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Original Research Article

Epidemiology of viral components causing respiratory problems in broilers in six Egyptian Governorates

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ABSTRAC

Infectious bronchitis (IB), Newcastle disease (ND) and Avian influenza (AI) are highly contagious and the most economically important diseases of the poultry affecting the respiratory tract and causing economic losses in the poultry industry throughout the world. In the present study, 180 broiler flocks were sampled from 6 different Egyptian provinces (Giza, Qaluobia, Sharqia, Menofia, Al Behira and Fayoum) during 2014 to 2015. The birds showed respiratory illness and they were examined for 4 respiratory viral diseases; avian influenza (AI subtype H5 and H9), vNDV and IBV. All farms were vaccinated against IBV, ND and AI and were investigated using RT-PCR. The results showed that 41 out of 180 broiler farms were positive for either IBV or vND or AI-H5 and AI-H9 as a single infection as follows: 24, 10, 5 and 2 farms respectively. There were 62 farms detected as mixed infection, the highest incidence was shown in 40 farms co-infected with IBV and AI (H9) and 11 with IBV and vNDV, rRT-PCR results for each governorate separately go more or less parallel to that of all governorates collectively, There was no clear geographical preferences in positive viruses among governorates. ,Mortality rate and clinical signs incidence showed the highest percentage for birds reared in winter and Autumn compared with the other seasons. The results revealed that IBV as a single or a mixed infection had a major role in the respiratory problem in the field.

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1. Introduction

Respiratory diseases in poultry are caused by several pathogens that act either singly or in combination with each other. In Egypt, respiratory diseases represent a huge problem in the poultry industry because of their multifactorial nature. Clinical signs elicited by some poultry respiratory pathogens are similar and may be confused. Different clinical manifestations have been increasing in Egyptian commercial chicken flocks during the last few years. These pathogens are of major significance and have a large economic impact because they are able to induce disease independently or in association with each other (Roussan et al., 2008). Avian influenza (AI) either highly pathogenic ad H5 o low pathogenic as H9, Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) are the main respiratory causes of high mortality rates in broiler chicken flocks (Haghighat-Jahromi et al., 2008).

One of the major components of mixed infections is Infectious Bronchitis (IB) that is a highly contagious disease results in a significant economic losses to the commercial chicken flocks. Chickens are susceptible to IBV infection. In broilers, economic loses are produced by a decrease in weight gain with low feed efficiency and increased condemnations in the carcasses specially when infectious bronchitis is complicated with secondary as bacterial infections (Pan et al., 2012).

The disease frequently causes respiratory signs including gasping, coughing, sneezing, tracheal rales, and nasal discharge (Gelb et al., 1991). In addition, some strains have been associated with kidney lesions (Liu and Kong, 2004). The severity of the symptoms in chickens is related to their age and immune status. Other signs of IB including wet droppings are due to increased water consumption. The type of virus strain infecting a flock determines the pathogenesis of the disease and the degree and establishment of lesions in different organs. The upper respiratory tract is the primary site of infection, but the virus can also replicate in the reproductive, renal, and digestive systems (Stachowiak et al., 2005). To monitor the existing different IBV in a geographical region, PCR on the reversely transcribed RNA is a potent technique for the detection of IBV. Comparing with classical detection methods, PCR-based techniques are both sensitive and fast (Zwaagstra et al., 1992).

Newcastle disease (ND) is one of the most devastating viral diseases of poultry and has great economic impact in the poultry industry causing bird mortality reaches 100% between the infected flocks in case of infection by velogenic strains of the virus (Alexander et al., 2003). ND virus is regarded as being endemic in many countries including Egypt (OIE, 2009, 2012).

Exotic Newcastle Disease Virus (ENDV) is very virulent strain causes severe losses in pet and game birds in USA and these birds are considered a good reservoir for transmitting the virus between domestic commercial flocks causing severe losses in poultry production (OIE, 2008). Infection by NDV is categorized into different pathotypes according to the strain affecting the flock and clinical signs appear; lentogenic strains which cause mild respiratory symptoms and used as secondary live vaccines, mesogenic strains which are fatal only for young chicks, viscerotropic velogenic strains which are fatal for all ages of chicken and almost characterized with enteric signs and neurotropic velogenic strains which are characterized with nervous signs (Alexander and Allan, 1974; Beard et al., 1984).

