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The Role of Infectious Bronchitis Virus in Respiratory and Renal Problems in Broiler Chickens

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Abstract

Broiler chickens in Egypt represent the main source of animal protein. Unfortunately, they usually are hit by many disease problems. Respiratory and renal affections are investigated in forty four broiler chicken flocks aged (19-54) days and suffering from respiratory and renal distress during 2014-2015 in three large Governorates; Sharkia, Dakahlia and Gharbia Variable mortality rates (0.5-30%) were recorded. On necropsy, tracheitis, bronchitis and congestion of lungs were recorded in all examined flocks. Caseated plugs at tracheal bifurcation 28/44, fibrinous pericarditis, perihepatitis and air sacculitis 19/44 were also observed. Nepherosis-nephritis 22/44, and General congestion with hemorrhage on proventriculus 9/44 and cecal tonsil 4/44 were seen among affected birds. Chicken embryo inoculation for IBV isolation revealed reduction in embryo size was recorded in 20%, death within 72h in 54.5% while 11% of flocks required successive blind passage to show reduction in embryo size up to 2cm compared to 5cm in negative control. Thirty three out of forty four harvested allantoic fluids were positive with Heamagglutination test (75%). The reverse transcriptase polymerase chain reaction (RT-PCR) was carried out on both original tissues and allantoic fluids using specific primers for S1 gene. Nine positive infectious bronchitis viruses (IBVs) (20%) were detected in original samples compared with only 2 (4.5%) in allantoic fluids of the same samples. It could be concluded that the IB virus infection did not exceed 20% of the causative agents in disease condition in question and usually in mixed infection. In turn the use of variant vaccines did not solve the problem but exacerbated the emergence of new IB viruses. There were clear evidence that HA virus did the main role in such disease problems. The direct RT-PCR could be reliable tool for appropriate IBV diagnosis to estimate the real situation in chicken diseases.

Key word: IBV, Respiratory, Renal, RT-PCR, HA viruses.

Introduction

Poultry industry is considered an important pillor in Egyptian economy. Broiler chickens as a major source of animal proteins are threatened by many pathogens that substracte the profit of this sector. Respiratory and renal troubles are commonly recorded among flocks [1-3]. Infectious bronchitis is one of important disease affecting respiratory and /or urogenital systems in broilers all over the world [4] In addition, morbidity as high as (100%) and mortality (≤ 30%) occurs in young chickens in nephropathogenic strains. The disease is exaggerated by secondary bacterial infections leading to increased mortality rate in broiler flocks [4].Infectious bronchitis virus (IBV) is one of genus corona viruses, family coronaviridae, order nidovirals**,** more than 20 serotypes within IB viruses have been identified worldwide [5,6].

Variant IB viral strains emergence has complicated the problem. Outbreaks with these variant strains usually differ serologically from the vaccine strains. Despite using both classic and variant types of IB vaccines, economic losses still occurred continuously by IB virus

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causing clinical disease and production problems in vaccinated flocks [7-11]

The IB virus has two spike - glycopolypeptides S1 and S2. Neutralizing and serotype specific antibodies have mainly bounded for the S1 glycoprotien [12]. Consequently, the identification of IB viruses mostly focused on S1 gene analysis [13].

Materials and Methods

Clinical and postmortem examination

Broiler chicken farms (n=44) suffering from respiratory and renal distress were investigated during 2014-2015. The study included three Governorates Sharkia, Dakhalia and Gharbiathe. Gharbia. A comprehensive descriptive data were obtained (Table.1).Clinical and postmortem examination the birds were (n=5-10) examined clinically and postmortem lesions were recorded.

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Table 1: Data of the Chicken Broiler Flocks examined in Sharkia, Dakhlia and Gharbia Governorates during 2014-2015

