EPIDEMIOLOGICAL HIGHLIGHTS ON SOME VIRUSES ASSOCIATED WITH INCREASED MORTALITY IN CHICKENS BRED IN SHARKIA GOVERNORATE.

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ABSTRACT

In the present study 1317 serum samples obtained from 6 broiler flocks, 2 broiler breeders and 28 commercial layer flocks were examined for HI antibodies of {NDV, IBV (Mass-41, 4/91, and D-274), AIV (H5N1, H5N2, H5N3, H9, H7) and Adenov (EDS_{76})}. Studying the vaccination protocols in flocks under investigation and their seroconversion had led us to speculate and conclude viral affections in the different localities of Sharkia governorate. Speculating viral affections was a very hard task because the Egyptian market is jammed with a great variety of protective vaccines this was conflicting during result interpretation. Positive immune titers for AIV-H7 in sharkia governorate was detected at June /2014 in (El-salhia, 10th of Ramadan, El-ibrahemia and Abo-hammed) in commercial layer flocks only. The seropositive samples that exceeded the cutoff values were 63 out of 1317 (4.8%). AIV-H9 high seropositive immune titers was constantly found in examined samples although their protective vaccines were neglected. AIV-H_5N_3 seropositive results was recorded in a totally non vaccinated flocks against H_5 which reflect virus circulation in the poultry premises. Seropositive titer for IBV-D_{274} and EDS_{76} was recorded in a totally non vaccinated flocks against such antigens., which refer to their role in the total simultaneous incidence of disease and consequent mortality. From another point of view it should be noted that. Vaccinating chicken flocks following a ready made manuscript of the producing companies without prior evaluation of the maternally derived antibodies (MDA) or evaluating the immune titers before taking the decision of vaccination or even considering the disease situation in the area is possible cause for vaccine failure. Sentinel birds inclusion in poultry patches should be taken seriously to give a mirror for the circulating viral agents in the poultry premises. It worth to mention that a parallel bacteriological work was running during investigation of the causes of increased mortality or dropped egg production. This work revealed the isolation of a resistant bacteria of the (Kebseilla spp).

Key words: *NDV, AIV, IBV, EDS_{76}, Epidemiological.*

INTRODUCTION

Eid (1994) stated that avian viruses causes severe economic losses in poultry beside other identified causative agents (Bacterial, mycotic, intoxication, etc.) and that the outcome of infection is influenced by many factors associated with the host، organisms and environment Bradbury (1984), Dhillon and Kibenge (1987), and Gelb (1989).

Morrow (2008) stated that demonstration of antibody simply shows the antigen that a bird has been in contact with at some time in the past., but this does not prove that a clinical disease syndrome is caused by the organism associated with the particular antigen., because vaccinated flocks will have antibody from vaccination., and because natural infection could have occurred earlier. For this Paired serum samples (taken at the time of clinical disease and then in convalescence) will provide a convincing

evidence of seroconversion and association of an agent to the clinical signs seen. As for broilers testing the seroconversion is difficult because of their short life, and for possessing maternally derived antibodies which may be from vaccination of parent stock rather than wild strain infection. For this reason sentinel birds should be grown on to allow clearer seroconversion demonstration.

Comin *et al.* (2013) stated that the serological diagnosis of avian influenza (AI) can be performed using different methods, yet the haemagglutination inhibition (HI) test is considered the 'gold standard' for AI antibody subtyping.

This study aimed to highlight the potential existence avian of influenza infections circulating among chicken flocks in Sharkia beside NDV, IBV and EDS_{76}.

MATERIALS

Chicken flocks and Serum samples
1317 Serum samples were collected from different localities in Sharkia Governorate from 36 poultry flocks during the period of January 2014 to November 2014., they represent (6) broiler, (2) breeder, and (28) layer chicken flocks. These flocks were suffering increased mortality, drop in egg production or assessing immune titers post vaccination. The obtained sera were stored at −20 °C in HI plates until used. Table (1) shows the vaccination history, source and number of the collected samples.

Washed CRBCS
Chicken RBCS were obtained from 28 day old specific antibody negative chickens (SAN).

Saline
Sodium chloride 0.9% (ADWIC) ®.

Viral antigens
IB viral antigen
IB viral antigens for HI test were obtained from GD Holland,

- (mass- 41- VLDA 035) lot 12639-020412 exp. 4/2022,
- (793 B Designated 4/91 – VLDIA 186) lot 13695-280613 exp.6/2023,
- (D-274 VLDIA 032) lot 12645-020812 exp. 8-2022,

ND viral antigen
Allantoic fluid from chicken embryos inoculated with Lasota NDV{Intervet, batch-12636jj01, Exp.1-2015 }was used as HA antigen for HI-test.