Avian influenza (AI) is a disease of poultry that resulted in high economic losses beside its zoonotic potential. The disease is caused by type A influenza viruses that belong to family Orthomyxoviridae (Palese and Shaw, 2007). Avian influenza is further classified into highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI), depending on the severity of the disease in susceptible birds. HPAI outbreaks in chickens and turkeys have been caused mainly by the H5 and H7 subtypes, however some of the H5 and H7 subtypes have been characterized as HPAI and many strains of these subtypes have been shown to be LPAI (Zhou et al., 1999).The silent spread of LP H9N2 has been recorded in the Middle East and the Far East regions for several years indicated additional risk factor to the poultry industry. Although H9N2 viruses were characterized as low pathogenic avian influenza (LPAI) viruses, they caused high morbidity and mortality (Naeem et al., 2007). The recent emergence of H9N2 virus in Egypt was from clinically healthy commercial bobwhite quail flock in May 2011 (El-Zoghby et al., 2012) then the virus co-circulated with HPAIV subtype H5N1 in which the silent spread of H9N2 viruses could affect the normal spread of HP H5N1(Arafa et al., 2012).

This work aims primarily to describe the current field problems facing the poultry industry with special reference to avian influenza, Newcastle disease and infectious bronchitis viruses in commercial broiler chicken flocks. The exact field situation of both single and mixed viral infections will be determined and their role in enhancing severity of respiratory affections and subsequent high mortality rates. That may help us to raw epidemiological map that help the authorities the circulating viruses in the field that share in accomplishment the applied scientific solutions among poultry flocks.

2. Materials and methods

Sampling and collection of epidemiological data

Tracheal swabs, Kidney, proventriculus, cecal tonsils, trachea and lungs from each bird were collected from a total of 180 broiler flocks then prepared for RT-PCR. The prevalence of four respiratory viral diseases (IBV, vNDV and AIV subtype H5 and H9) in commercial Broiler chickens with respiratory tract abnormalities was studied for a period of 2 years (January 2014 to December 2015) in six different governorates. One hundred and eighty broiler flocks collected from six different governorates with thirty flocks per each, with history and symptoms suggestive of Respiratory tract infection were inspected and the information regarding flock strength, age, method of rearing, vaccination schedule, production performance, symptoms manifested and mortality were collected.

RNA isolation and real-time RT-PCR

RNA was extracted using RNA Extraction: QIAamp Viral RNA Mini Kit (Qiagen, Valencia, Calif., USA, Cat. no. 52904). The kit possesses the selective binding properties of a silica- gel-based membrane with the speed of micro-spin technology, the simultaneous detection and differentiation of causative agents of these diseases were investigated using Real –Time Reverse Transcriptase PCR (RRT-PCR), Quantitect probe RT-PCR kit (Qiagen, Inc. Valencia CA, Cat no 204443): reactions. It was used for performing RRT-PCR using Stratagen MX3005P machine (Stratagene, USA).

Primer and probe used for RT-PCR were as follows (Table 1); For IBV: IBV5_GU391, IBV5_GL533, IBV5-G probe (Callison et al., 2001). For vNDV: ND-For, ND-Rev, ND-probe-(VFP-1) (Wise et al., 2004). For AI (H5): H5LH1, H5RH1, H5PRO (Slomka et al., 2007). For AI (H9): H9-FOR, H9-Rev, H9-probe (Ben Shabat et al., 2010).

3. Results

In the present study, 180 broiler flocks were collected from 6 different Egyptian governorates (Giza, Qalyobia, Sharqia, Menofia, Al Behira and Fayoum) during the period from 2014 to 2015. Birds showed respiratory illness and they were examined for some respiratory viral diseases like avian influenza (AI) for the 2 most common subtypes (H5 and H9), vNDV and IBV. All farms were vaccinated against IBV, vND and AI. The cardinal signs of the examined broilers were respiratory distress, tracheal rales, coughing and sneezing with or without nasal discharge and generalized weakness was observed accompanied by depression. Feed consumption and body weight are markedly reduced, greenish diarrhea, renal ureate deposition and death beyond three to four weeks of age (late mortality), Incidence of clinical signs and mortality rate was the highest in birds aged from 20-35 days, Mortality rate in the flock under investigation ranged from 2.5% to 43% with higher mortality rate in a vaccinated flock suffer from triple infection of vNDV, IBV and AI-H9 viruses. Mortality rates in flocks co-infected with IBV and AI-H9 were as high as 27%, even in flocks vaccinated against both pathogens. The simultaneous detection and differentiation of causative agents of these diseases were investigated using RT-PCR.