Flock number	Date of sampling	Locality	Breed	Age (days)	Total number	Mortality*	Previous vaccination
1	15-12-2014	Gharbia	Cubb	30	1400	10%	IB primer 1d- ND+H9 inactivated 5d- IBMA5+Clone 18d
2	15-12-2014	Gharbia	Sasso	45	6000	3%	IB primer 1d- ND+H9 inactivated 5d- IBMA5+Clone 18d
3	15-12-2014	Gharbia	Sasso	29	8000	0.5%	Not vaccinated
4	19-12-2014	Gharbia	Sasso	38	6000	2%	IB primer 1d- ND+H9 inactivated 9d- IBMA5+Clone 17d
5	20-12-2014	Gharbia	Cubb	30	2000	2%	IB primer 1d- LaSota+ H9 7d- ND inactivated 15d- IBMA5+Clone 19d
6	22-12-2014	Gharbia	Cubb	33	2400	12%	IB primer 1d- ND+H9 inactivated 8d- Clone 17d
7	1-1-2015	Gharbia	Cubb	35	10000	4%	IB primer 1d- ND+H9 inactivated 5d- IBMA5+Clone 18d- LaSota 30d
8	6-1-2015	Gharbia	Sasso	26	5000	5%	IB primer 1d- ND+H9 inactivated 5d- IBMA5+Clone 18d
9	6-1-2015	Gharbia	Cubb	23	2000	9%	IB primer 1d- ND+H9 inactivated 8d- IB 4/91 15d Clone 30d
10	12-1-2015	Gharbia	Cubb	25	700	8.5%	Not Vaccinated
11	10-2-2015	Gharbia	Cubb	30	2200	2.5%	IB+ Hitchener B11d
12	10-12-2014	Dakahlia	Cubb	27	1900	6%	IB primer 1d- IB 4/91 14d
13	12-1-2015	Dakahlia	Sasso	54	7700	2.5%	IB primer 1d- ND+ H9 inactivated 5d IBMA5+Clone 18d- LaSota 42d
14	6-1-2015	Sharkia	Cubb	30	1000	30%	Not Vaccinated

15	17-1-2015	Sharkia	Sasso	28	220	20%	Not Vaccinated
16	18-1-2015	Sharkia	Sasso	35	10000	1%	IB primer 1d- ND+H9 inactivated 8d-Clone 17d
17	22-1-2015	Dakahlia	Avian 48	28	2000	12%	IB primer 1d- ND+H9 inactivated 8d- IB 4/91 15d Gumboro 19d
18	22-1-2015	Dakahlia	Cubb	27	17000	6.5%	IB primer+Gumbro 1d- LaSota+H9 5d- ND+ IB primer 15d Clone 30 19d
19	26-1-2015	Dakahlia	Sasso	36	11000	1.5%	IB primer1d LaSota18d- LaSota28d
20	26-1-2015	Dakahlia	Cubb	36	19000	1%	IB primer 1d- ND+H9 inactivated 8d- IB 4/91 15d Clone 30 19d
21	26-1-2015	Sharkia	Cubb	25	6000	5%	Not Vaccinated
22	27-1-2015	Sharkia	Cubb	30	7000	1%	IB primer 5d
23	9-2-2015	Dakahlia	Cubb	30	20000	1%	Vaccitec+ IB primer 1d H5N1 7d- ND inactivated 10d IBMA5 15d- LaSota 21d
24	9-2-2015	Dakahlia	Sasso	40	26000	1%	IB primer 1d- H5N1 7d ND inactivated 10d IB primer 14d
25	9-2-2015	Dakahlia	Sasso	40	6000	5%	IB primer 1d- ND inactivated 5d- Gumboro 14d-LaSota 21d - H5N1 35d
26	16-2-2015	Dakahlia	Cubb	30	6800	4%	Vaccitec+ IBprimer+ ND1d- H5N1 6d- LaSota8d- ND inactivated 12d IB primer 14d
27	23-2-2015	Dakahlia	Sasso	37	16000	2%	IB primer+ H9 1d IBMA5 9d-Gumboro 12d-Vitabron 22d
28	23-2-2015	Dakahlia	Sasso	38	15000	1.5%	Hitchener+ Gumboro7d ND inactivated 9d- Gumboro14d- H5N1 15d- LaSota+IB 18d- LaSota 28d
29	8-3-2015	Sharkia	Sasso	34	23	4%	Not Vaccinated
30	25-3-2015	Sharkia	Cubb	23	17000	5%	IB primer1d- Clone 7d- ND+AI inactivated 9d-Gumboro 12d
31	25-3-2015	Sharkia	Cubb	25	5000	0.5%	IB primer1d- ND+AI inactivated 1d- H5N2 9d- IBD 13d
32	26-4-2015	Sharkia	Cubb	40	500	2%	Hitchener B1 1d
33	30-4-2015	Dakahlia	White	29	9000	5%	IB primer 1d- Hitchener+ H5N2 5d H5N2+ND inactivated 9d IBMA5 10d

