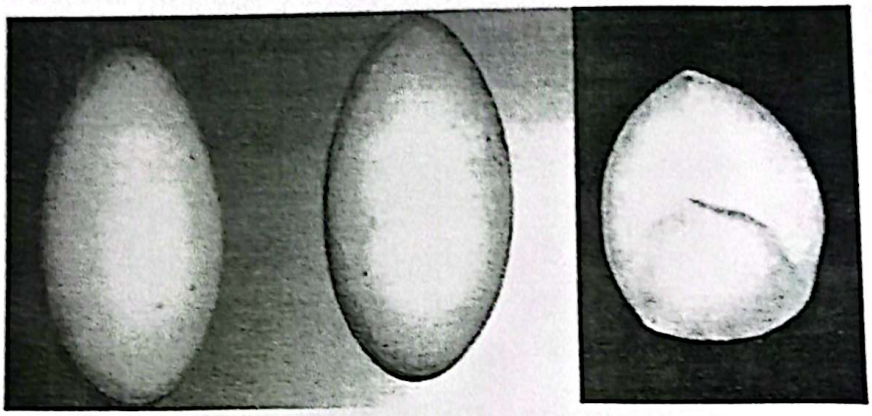

Fig. 8: Changes in egg shell due to IBV infection:
(left) Normal.(middle) Rough shell egg (right) Soft shell.

Fig. 9: Congested kidneys with tubules and ureters distended with urates. (14 days P.I.).

DISCUSSION

Infectious bronchitis (IB) is a highly contagious viral disease affecting all ages of chickens and causes a number of economically significant problems in commercial chickens. (Cumming 1962), reproductive tract infection (Bisgaard 1976 ; Cook et al., 1986), enteric infection (Karaca et al., 1990) and more recently proventricular infection (Fang et al., 1998). Since the description of ELISA by (Engvall and Perlmann in 1971), it has been adapted for detecting antibodies to a variety of disease causing agents including IBV antibody (Soula and Moreau 1981; Nadapalan et al., 1982; Case et al., 1983; Snyder et al., 1984). (Snyder et al., 1983a and b) found that S/P ratio is the most commonly adopted in ELISA kits. In this study the regression line equation: S/P ratio = 0.591(log titer)-1.167. Regression analysis yielded a regression line (standard curve) with a correlation coefficient of (r=0.9236). Seroprevalence of IBV ELISA antibody among broiler chickens age ranged from 21-45 days of age, so it can be assumed that the antibodies being measured were due to IBV exposure as the maternal antibodies decline about 2 weeks following hatching (Andrade et al., 1983 ; Abdel-Moneim 2003). All examined broiler flocks were found positive for IBV (100%). This result agrees with that of (Abdel-Moneim 2003) in Beni-Suef governorate. We found high percentage of infection, while the previous studies in Egypt showed lower positive reaction in chicken flocks by (Ahmed et al., 1968; Moustafa 1977; Ismail et al., 1980; Amer 1984; Sheble et al., 1986; and EL-Kady 1989) where the prevalence of IBV precipitating antibodies were 11.86%; 13.7%; 15.57%; 25.9%; 14.65% and 42.4%; respectively. These lower percentage may be related to either increasing the prevalence of IBV infections in El-Menia Governorate or due to test sensitivity. The percentage of positivity within the examined samples per flock ranged from 18.42% to 75 %. The high percentage of positivity may denote the frequency of IBV infection among the broiler flocks in El-Menia Governorate. Most examined samples showed moderate percentage of positivity (more than 30 %) with only one farm in Dear-Moass showed low positivity 18.42% (Table 2). Very high titers profile (more than 9% of the samples are >6000) were noticed in farm 5 (9/40), farm 13 (7/40), farm 14 (7/35), and farm 25 (9/41) (Table 1&2 Fig.2) that may suggest that chickens in such farms were repeatedly infected with IBV. Expected repeated IBV infection from very high IBV antibody titers was also suggested with other investigators (Takase et al., 2000). Concerning virus isolation attempts, earlier attempts of IBV isolation was almost unsuccessful (Salama 1976, Moustafa 1977). The first virus isolation was accomplished by (Amin and Mostageer 1977) who isolated a nephrogenic strain of IBV from broiler chicken but its serotypes was not defined. (Bastami et al., 1987) also isolated a nephrogenic IBV strain. Isolates related to Massachusetts, D3128, D274 and the novel genotype Egypt/Beni-Suef/