In this study, we investigated 30 broiler flocks from each governorate (Giza, Monofia, Behira, Sharqia, Fayiom and Qaliobia). The results of rRT-PCR showed that IBV infection had a major role in the respiratory problem in the field with 82 flocks (45.6%), followed by AIV-H9 with 52 flocks (28.9%),where the incidence of vNDV and AIV-H5 was 27 flocks (15%) and 10 flocks (5.6%), respectively. The Mixed infection including more than one causative agent was commonly shown in recent respiratory affection in poultry in Egypt, Where it represents 34.4% (62 flocks), while single viral infection of the tested viruses was 22.8% (41 flocks) of total 180 investigated flocks collectively. Moreover, results showed that mixed infection with IBV and AIV-H9 viruses was the highest incidence as mixed infection in the examined flocks with positivity in 40 flocks (22.2%). This was followed by 11 flocks (10.8%) showed mixed IBV and NDV infection, 6 flocks (5.8%) suffering triple IBV, vNDV and AIV-H9 infection. There were 4 flocks

showed double AIV-H5, AIV-H9 infection and one flock showed double IBV and AIV-H5 infection. The highest incidence of single infection was IBV with positivity in 24 flocks (13.3 %) followed by 10 flocks (9.7%) showing single vNDV, 5 flocks (4.85%) showing single AIV-H5 infection, 2 flocks (1.9%) showing single AIV-H9 infection. There was no record of mixed triple IBV, AIV-H9 and AIV-H5 infection as well as the co-circulation of the 4 investigated viruses (Table 2).

According to the geographical distribution, we found that the mixed infection with both IBV and AI-H9 was the highest incidence at Sharqia governorate (9 flocks) represent 30% of tested flocks (30), followed by Behira governorate with (8 flocks) represent 27.6%, while the lowest incidence was at Monofia governorate with (5 flocks) represent 16.7% of tested flocks. In all examined governorate the most prevalent was IBV and AI-H9 viruses with total (40 flocks) represent 22.2% from total investigated flocks. Then, we found IBV and vNDV with the same incidence percent to all governorate with (6.6%), except for Monofia governorate with only one flock representing 3.3%. for triple infection with IBV, vNDV and AI-H9, we found that Giza and Behira governorates was the highest incidence with (6.6%), followed by Qalubia and Monofia governorates with (3.3%) and negative to other governorates. The double infection with AI-H5 and AI-H9 was the highest at Behira (6.6%) while Giza and Qalubia was lower (3.3%) and negative for other governorates. The double infection with IBV and AI-H5 was only one flock in Fayoum. On the other hand we found the highest incidence with IBV as a single infection was at Giza with (6 flocks) represent 20%, followed by Behira, Sharqia and Qalubia (4 flocks) represent 13.3%, and the lowest incidence was in Fayoum and Monofia (3 flocks) represent 10%. Moreover, In all surveyed governorates the most prevalent single infection was IBV with total (24 flocks, 13.3%). Then vNDV with the highest incidence in Sharqia and Monofia (3 flocks each, 10%) and the lowest incidence was at Behira and Qalubia (3.3%) and it was negative in Giza.

For AI-H5 as a single infection the highest incidence was at Behira (6.6%), then Sharqia, Qalubia and Monofia (3.3%) and negative in Giza and Fayoum. AI-H9 was recorded as a single infection in Giza and Sharqia in only one flock for each governorate (3.3%) (Table 2, Fig. 1)

In summary, rRT-PCR results for each governorate separately go more or less parallel to that of all governorates collectively. Mixed infection with IBV and AIV-H9 virus predominates, IB and vNDV infection comes in the second level and triple IBV, NDV and AIV-H9 virus follows, while the highest incidence of single infection was IBV, vNDV comes in the second level and AIV-H5 virus follows (Table 2).

According to seasonal distribution, the number of samples collected was varied between seasons with the largest winter (52/90 flocks; 28.9%) of 2014-2015. While positive samples collected during autumn were (23/40 flocks; 12.8%), then summer (19/33 flocks; 10.6%) then spring with (9/17 flocks; 5%) (Table 3, Fig. 2)

4. Discussion

Poultry industry represents a major economic activity; in addition, it is considered one of the most important sources of relatively low-price animal protein, more than 300 million broilers are fattened annually throughout the country (Abdelwhab and Hafez,2011), Respiratory affections represent a great problem to the poultry industry because of their multifactorial nature. Respiratory affections in poultry are very complex especially whenever viral ones are incriminated, as they usually involve more than one pathogen (Roussan et al., 2008).