								LaSota19d
34		30-4-2015	Sharkia	Sasso	22	6000	8%	IB primer1d H5N1 5d ND inactivated 9d
35		30-4-2015	Dakahlia	Sasso	36	4800	4.3%	Vaccitec+ IB primer+ Avenu 1d- H9+ND inactivated 5d- H5+LaSota 12d IB primer 18d
	36	30-4-2015	Dakahlia	Cubb	19	5000	16%	Hitchener 6d- Gumboro 12d
37		12-5-2015	Sharkia	Balady	40	45	2%	Not Vaccinated
	38	12-5-2015	Sharkia	Ross	27	2500	6%	Hitchener 7d- Gumboro 14d
39		28-5-2015	Dakahlia	Balady	45	8000	1.5%	IB primer1d Hitchener 7d Gumboro 12d Clone30 20d LaSota 45d
40		4-6-2015	Dakahlia	Cubb	29	20000	8%	Vaccitec 1d H5N1+ LaSota 10d LaSota 20d
41		4-6-2015	Dakahlia	Cubb	26	10000	1%	Vaccitec+ ND +IB primer 1d – H5N1 7d- LaSota 10d- Clone + IB 17d
42		14-6-2015	Sharkia	Avian48	26	11000	10%	IB H120 1d- ND+H9 inactivated 7d IBD 13d – Clone30 18d
43		14-6-2015	Sharkia	Cubb	24	5000	3	Hitchener + IB 1d ND inactivated 5d IBD 12d Clone 30 17d
44		20-6-2015	Dakahlia	Cubb	35	11500	10%	IB primer 1d H5N1 6d LaSota 9d ND inactivated 11d

Mortality rate was calculated till submission day.

Sample preparation

Tissue pools of respiratory organs (tracheas, bronchi, lungs) and kidneys (3/each) were taken separately from the examined flocks (n=44)**.** The tissue suspension was prepared and antibiotics (penstrept Biowhit taker) were added to supernatants [14].

Virus isolation

Tissue supernatants (0.2 mL / each) were inoculated into five (9-11days) ECE via allantoic cavity route. The dead embryos were collected and left to be chilled at 4°C for overnight then allantoic fluids were harvested aseptically and examined for HA activity. Embryos examined for any lesions indicate of IBV infection [15].

Detection of IBV by RT-PCR

RNA Extraction

Total RNA was extracted from suspected original tissues and allantoic fluids by Gene JET RNA Purification Kit (Thermo Scientific ^{TM} # K0731).

cDNA synthesis

The cDNA synthesis was carried out in 20 µL Vol. using revert Aid first strand cDNA

synthesis kit (Thermo Scientific ™ # K0731). The reactions were incubate at 37°C for 60 min then at 42°C for 60 min later on it was terminated by heating at 70°C for 5min. followed by cooling at 4°C for 15min.

RT-PCR reaction

The RT-PCR was performed in a total of 25 µl in a sterile 0.2 mL RNase free PCR tubes. For one reaction, the assay optimized to 12.5µL of Dream Taq Green PCR Master Mix (2X), 0.25µLof selected forward and reverse primers (100pmole/µl), 2µL of cDNA and 10µL of RNase- free water [16].

The optimized PCR cyclic reaction condition preformed in MWG-Biotech Thermal cycler and described as following: PCR cycle for detection of IBV using a pair of primers F: 5'- GCT TTT GAG CCT AGC GTT-3', R: 5'-GCC ATG TTG TCA CTG TCT ATT-3' [16]

Each cycle of PCR includes steps for template denaturation, primer annealing and primer extension. The initial step denatures the target DNA by heating it to 94°C for 3 min. In the denaturation process, the two intertwined strands of DNA separate from one another, producing the necessary single-stranded DNA template for replication by the thermostable DNA polymerase. In the next step of a cycle, the temperature is reduced to approximately 52°C. At this temperature, the oligonucleotide primers can form stable associations (anneal) with the denatured target DNA and serve as primers for the DNA polymerase. This step lasts approximately 30 s. Finally, the synthesis of new DNA begins as the reaction temperature is raised to the optimum for the DNA polymerase to approximately 72°C. The extension step lasts approximately 30 s.

Agrose gel electrophoresis of RT-PCR

Electrophoresis was done at 100 voltes for 40 min and the bands were viewed by UV trans-illuminator [16].

Results

Clinical Findings

Clinically examined chickens showed general signs of illness and respiratory troubles including (sneezing, coughing, rales, gasping and nasal discharges) in all flocks. Whitish diarrhea was observed in Eighteen flocks (40%) while Greenish diarrhea was observed in thirteen flocks (29%). Mortality rates ranged from (0.5-30%); in investigated flock suffering from respiratory troubles (caseated plug) it ranged from (0.5-20%); and increased to 30% when associated with renal affection. It ranged from (0.5-30%) in positive HA flocks. In positive single IB cases the mortality rates ranged from (5-10%) while in cases IB mixed with hemagglutinating agent was (1-16%)

Necropsy revealed tracheitis, bronchitis and congestion of lungs in all flocks. Caseated plugs at tracheal bifurcation (n= 28) (Figure 1 A,B). Beside CRD lesions (fibrinous pericarditis, perihepatitis, air saccculitis) (n=19). Kidney damage in the form of nephritis -nepherosis with urate deposition in the ureters (n=22). Nephritis (Figure 1 C) as well as swollen and pale kidneys with prominent tubules was observed (Figure 1 D). General congestion and hemorrhages were noticed on Proventriculus, Cecal tonsils were enlarged and ulcerated.