EDS_{76} viral antigen
Adeno 127 Designated EDS_{76} viral antigens for HI test were obtained from GD Holland. (VLDIA 038) lot 13773-020813 exp. 8-2022.

AIV viral antigens

- H5N1 (Kindly obtained from Dr. Souzan Tolba NDV department El-Abassia)
- H5N2 (lot 101111A pro. 10/11/2011 exp. 10/11/2015). Kindly obtained from Profarm for vaccine distribution.
- H5N3 (VLDIA 240 GD Holand) lot 7605-010607 exp. 11-2023.
- H9 (VLDIA 113 GD Holand) lot 14672-080414 exp. 4-2024.
- H7 (VLDIA 98 GD Holand) lot 10604-260110 exp. 1-2020.

Negative serum
Sera from one day old SPF chicks were used.

METHODS

HI for IBV, NDV, EDS $_{76}$
HI tests, for the fore mentioned antigens were performed as described by Villegas (2006).

HI for AIV.
OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2010.

Bacteriological Examination:
Bacteriological samples were examined according to Osbaldiston (1973).

Statistical analysis.
Data were statistically analyzed as described by Snedecor and Cochran (1967) using SPSS.16 computer program, value was used to determine significance.

Fig. 1: location of Sharkia governorate in Egypt

NO	Localities of Sharkia	Designation	Samples Per Locality	Flock Sample Distribution
1	10th of Ramadan	●	5	28 Layer Flock ------- 6 Broiler Flock ------- 2 Broiler Breeder Flock
2	Abo Hammad	■	13	
3	Bilbees	▲	2	
4	Zagazig	★	2	
5	El-Salhia	⬢	12	
6	El-Ibrahemia	⌐	1	
7	Kafer Sakker	◆	1	

Fig. 2: Some Localities of Sharkia

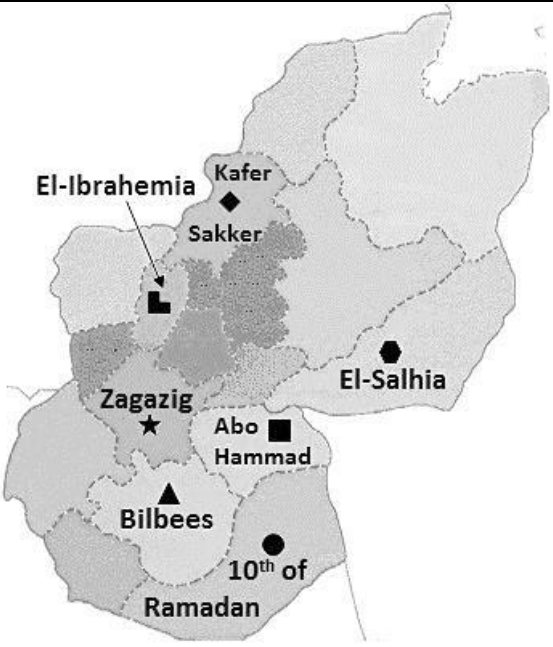

Table 1: History of investigated samples.

FLOCK SAMPLE	Date	Locality	No. of samples	Housed Birds	Breed	Age at Sample collection	Complain	Vaccination history	
								Age In Days	vaccines
1	12-1-2014	◆	23	6000	L.	28W	⇩ Egg Prod.	5D	Killed NDV , Clone – IBV(H_{120})
								13 D	H_5N_1
								16 D	Clone – IBV(H_{120})
								28 D	IBV(H_{120}) - Lasota
								48 D	Pox – AIV
								65 D	IBV(H_{120}) - Lasota
								80 D	IBV(H_{120}) - Lasota
								100 D	H_5 - H_9
								105 D	IBV(H_{120}) - Lasota
								125 D	Killed (NDV + IBV + EDS_{76})
2	19-1-2014	★	10	5000	B.	3W	⇧ Mort. + Colisepticaemia	1 D	HB_1 - IBV (H_{120})
								9 D	IBV $_{MA-5}$, Clone
								10 D	killed (NDV –AIV-H_5)
								14 D	$IBV_{4/91}$
								19 D	$NDV_{6/10}$
3	10-3-2014	⬣	75	75000	L.	13W	⇩ Prod.	1 D	IB (H_{120})
								6 D	HB_1
								8 D	IBV(H_{120}) - Lasota
								15 D	IBV $_{4/91}$
								22 D	Lasota
								40 D	IBV $_{MA-5}$
								45 D	Lasota
								65 D	Lasota
								80 D	$IBV_{4/91}$
								85 D	Lasota
4	28-3-2014	★	40	13500	B.	4W	⇧ Mort.	7 D	HB_1
								18 D	H5N1
								20 D	Clone
5	31-3-2014	⬣	75	75000	L.	61W	⇩ Prod.	1 D	IB (H_{120})
								6 D	HB_1
								8 D	IBV(H_{120}) - Lasota
								15 D	$IBV_{4/91}$
								22 D	Lasota
								40 D	IBV $_{MA-5}$
								45 D	Lasota
								65 D	Lasota
								80 D	IBV $_{4/91}$
								85 D	Lasota
								102 D	IBV(H_{120}) - Lasota
								103 D	Killed (NDV + IBV + EDS_{76})
								110 D	H5
6	12-4-2014	⬣	75	75000	L.	20W	⇩ Prod. &Egg deformity	↳ S-9	
7)	26-4-2014	▲	35	5000	L.	26W	⇧ Mort.	1 D	IB (H_{120})
								6 D	HB_1
								8 D	IBV(H_{120}) - Lasota
								15 D	IBV $_{4/91}$