In the current study, investigation of some viral pathogens with special focus on AIV, IBV and vNDV, was conducted in broiler chickens suffering respiratory disease problems. Hundred and eighty broiler chicken flocks from Giza, Monofia, Behira, Sharqia, Fayiom and Qaliobia governorates were examined during the period from January 2014 till December 2015. For each flock, clinical data were collected. The prevalence of AIV, IBV and vNDV viruses was determined using rRT-PCR. Clinical manifestations and postmortem lesions varied among naturally infected birds based on the infecting virus strain, the immune status of the flock and whether the disease is due to single or multiple infections. Mortality rates in the flocks under investigation ranged from 2.5% to 43% with the highest mortality rate found in a flock suffering triple IBV, vNDV, AIV-H9 infection. The mixed infection with 3 respiratory pathogens could be the

Table 1. Primers for RT-PCR.

Primer	Type	Sequence (5′-3′)	References
IBV5_GU391	Forward	5-GCT TTT GAGCCT AGC GTT-3	Callison et al. (2001)
IBV5_GL533	Reverse	5-GCC ATG TTG TCA CTG TCT ATT G-3	Callison et al. (2001)
IBV5-G-probe	Probe	5-FAM-CAC CAC CAG AAC CTG TCA CCT C-BHQ1-3	Callison et al. (2001)
H9-FOR	Forward	5-GGA AGA ATT AAT TAT TAT TGG TCG GTA C-3	Ben Shabat et al. (2010)
H9-Rev	Reverse	5-GCC ACC TTT TTC AGT CTG ACA TT-3	Ben Shabat et al. (2010)
H9-probe	Probe	5-FAM- AAC CAG GCC AGA CAT TGC GAG TAA GATCC –Tamra-3	Ben Shabat et al. (2010)
ND-For	Forward	5′-GGTGAGTCTATTCGGARGATACAAG-3′	Wise et al. (2004)
ND-Rev	Reverse	5′-AGCTGTTGCAACCCCAAG -3′	Wise et al. (2004)
ND-probe	Probe	5-[FAM]AAGCGTTTCTGTCTCCTTCCTCCA[TAMRA]-3	Wise et al. (2004)
H5LH1	Forward	5-ACATATGACTAC CCACARTATTCA G-3	Slomka et al. (2007), VLA (2007)
H5RH1	Reverse	5-AGACCAGCT AYC ATGATTGC-3	Slomka et al. (2007), VLA (2007)
H5PRO	Probe	5-[FAM]TCWACA GTGGCGAGT TCCCTAGCA[TAMRA]-3	Slomka et al. (2007), VLA (2007)

Table 2. Total and detailed rRT- PCR results for tested samples in six different governorates.

		Real Time- PCR results									
Samples	Mortality range	**Single infection**				**Mixed infection**					
Giza	**6%-43%**	**IB**	**ND**	**H5**	**H9**	**IB+ND**	**IB+H9**	**IB+H5**	**H5+H9**	**IB+ND+H9**	**Total**
No.		6	-	-	1	2	6	-	1	2	**18/30**
%		20%	0%	0%	3.3%	6.6%	20%	0%	3.3%	6.6%	**60%/100%**
Behira	**4%-38.9%**										
No.		4	1	2	-	2	8	-	2	2	**21/30**
%		13.3 %	3.3%	6.6%	0%	6.6%	26.7%	0%	6.6%	6.6%	**70%/100%**
Fayiom	**3%-36%**										
No.		3	2	-	-	2	6	1	-	-	**14/30**
%		10%	6.6%	0%	0%	6.6%	20%	3.3%	0%	0%	**46.7%/100%**
Sharqia	**3.4%-27.4%**										
No.		4	3	1	1	2	9	-	-	-	**20/30**
%		13.3 %	10%	3.3%	3.3%	6.6%	30%	0%	0%	0%	**66.7%/100%**
Qalybia	**2.5%-23.8%**										
No.		4	1	1	-	2	6	-	1	1	**16/30**
%		13.3 %	3.3%	3.3%	0%	6.6%	20%	0%	3.3%	3.3%	**53.3%/100%**
Monofia	**7.3%-25.3%**										
No.		3	3	1	-	1	5	-	-	1	**14/30**
%		10%	10%	3.3%	0%	3.3%	16.7%	0%	0%	3.3%	**46.7%/100%**
Total		**24**	**10**	**5**	**2**	**11**	**40**	**1**	**4**	**6**	**103/180**
		13.3 %	**5.6%**	**2.8%**	**1.1%**	**6.1%**	**22.2%**	**0.55%**	**2.2%**	**3.3%**	**57%**
Total IBV		**24**	**0**	**0**	**0**	**11**	**40**	**1**	**0**	**6**	**82(45.6%)**
Total NDV		**0**	**10**	**0**	**0**	**11**	**0**	**0**	**0**	**6**	**27(15%)**
TotalAI-H5		**0**	**0**	**5**	**0**	**0**	**0**	**1**	**4**	**0**	**10(5.6%)**
TotalAI-H9		**0**	**0**	**0**	**2**	**0**	**40**	**0**	**4**	**6**	**52(28.9%)**
			41/180 (22.8%) Single infection				**62/180 (34.4%) Mixed infection**				