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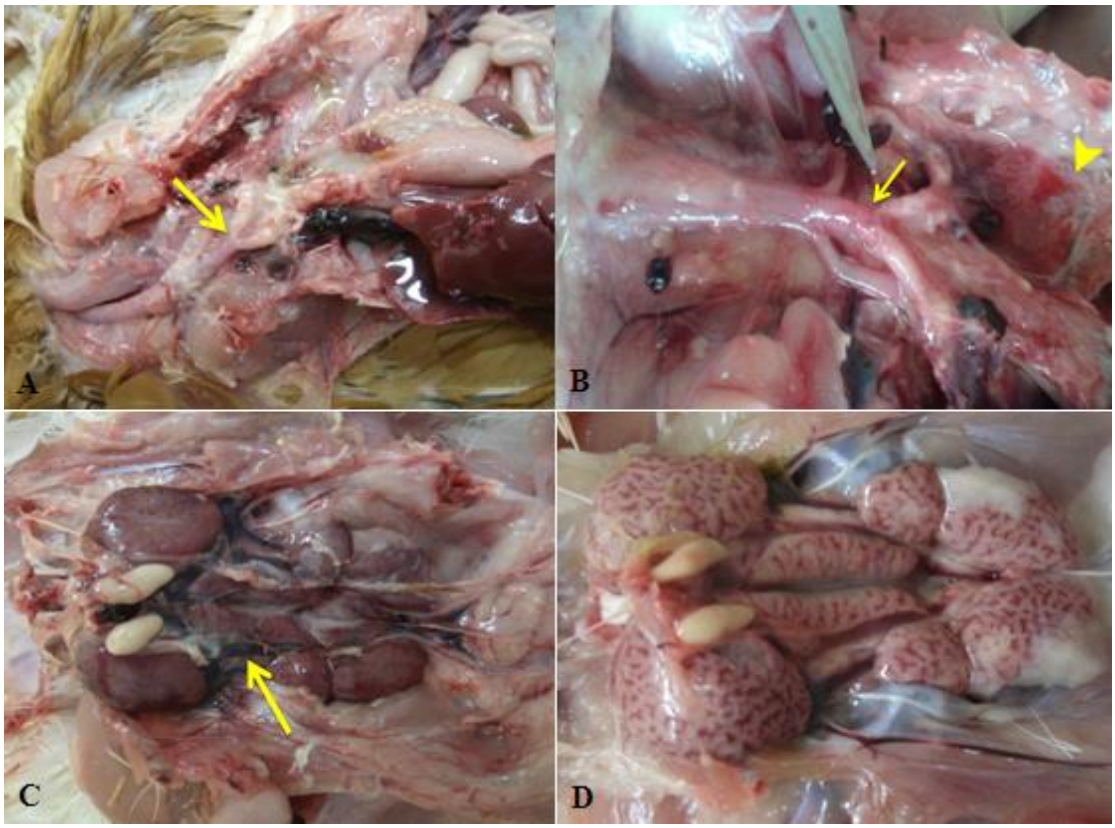

Figure 1: Postmortem lesions of examined flocks. A. Trachea of 29 day old broiler chicken (farm No.33) containing caseated plug in tracheal bifurcation. B. Trachea of 26 day old broiler chicken (farm No.42) containing caseated plug in tracheal bifurcation and Cloudiness of air sacs (arrow head). C. Kidney of 28 day old broiler chicken (farm No.17) showing Nephritis. D. Kidney of 29 day old broiler (farm No.40) showing severe Nephrosis (swollen and pale kidneys with prominent tubules).

Isolation on ECE and HA results

Embryo death with congestion within 1st 72 hrs (n=24) were recorded and size of embryo was reduced during first passage (n=9). While, five flocks required 3-6 blind passages to show reduction in embryo size up to 2 cm in comparison to its negative control 5 cm (Table 2).

Thirty three out of forty four harvested allantoic fluids showed agglutination of washed chicken RBCs 10% with percentage of (75%) while eleven failed to agglutinate RBCs (Table 2).