FLOCK SAMPLE	Date	Locality	No. of samples	Housed Birds	Breed	Age at Sample collection	Complain	Vaccination history	
								Age In Days	vaccines
								22 D	Lasota
								40 D	IBV MA-5
								45 D	Lasota
								65 D	Lasota
								80 D	$IBV_{4/91}$
								85 D	Lasota
								102 D	$IBV(H_{120})$ - Lasota
								103 D	Killed (NDV + IBV + EDS_{76})
								110 D	H_5
8	30-4-2014	⬣	75	75000	L.	64W	⇩ Prod.	↳ S-9	
9	1-5-2014	⬣	15	25000	B.	5W		5 D	HB_1
								6 D	H_9 – ND
								9 D	H_5N_1
								14 D	IB Primer (H_{120}+D_{-274})
								17 D	$NDV_{Avi\ new}$
								27 D	HB_1
10	16-5-2014	■	15	10000	L.	15W	⇧ Mort.⇩ Prod.	Unknown	
11	18-5-2014	⬣	14	5000	B.	4W	⇧ Mort.	7 D	HB_1 – IB (H_{120})
								24 D	Clone – IBV (H_{120})
12	20-5-2014	■	25	10000	L.	3W	⇩ Prod.	7 D	HB_1 – IB (H_{120})
								13 D	IBV(H120) - Lasota
13	21-5-2014	▲	6	11000	B.	3W	⇩ Prod.	1 D	1B Primer (H_{120}+D_{-274})
								7 D	HB_1 – IB (H_{120})
								9 D	Clone
14	1-6-2014	⬣	75	75000	L.	35W	⇩ Prod.	↳ S-9	
15	3-6-2014	■	14	4000	B.	4W	⇩ Prod.	Unkown	
16	5-6-2014	●	35	58000	L.	20W	⇩ Prod.	1 D	IB (H_{120})
								6 D	HB_1
								8 D	$IBV(H_{120})$ - Lasota
								15 D	$IBV_{4/91}$
								22 D	Lasota
								40 D	IBV_{MA-5}
								45 D	Lasota
								65 D	Lasota
								80 D	$IBV_{4/91}$
								85 D	Lasota
								102 D	$IBV(H_{120})$ - Lasota
								103 D	Killed (NDV + IBV + EDS_{76})
								110 D	H_5
17	22-6-2014	●	15	11000	L.	22W	⇩ Prod.	Unknown	
18	15-6-2014	●	50	5800	L.	22W	⇩ Prod.	Unknown	
19	16-6-2014	⬣	50	75000	L.	21W	⇩ Prod.	↳ S-9	
20	23-6-2014	⬣	75	75000	L.	51W	⇩ Prod.	↳ S-9	
21	23-7-2014	⬣	75	75000	L.	26W	⇩ Prod.	↳ S-9	
22	27-8-2014	⬣	75	75000	L.	31W	⇩ Prod.	↳ S-9	
23	31-8-2014	■	20	20000	L.	10W	⇩ Prod.	7 D	HB_1 – IB (H_{120})
								10 D	KILLED (NDV + IBDV)
								21 D	Clone – $IBV(H_{120}$)