Table 3. Seasonal relationship of examined samples according to rRT- PCR during year 2014 and 2015.

Year	Season	IBV	NDV	AI-H5	AI-H9	IB-H9	IB-ND	IB-H5	H5-H9	IB-H9-NDV	Total	Months
2014	**Winter**	6	2	1	-	9	1	-	1	-	**20/40**	December January February
	Spring	-	-	1	-	2	1	-	-	-	**4/7**	March April May
	Summer	3	1	-	-	2	2	-	-	-	**8/15**	June July August
	Autumn	1	-	1	1	5	1	-	-	2	**11/21**	September October November
2015	**Winter**	8	3	2	1	11	3	-	2	2	**32/50**	December January February
	Spring	1	1	-	-	2	1	-	-	-	**5/10**	March April May
	Summer	2	1	-	-	4	1	-	1	2	**11/18**	June July August
	Autumn	3	2	-	-	5	1	1	-	-	**12/19**	September October November
Total		**24**	**10**	**5**	**2**	**40**	**11**	**1**	**4**	**6**	**103/180**	
%		**13.3%**	**5.6%**	**2.8%**	**1.1%**	**22.2%**	**6.1%**	**0.55%**	**2.2%**	**3.3%**	**57%**	
Total Winter		**14**	**5**	**3**	**1**	**20**	**4**	**0**	**3**	**2**	**52**	28.9%
Total Spring		**1**	**1**	**1**	**0**	**4**	**2**	**0**	**0**	**0**	**9**	5%
Total Summer		**5**	**2**	**0**	**0**	**6**	**3**	**0**	**1**	**2**	**19**	10.6%
Total Autumn		**4**	**2**	**1**	**1**	**10**	**2**	**1**	**0**	**2**	**23**	12.8%