Table 2: IBV isolation and molecular identification from affected broiler chickens at Sharkia, Dakhalia and Gharbia Governorates during 2014-2015

Sample No.	ECE Lesions	HA test	IBV Detection		
			RT-PCR (Trachea)	RT-PCR (Kidney)	RT-PCR Allantoic fluid
1	Small	-	-	-	-
2	Congested*	+	-	-	-
3	congested	+	-	-	-
4	congested	+	-	-	-
5	congested	+	-	-	-
6	small	-	-	-	-
7	congested	+	-	-	-
8	congested	+	-	-	-
9	congested	+	-	-	-
10	small	-	-	-	-
11	congested	+	-	-	-
12	small	-	-	+	+
13	small	-	-	-	-
14	congested	+	-	-	-
15	congested	-	-	-	-
16	small	-	-	-	-
17	congested	+	-	+	-
18	congested	+	-	+	-
19	congested	+	-	-	-
20	congested	+	-	-	-
21	congested	+	-	-	-
22	congested	+	-	-	-
23	congested	+	-	+	-
24	congested	+	-	-	-
25	congested	+	-	-	-
26	congested	+	-	-	-
27	congested	+	-	-	-
28	congested	+	-	-	-
29	congested	+	-	-	-
30	congested	+	-	-	-
31	congested	+	-	-	-
32	congested	+	-	-	-
33	small	-	+	+	-
34	congested	+	-	-	-
35	small	-	-	-	-
36	congested	+	+	+	-
37	congested	+	-	-	-
38	congested	+	-	-	-
39	congested	+	-	-	-
40	small	-	-	+	-
41	congested	+	-	-	-
42	congested	+	+	+	+
43	congested	+	-	-	-
44	small	-	-	+	-
Total %	20%**	75%	20%		4.5%

*Congested embryos were small due to earlier death

** Percentage of ECE showing small and dwarfed embryos.

Molecular identification using RT-PCR

All samples suspected to be infected with IBV were identified by RT-PCR using specific primers. The results revealed 9 out of 44 (20%) tissue samples were IBV Positive. However, Allantoic fluids of the same flocks only 2 flocks were IBV positive (Table 2 and Figure 2).

The analysis of the relatedness of clinical findings, virus isolation and Rt-PCR results was illustrated in Figure (3).

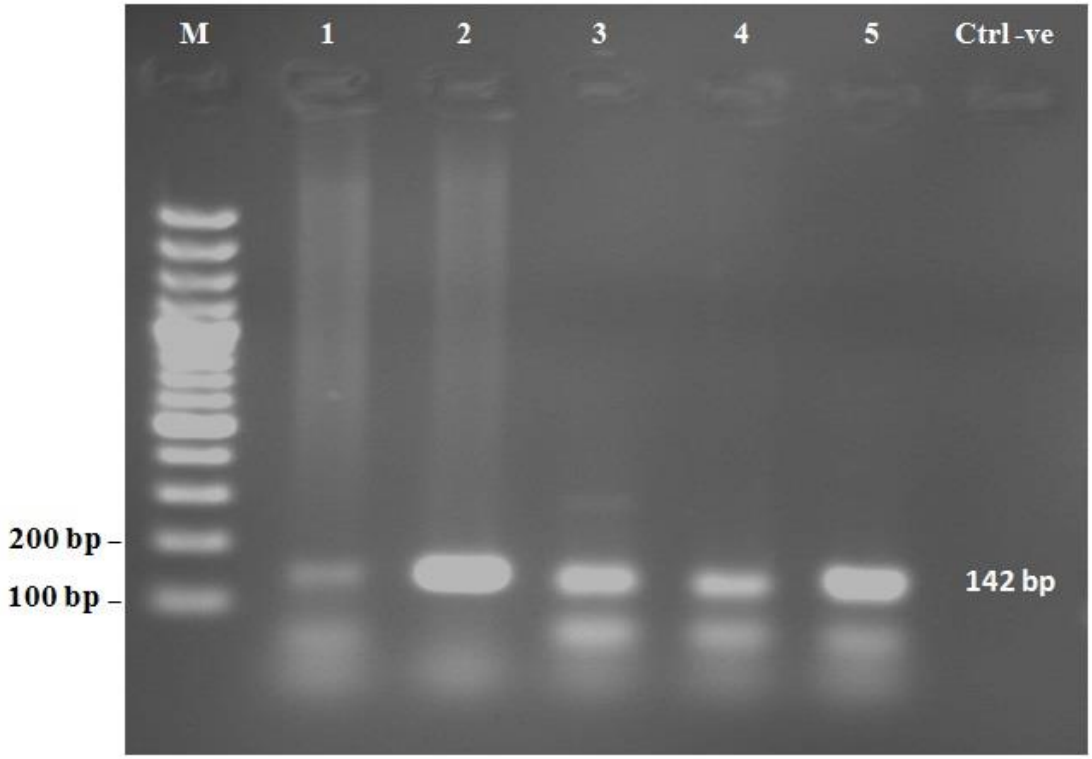

Figure 2: Molecular identification of IBV using RT-PCR: Agarose gel electrophoresis of the RT-PCR products of IBV. Lane(M): DNA ladder (marker) 100pb. Lane (1) positive IBV represents the tested sample No 12(kidney). Lane (2) positive IBV represents the tested sample No 40(kidney). Lane (3) positive IBV represents the tested sample No 42(trachea). Lane (4) positive IBV represents the tested sample No 42(kidney). Lane (5) positive IBV represents the tested sample No. 33 (trachea). All positive samples showed band at 142 bp Lane (ctrl -ve): negative control.