FLOCK SAMPLE	Date	Locality	No. of samples	Housed Birds	Breed	Age at Sample collection	Complain	Vaccination history	
								Age In Days	vaccines
24	9-9-2014	●	40	40000	L.	35W	⇩ Prod.	Unknown	
25	17-9-2014	●	20	58000	L.	33W	⇧ Mort. ,⇩ Prod.	Unknown	
26	21-9-2014	▙	15	2000	L.	30W	*	7 D	HB_1 – IB (H_{120})
								19 D	Clone
								40 D	Clone- IB (H_{120})
								60 D	Clone
								90 D	Clone – IB (H_{120})
								100 D	Killed (NDV + IBV + EDS_{76})
								110 D	Clone
								130 D	Lasota
27	10-9-2014	■	20	10000	L.	42W	⇩ Prod.	7 D	HB_1 – IB (H_{120})
								10 D	KILLED (NDV + IBDV)
								21 D	Clone – IBV (H_{120})
								65 D	Lasota
								80 D	$IBV_{4/91}$
								85 D	Lasota
								102 D	IBV(H_{120}) - Lasota
								103 D	Killed (NDV + IBV + EDS_{76})
								110 D	H_5
28	27-9-2014	■	15	10000	L.	15W	⇩ Prod.	7 D	HB_1 – IBV(H_{120})
								10 D	KILLED (NDV + IBDV)
								21 D	Clone + IBV (H_{120})
29	30-9-2014	■	45	13000	B.B.	32 W	*	Unknown	
30	6-10-2014	⬣	75	75000	L.	21 W	⇩ Prod.	↳ S-9	
31	12-10-2014	■	20	18000	B.B.	32 W	*	20 W	KILLED (ND, IB, VA, IBDV)
								23 W	Lasota
								25 W	H_5N_1
32	15-10-2014	■	20	4000	L.	11W	*	Unknown	
33	12-10-2014	■	20	3000	L.	15 W	⇩ Prod.	Unknown	
34	21-10-2014	■	20	5000	L.	20W	⇩ Prod.	Unknown	
35	29-10-2014	■	20	5000	L.	25 W	⇩ Prod.	Unknown	
36	3-11-2014	■	20	3000	L.	31 W	⇩ Prod &⇧ Mort.	Unknown	

L= Layer	B.= Broiler	B.B.= Broiler Breeder
↳ S-9	An integrated egg producing company applying a fixed vaccination protocol	
Unknown = unknown vaccination history		
* = Evaluation of vaccination titers.		
Vaccines that are not related to the investigation such as (IBDV, POX, ILTV,BACTERIAL Vaccine,….etc. weren't mentioned		

RESULT

Results of the present work are illustrated in tables (2-4).

Table 2: Results of Serological investigations (After Cut-off values)