01 were isolated from different poultry farms (Sheble et al., 1986; Elkady, 1989; Abdel-Moneim 2003). Dot-ELISA using the reference serum on the chorioallantoic homogenate of suspected positive sample was found positive that confirms IBV virus identity.

The pathogenicity of the isolated strain was in both broiler resulted in mild clinical signs including conjunctivitis, rales and nasal discharge. (Beaudette and Hudson 1937,Van Roekel 1939 and Fabricant 1949).

Gross lesions included: petecheal haemorrhages in the larynx, mild airsacculitis, congestion of kidney, lung, thymus spleen, as well as liver especially over its lower border. Very sever congestion of the kidneys in comparison to kidneys of control uninfected birds were detected all over the experiment. No marked gross differences were observed in lesions in different age groups. Interestingly, lung histopathology was different markedly in different age group. The most sever finding was detected in birds infected at 1day of age while birds infected at 7 and 14 days of age showed moderate and mild lesions respectively. The results are in agreement with findings of other investigators who found IBV antigen in larynx, trachea, thymus, lung, spleen, liver, bursa and kidney (Cumming 1969; Ambali and Jones 1990; Abdel-Moneim 2003). The microscopic findings of the trachea agree with those of (Dutta 1975; Purcell et al., 1976; Toro et al., 1988). The renal tubules showed hydropic degeneration, cloudy swelling, and coagulative necrosis as well as hyaline casts, focal lymphocyte infiltration, hypercellularity of the renal glomeruli. Microscopic examination of the ureter revealed, lymphocytic infiltration in the Lamina propria, fibroblastic proliferation of the Lamina propria of the ureter appeared at 14 day P.I. These findings are match the general findings recorded with nephrogenic IBV strains (Purcell et al., 1976; Albassam et al, 1986). The most prominent histopathological lesions observed in lung was included atelectasis, emphysema, oedema, intrabroncheal and alveolar haemorrhages with haemosiderosis as well as lymphocytic infiltration. At 14 days PI changes in the pulmonary blood vessels included fibroblastic proliferation, hyalinosis of tunica media, swelling of tunica intema as well as perivascular oedema. Fibroplasia was also detected in the bronchi. Different ranges of severity in different age group were also detected as mentioned in lung lesions at 4 days PI.

In layer chicken, infection with isolated IBV strain resulted in mild respiratory symptoms (sneezing, cough and rales) and singes of lower egg production and deterioration of egg quality (rough shell and shell less egg) that appeared after 2 weeks PI. This result agrees with that recorded by (Bisgaard 1976) who reported similar findings due to IB outbreaks. A total of 12 birds were dead after infection with sever kidney congestion, urates deposition and mucous in the

tracheal lumen. Although mild respiratory manifestations were recorded after infection of layer chicken with the isolated IBV strain, high mortality was observed (12/60). Absence of gross lung P/M findings and the presence of excessive urates deposition in the kidneys that were severely congested may denotes that deaths in layer birds may be related to renal failure. High level of dietary calcium in layer ration in conjunction with experimental IBV infection may predispose to the observed mortalities in experimentally infected layer chickens that succumbed with urolithiasis and kidney lesions. A condition recorded with the finding of other investigators (Brown et al., 1987; Glahn et al., 1989).

The high prevalence of IBV infection in EL-Menia Governorate as indicated by seroprevalence studies in conjunction with the success of isolation of nephrogenic IBV strain from broiler chickens that has the capacity to affect also layer chickens with considerable mortalities and decreased egg production and quality necessitate further isolation and serotype identification of IBV in a regular manner and defines the predominant serotype to develop suitable vaccination program to control IBV in Egypt.

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