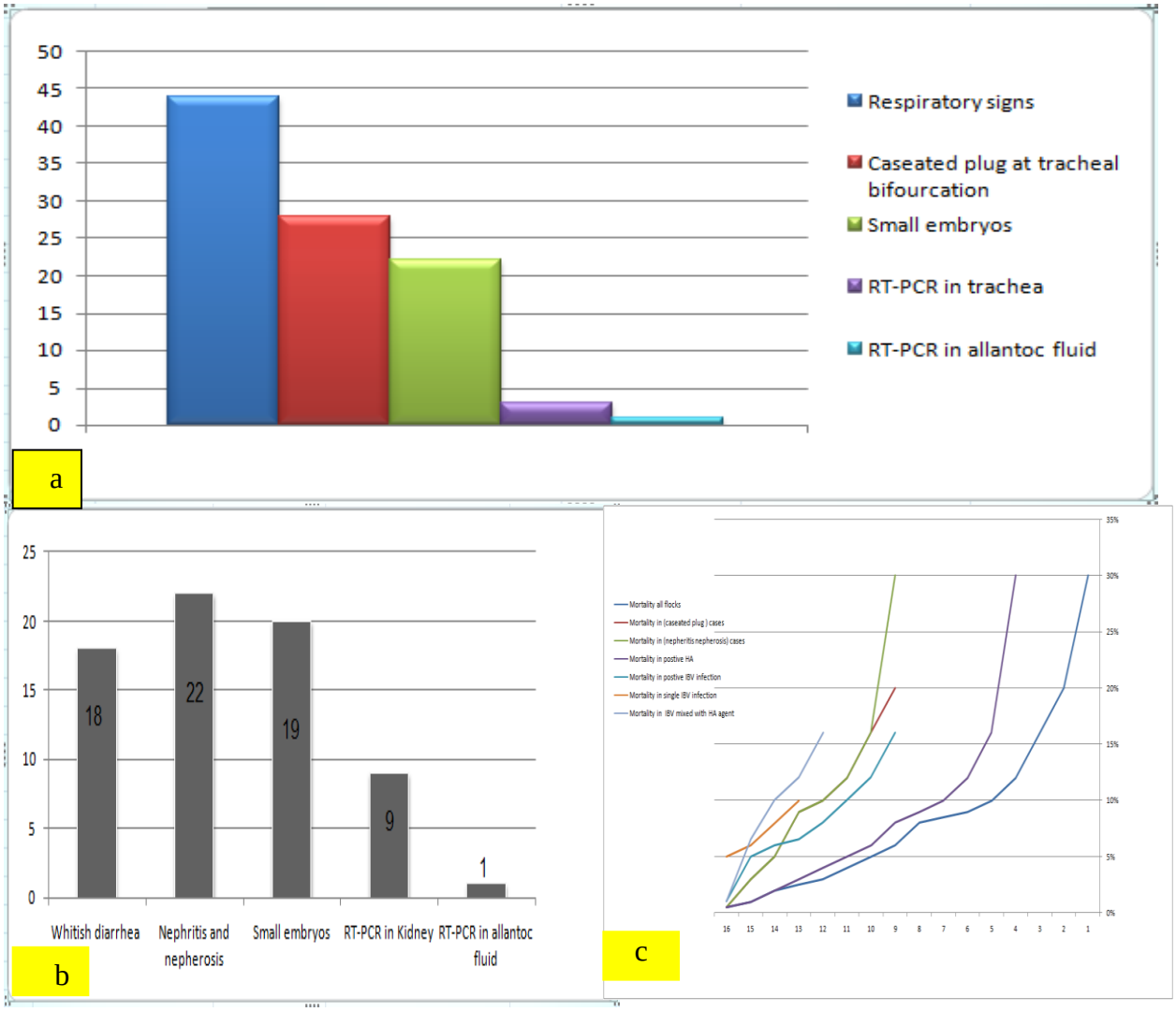

Figure 3: Relatedness between clinical and laboratory diagnosis of IB; a. Respiratory cases, b. Renal cases and c. mortality pattern

Discussion

Infectious bronchitis (IB) has occurred frequently in vaccinated as well as non vaccinated chicken farms causing harsh economic losses so this study aimed to throw light on the responsibilities of IBV on respiratory and renal troubles in broiler chickens in Sharkia, Dakalia and Gharbia Governorates. The obtained results revealed that the frequently observed clinical signs among examined broiler flocks were depression, huddling together under a heat source with profound reduction in feed consumption. The respiratory signs were the main, and ranged from mild to marked gasping, sneezing, coughing, nasal discharges, with sinus swelling. Other examined birds showed renal signs in the form of whitish diarrhea in 41% of the examined flocks. The obtained results were analogous to that previously recorded in IBV infection [17-20].