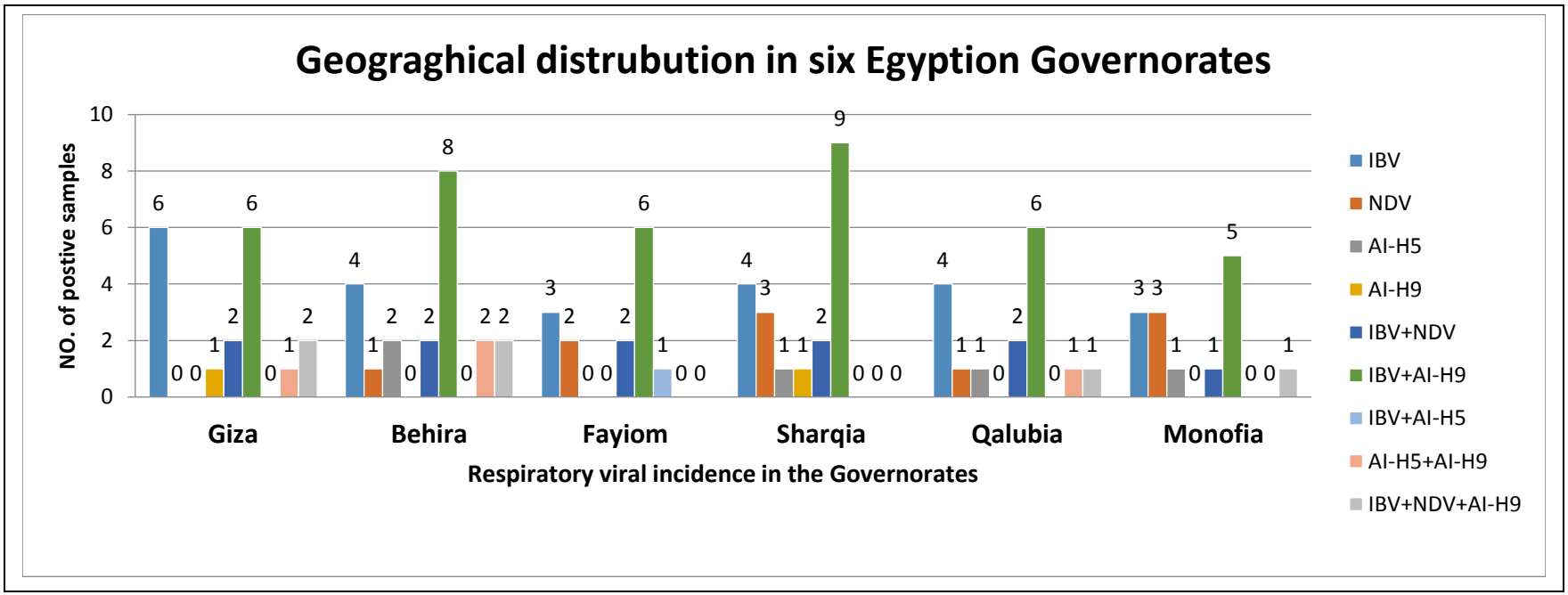

Fig. 1. Total and detailed rRT- PCR results for tested samples in six different governorates.