Mortality rates varied from 0.5 to 30%. There was a positive relation between the severity of clinical picture, and number of detected agents. Also the association of renal signs exacerbated the mortality (30%) among affected flocks. These findings are not dissimilar to the previously reported by Chone and Apostolov [21] Bastami *et al.*[22] and Ziegler *et al.*[23] In Egypt, Commercial

poultry flocks have highly regarded mortalities associated mainly with respiratory and renal troubles [13,24,25]

The postmortem lesions included tracheitis, bronchitis and congestion of lungs in all flocks followed by caseated plugs at tracheal bifurcation occurred in 28 flocks. Kidney damage in the form of nepherosis-nephritis with urate deposition in the ureters was seen in 22 flocks. Additionally some birds exhibited hemorrhages in proventriculus and general congestion, Also fibrinous pericarditis, perihepatitis, air sacculitis Similarly, variable lesions were recorded in former IBV single and mixed infections in chickens [23,26-28]. The presence of fibrinous inflammation indicated complication with bacterial infection. The finding was in agreement with that of Awad *et al*. [29] who recorded that IBV infection is commonly followed by secondary bacterial infection however the hemorrhagic lesions in visceral organs may be pointing to concurrent severe viral agents. Therefore in flocks under field conditions it is thought that infection with these isolates would also predispose chicks to bacterial and or viral infections, resulting in complicated morbidity and increased mortality [28-30]

Embryo death within 72 hrs PI was recorded in 54.5% of inoculated samples. The allontoic fluids of these embryos revealed positive HA activity that suggesting virulent hemagglutinating viral infection which may be of ND and /or AI viruses in the same way Eid [31] recorded the rapid embryo death with positive HA activity in both ND and AI virus infections in Egyptian chicken flocks.

Reduced Embryo size was evidenced during first embryo inoculation 20% and 11% required 3-6 blind passages to show reduction in chicken embryo size up to 2 cm in comparison to its negative control 5 cm. The recorded results were considered evocative to IBV infection as recorded by Jordan and Nassar [32]

Using specific primers, 9 flocks were IBV Positive to IBV including 6 from renal the examined tissues but only 3 were detected in both renal and respiratory tissues. The examination of their allantoic fluids with the same primers revealed dramatic decrease in IBV detection to be in only 2 flocks (12, 42) with a percentage of 4.5%. Correspondingly the IBV was evidenced positive using the same primer band at 142 bp By Callison *et al*.[16] and Awad *et al.* [29] who stated that the Taqman RT-PCR assay using a specific primer succeeded in detection of infectious bronchitis virus from infected chickens.

The hemagglutinating agents were detected in 75% of the examined flocks comparable results were recently recorded by Hassan [28] who reported the occurrence of HA viruses in 86% of the investigated flocks with respiratory and or renal affections in north Shrakia during 2012-2013.

Mixed virus infections were recorded in 5 out of 9 (55.6%) of IBV positive samples. likewise many authors stated that IBV was frequently isolated with other viral and /or bacterial infection [30,33]

In a trial to analyze the role of different vaccine protocols applied among investigated flocks to protect birds from IB virus infection, the obtained information were as follows 1: The IBV was isolated from both classic and variant virus vaccinated flocks; 2: Neither once nor twice vaccination prevent IBV infections e.g. the virus was isolated in birds vaccinated only with classic virus at day 1 of age as in flock 42 and in flocks received both classic (Ma5) and variant (primer vaccine) or 4/91 vaccines as in 12,17,18, 23, 33, 36 and 44 flocks. On the same level non vaccinated flocks (36 and 40) revealed positive IBV detection. Correspondingly, many authors explained their findings by little or no cross protection of vaccinal strains against field circulating ones [24,34].Vaccine failure may be attributed also to misapplication of vaccine or impaired immune status of the birds and concurrent infections as previously mentioned [12,35]

The correspondence of field observations and laboratory IBV isolation and identification was analyzed. It was found that 44 chicken flocks suffering from respiratory signs and have caseated plugs in tracheal bifurcation among 28 ones. Only three IB virus isolates were identified in their tissues which were reduced to one after embryo passage. Similar pattern was observed among renal affections in

22 flocks and virus isolation was evidenced in 9 while only one allantoic fluid sample positive for IBV. The obtained findings could be explained by; 1: the high incidence of concurrent HA viruses detected in allantoic fluids of 33 samples; 2: presence of latent virus infection and /or maternal derived antibodies (MDA) in chicken embryos which may interfere with IBV replication [28,36]

It is clear that, despite the different vaccine program against IB and hemagglutinating (ND and AI) viruses, concurrent virus infections were commonly recorded among chickens and still causing bad economic impact on this industry. The HA virus spread interfered with the routine protocol of IBV diagnosis.

Conclusion

It could be concluded that; The IB virus infection did not exceed 20% of the causative agents in disease condition in question and usually in mixed infection. In, turn the use of variant vaccines did not solve the problem but exacerbated the emergence of new IB viruses, There were clear evidence that HA virus did the main role in such disease problems and the applied vaccine programs against ND and AI viruses succeeded only to minimize rather than to prevent them.

Conflict of interest

The authors have no conflict of interest to declare.

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الملخص العربى

دور فيروس التهاب الشعب الهوائية المعدي في مشاكل الجهاز التنفسي والكلوي في دجاج التسمين

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يمثل دجاج التسمين في مصر المصدر الرئيسي للبروتين الحيواني و لسوء الحظ ، عادة ما يصاب بالعديد من المشاكل المرضية. ولقد تم فى هذا البحث دراسة التأثيرات التنفسية والكلوية في أربع وأربعين من قطعان دجاج التسمين (١٩-٥٤) يومًا ، و التى تعاني من اعراض تنفسية وكلوية خلال ٢٠١٤-٢٠١٥ في ثلاث محافظات كبيرة هى الشرقية والدقهلية والغربية وقد كانت معدلات النفوق متغيرة (٠.٥-٣٠٪). واظهرت نتائج فحص الصفة التشريحية للطيور المصابة التهاب فى القصبات والشعب الهوائية مع تجمع الافرازات المتجبنة فى شعب القصبة الهوائية واحتقان الرئتين في جميع القطعان التي تم فحصها. مع وجود سدة متجبنة عند نهاية القصبة الهوائية وبداية الشعبتين الهوائيتين في ٢٨/٤٤ قطيع والتهابات فبرينية على غشاء التامور والكبد والأكياس الهوائية في ٤٤/١٩ قطيع كما وجدت التهابات بالكلى مع تضخم وشحوب فى لونها فى ٤٤/٢٢ قطيع. واحتقان عام مع نزيف على ٤٤/٩ بالمعدة الهاضمة ولوزتين الأعورين فى عدد ٤٤/٤ بين الطيور المصابة. وقد كشف حقن الاجنة عن نفوق الأجنة خلال ٧٢ ساعة (٥٤.٥٪)، وصغر حجم الجنين فى ٩ قطعان، بينما تطلب ١١٪ من القطعان متعاقبة لإظهار صغر فى حجم الاجنة لتصل إلى ٢ سم بالمقارنة مع الضابط السلبي ٥ سم . وباستخدام اختبار تلزن الدم على سوائل الالونتيوس وجد عدد ٣٣ عينة (٧٥%) إيجابية ثم اجرى تفاعل البلمرة المتسلسل للاستنساخ العكسى (RT-PCR) على كل من الأنسجة الأصلية وسوائل اللانتيوس باستخدام بادئات محددة لجين S1. تم الكشف عن تسعة أنواع من فيروسات التهاب القصبات المعدية الإيجابية (٢٠٪) في العينات الأصلية مقارنة مع ٢ (٤.٥٪) فقط في السوائل الجنينية من نفس العينات. وعليه يمكن الاستنتاج أن عدوى فيروس التهاب الشعب المعدى(IB V) لا تتجاوز ٢٠ ٪ من العوامل المسببة في حالة المرض المعنية وعادة في العدوى المختلطة. وان استخدام اللقاحات االمتحورة لم يحل المشكلة بل يمكن ان يؤدى الى تفاقم ظهور فيروسات جديدة .و كان هناك دليل واضح على أن فيروسات اخرى ايجابية التلزن الدموى قامت بالدور الرئيسي في مثل هذه المشاكل المرضية وعليه يمكن أن يكون تفاعل البلمرة المتسلسل للاستنساخ العكسى المباشر أداة موثوق بها لتشخيص فيروس التهاب الشعبى المعدى (IBV) في الدجاج للوقوف على التمثيل الحقيقى لتواجد فيروس التهاب الشعبى المعدى بالحالات المرضية بالدجاج.