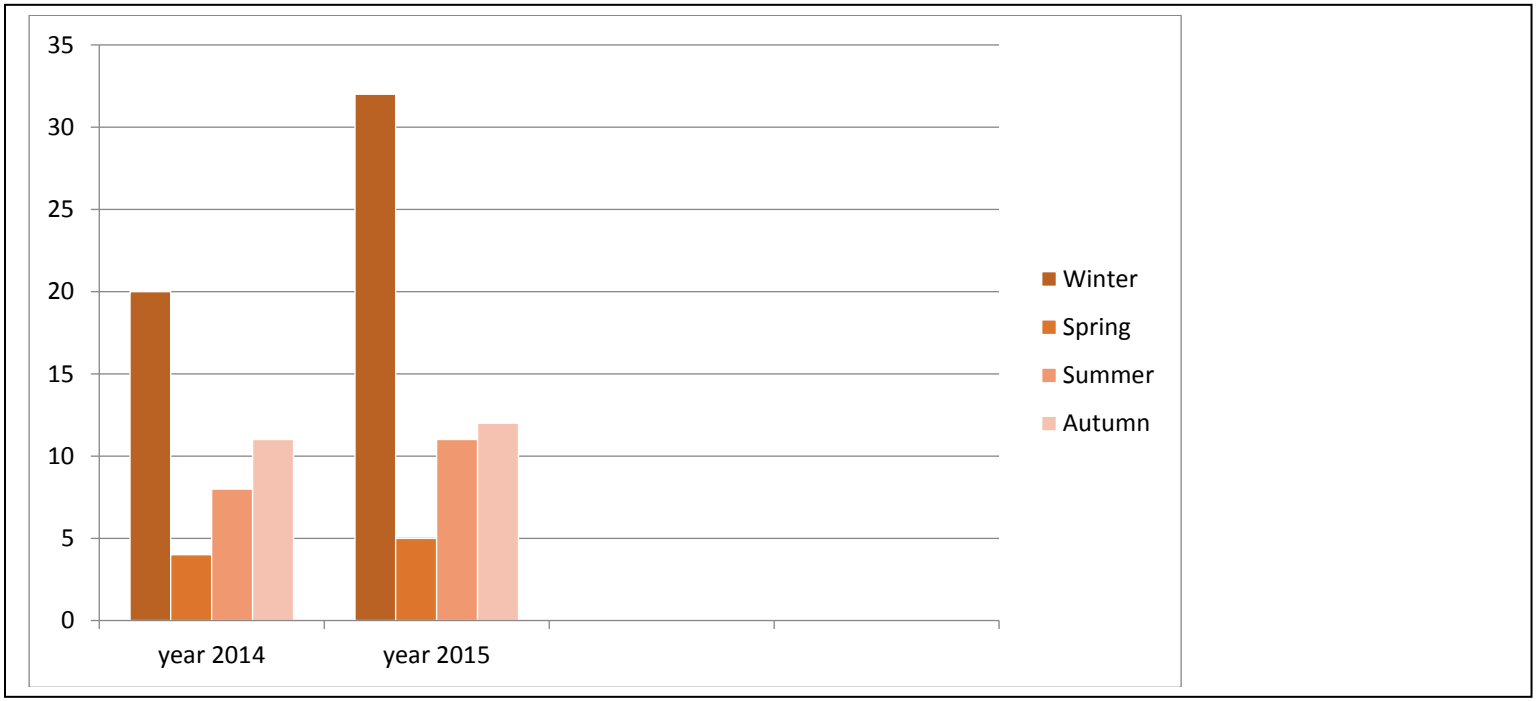

Fig. 2. Seasonal relationship of examined samples according to rRT- PCR during year 2014 and 2015.

explanation of this high mortality rate (Nili and Asasi, 2003; Hassan et al., 2015).

Data in the current study revealed that IBV can cause high mortality (26%) that is the highest percentage even in vaccinated flocks. Improper vaccination, secondary bacterial infection and environmental related stress could explain such high mortality (Hofstad, 1984). Co-infection with IBV and AIV-H9 has resulted in (27.4%) mortality rate and also highest incidence in mixed infection even in flocks vaccinated against both pathogens, this agreed with (Hassan et al.,2015) that AIV-H9 increases the mortalities and severity of the IBV infection.

Meanwhile, AIV-H9 single infection in some flocks resulted in respiratory signs, tracheal caseation and (13%) mortality rate. This data highlights the pathogenicity of AIV-H9 as previously reported (Aamir et al., 2007, Hassan et al., 2015). However, the role of secondary agents can't be neglected, including environmental and/or bacterial ones in the pathogenesis of AIV-H9N2 (Perk et al., 2006). Moreover, infection with vNDV has resulted in high mortality rate ranged from 11 to 24.3%, and it increased when mixed with IBV and AI-H9 to reach 43.2% which was the highest mortality rate in all flocks, this agreed with (Hussein et al., 2012) recorded that infection with IBV and NDV circulating among broiler flocks demonstrating high mortality which reached more than 60% in some flocks. Increased mortalities might be related to the occurrence of mixed infection. Moreover, velogenic genotype isolated from vaccinated broiler farms in Fayoum, Behira and Giza governorates, clustered and published NDV genotype VII that closely related to Middle East isolates, and concluded the spread of velogenic genotype strain to Egypt via Middle Eastern countries is likely to be a source of infection (Radwan et al., 2013). Also, high mortalities may be a result of the carelessness of some owners that appeared to be a major factor that increased the incidence of NDV infection among chickens especially the extensively raised one, this agree with (Eid, 1988) who reported that some outbreaks occurred due to the unawareness of the owner by vaccination scheme, while 70-80% mortality resulted from NDV infection in non-vaccinated chickens as reported by (Sparbrow, 1993). Furthermore, the narrow period between two successive vaccinations (every 10 days) lead to interference between the virus and antibody of the previous vaccination (Eid, 1988). This hypothesis also may explain the incidence of infection in the flocks that were vaccinated at 7 days with HB1 vaccine and received a second vaccination by Lasota NDV vaccine at 17-21 days old and revaccinated by LaSota NDV vaccine at age 30 days, they received NDV vaccine every 11 days which lead to interference and lowering the immune response and increase the susceptibility of birds to infection (Vindevogel et al., 1972).

In addition to, immune suppression in chickens is a frequent problem in poultry production (Jackwood, 1991). It may be caused by many viruses as lymphoid leucosis, IBD, Infectious anemia and Marek's or may be caused by mycotoxin that lead to decline in the immune response to the vaccines and rendered these birds highly susceptible to infection as mentioned by Ragland et al. (1998).

In AIV-H5 infected flocks; typical postmortem lesions were observed in the form of blue comb and wattles (Alexander, 2000), severe hemorrhage in shank (Naeem et al., 2007) and generalized congestion (Pantin-Jackwood and Swayne, 2009). In one of the examined flocks, mortality rate of 35% was observed at 27 day old, though the flock was vaccinated against H5N1 at 8 day old, early exposure to field infection in the flock probably gave a chance for the mortality to increase before the action of vaccine to develop a higher antibody titer and subsequently aggravation of disease manifestations and mortalities (Tian et al., 2005).

Interestingly, co- infection with AI-H5 and H9 has not resulted in very high mortality rate as expected. Currently, approximately 4 flocks were found co-infected with these both subtypes. Co-infection of both subtypes in investigated flocks has resulted in 19.2%-35% mortality, thus might revealed to the early exposure of H9N2 could provide cell-mediated immunity against H5N1 due to their similar internal genes leading to partial protection by what is called protected window after H9 infection and masking the fact that of chickens carrying lethal isolates of H5N1and appeared healthy ,thus make initial outbreak of AIV- H5N1 may go unnoticed while the virus is shed predominantly in feces by protected birds and becomes more widespread .This potentially important problem for countries like Egypt that use H5N1 vaccines and cull flocks only when clinical disease is evident (Khalenkov et al., 2009; Hassan et al., 2015). All those are speculations and need

further investigations and experiments to prove or disclaim interference between AI-H5 and AI-H9.

The results of rRT-PCR highlighted that mixed infection with IB and AIV-H9 viruses was the most common and was more predominant than other mixed infections in the examined flocks with positivity in 40/103 flocks (22.2%). This was followed by 24 flocks (13.3 %) suffering from single IBV. The high prevalence of IBV was previously reported by (Abdel-Moneim et al., 2006), More or less similar results were previously reported by (El-Zoghby et al., 2012; Hassan et al., 2015).

In this study we investigated 30 from each broiler flocks from Giza, Monofia, Behira, Sharqia, Fayiom and Qaliobia governorates. rRT-PCR results for each governorate separately go more or less parallel to that of all governorates collectively. Mixed infection with IBV and AIV-H9 virus predominates, single IBV infection comes in the second place then IBV with NDV mixed infection follows. This was agreed with (Hassan et al., 2015).

According to seasonal distribution, number of samples collected varied between seasons with the largest number of postive samples (52 flocks) during winter of 2014-2015. Cold climate is thought to favor virus survival, higher ammonia level and bad ventilation in different farms and, at least in part, is associated with increased poultry stocking capacity in Egypt. All of these factors lead to increased risk of infection (Seififi et al., 2010) and this could explain the higher number of samples being collected in winter than other seasons followed by autumn season in regard to the number of cases (23 flocks) were positive, there was evidence of an increased stress response due to elevation of environmental gases and dust in winter and autumn seasons reflecting the increase of stress due to environmental conditions. Indeed, insufficient house environmental conditions were detected in winter and autumn, reducing the economic efficiency of enterprise due to lack of ventilation rate. Furthermore, mortality rate showed the highest percentage for birds reared in winter and Autumn compared with the other seasons this was agreed by (Wang et al., 2008) while, in spring and summer, flocks collected were less in number and in positivity with (9 flocks) and (19 flocks) respectively. Furthermore, mortality rate is low in comparison to the other different seasons (Abdel-Azeem et al., 2015).

Studying the prevalence of IB in Egyptian chicken broilers farm revealed that 82 samples were positive for IBV represent (45.6%). It denotes that IBV is widely prevalent in Egypt, since the initial description and isolation of the virus (El Kady, 1989). The emergence of new IBV variants with nephropathogenic property in most of them was the characteristic of the recent history of the disease in Egypt (Abdel-Moneim et al., 2012). The present study confirms that the epidemiology of IB in Egyptian chicken farms is a continuous problem, where none of the countries which have an intensive poultry industry is free from IBV. Although attempts have been made at the regional level to keep flocks free from IBV, none have been successful. Given the highly infectious nature of the virus, the strictest preventative measures are sometimes not sufficient (Ignjatovic and Sapats, 2000). Under normal flock management with “all-in/all-out” operations, cleaning and disinfections between batches will limit the level of infection to a minimum, however, exclusion of IBV has not been achieved through such measures (Ignjatovic and Sapats, 2000).

In conclusion, the co-infection of IBV and AI-H9N2 plays a major role in increasing the severity and the high mortality rates of field outbreaks of respiratory infections in broiler chickens in Egypt (Hassan et al., 2015). IBV in Egyptian chicken farms is a continuous problem. The AI, IBV, and NDV viruses’ interference and co-infections in terms of altering the severity of clinical signs and lesions need further investigation. Identification of factors that influence avian respiratory virus interference will provide new insights in the pathogenesis and subsequently improvement of control programs could be achieved.

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