FLOCK SAMPLE		Laboratory Works									
		NDV	AIV					IBV			EDS_{76}
			H5N1	H5N2	H5N3	H9	H7N1	Mass-41	4/91	D-274	
1	*	2.61 ± 0.41	2.43 ± 0.56	9.78 ± 0.11	0	9.16 ± 0.24	0	4.78 ± 0.11	2.35 ± 0.54	7.19 ± 0.19	0
	**	1.95	2.69	0.52		1.21		0.52	2.59	0.75	
	***	3.79	7.26	0.27		1.47		0.27	6.69	0.56	
2	*	7.6 ± 0.52	6.62 ± 0.18	4 ± 0.93	0	0.8 ± 0.53	0	1.9 ± 0.52	6.63 ± 0.18	8.75 ± 0.59	6.75 ± 0.16
	**	1.65	0.65	2.94		1.69		1.66	0.52	1.67	0.46
	***	2.71	0.42	8.67		2.84		2.77	0.27	2.79	0.21
3	*	7.84 ±0.23	8.04 ± 0.14	6.78 ± 0.21	9.98 ± 0.02	5.45 ± 0.4	0	8.68 ± 0.17	2.5 ± 0.34	5.52 ± 0.08	9.13 ± 0.21
	**	1.95	1.21	1.81	0.13	3.45		1.5	2.52	0.57	1.58
	***	3.81	1.47	3.27	0.02	11.93		2.25	6.33	0.33	2.51
4	*	3.25 ±1.11	2.75 ± 1.6	8.38 ± 0.46	0	5.25 ± 0.25	0	3.75 ± 1.25	0	0	0
	**	2.22	3.2	1.3		0.5		2.5			
	***	4.92	10.25	1.7		0.25		6.25			
5	*	8.74 ±0.14	6.39 ± 0.2	5.78 ± 0.29	0	9.25 ± 0.15	0	4.96 ± 0.02	0	0	0
	**	1.19	1.72	2.46		1.3		0.2			
	***	1.43	2.97	6.05		1.68		0.04			
6	*	9.45 ±0.13	6 ±0	7.79 ± 0.25	9.95 ± 0.05	9.18 ± 0.23	0	9.92 ± 0.08	1.88 ± 0.41	4.75 ± 0.18	7.98 ± 0.17
	**	1.14	0	2.16	0.32	1.47		0.4	2.62	1.17	1.07
	***	1.31	0	4.68	0.1	2.15		0.16	6.88	1.37	1.15
7	*	9.69 ± 0.1	7.31 ± 0.22	4.6 ± 0.22	8.04 ± 0.54	8.83 ± 0.19	0	2.56 ± 0.22	2.09 ± 0.34	5.02 ± 0.16	7.09 ± 0.3
	**	0.58	1.33	0.70	4.01	1.11		1.32	2.58	1.2	2.28
	***	0.33	1.76	0.49	16.07	1.23		1.74	6.66	1.44	5.21
8	*	3.53 ±0.41	6.4 ± 0.27	8.68 ± 0.16	9.5 ± 0.35	9.53 ± 0.28	0	3.79 ± 0.07	2.38 ± 0.41	5.2 ± 0.18	3.25 ± 0.45
	**	2.31	1.06	1.38	2.21	1.74		0.58	2.59	1.11	2.85
	***	5.35	1.11	1.9	4.87	3.03		0.33	6.7	1.24	8.14
9	*	4.93 ±0.07	6.33 ± 0.25	7.75 ± 0.41	7.7 ± 0.26	10 ±0	0	4.38 ± 0.33	1.93 ± 0.65	6.69 ± 0.21	0
	**	0.26	0.98	1.42	0.82	0		1.31	2.52	0.75	
	***	0.07	0.95	2.02	0.68	0		1.72	6.35	0.56	
10	*	4.73 ±0.12	5.8 ± 0.14	3.47 ± 0.7	3.5 ± 0.66	9.67 ± 0.16	0	6 ± 0.16	6.74 ± 0.27	7.88 ± 0.48	4.4 ± 0.16
	**	0.46	0.56	2.72	2.47	0.62		0.58	1.01	1.36	0.52
	***	0.21	0.31	7.41	6.12	0.38		0.33	1.02	1.84	0.27
11	*	6.75 ±0.63	10 ±0	7.5 ± 0.46	0	1 ±1	0	0	0	3.43 ± 0.14	5 ±0
	**	1.26	0	1.31		2				0.51	0
	***	1.58	0	1.71		4				0.26	0
12	*	6.88 ± 0.7	10 ±0	0	0	0.32 ± 0.22	0	1.32 ± 0.3	2.71 ± 0.54	0	0
	**	3.48	0			1.11		1.52	2.63		
	***	12.11	0			1.23		2.31	6.91		

FLOCK SAMPLE		Laboratory Works									
		NDV	AIV					IBV			EDS_{76}
			H5N1	H5N2	H5N3	H9	H7N1	Mass-41	4/91	D-274	
13	*	0	0	0	9.88 ± 0.12	1.67 ± 1.05	0	5 ±0	0	0	7 ±0.41
	**				0.35	2.58		0			0.82
	***				0.13	6.67		0			0.67
14	*	9.65 ± 0.1	6.25 ± 0.16	0	8.7 ± 0.38	9.66 ± 0.15	1 ±0.25	0	2.91 ± 0.35	4.64 ± 0.15	6.02 ±0.41
	**	0.89	0.46		2.85	1.15	1.86		2.62	1.15	3.1
	***	0.8	0.21		8.11	1.32	3.45		6.85	1.32	9.58
15	*	10 ±0	5 ±0	0	4.36 ± 0.34	0.83 ± 0.57	0	5 ±0	1.29 ± 0.57	3.18 ± 0.18	0
	**	0	0		1.28	1.99		0	2.13	0.73	
	***	0	0		1.63	3.97		0	4.53	0.53	
16	*	6.24 ±0.29	0.38 ± 0.18	0	0	4.95 ± 0.65	0	4.97 ± 0.03	2.71 ± 0.46	0	0
	**	1.88	1.19			4.24		0.17	2.74		
	***	3.55	1.41			17.95		0.03	7.5		
17	*	8.13 ± 0.6	7 ±0.29	6.53 ± 0.73	9.75 ± 0.16	10 ±0	3.88 ± 0.89	7 ± 0.36	2.13 ± 1.07	5 ± 0.27	4.13 ± 1.24
	**	2.33	1.13	2.83	0.46	0	2.53	1.15	3.04	0.76	3.52
	***	5.41	1.29	7.98	0.21	0	6.41	1.33	9.27	0.57	12.41
18	*	8.98 ±0.34	10 ±0	0	9.1 ± 0.24	9.64 ± 0.15	0.1 ± 0.01	5.85 ± 0.19	1.68 ± 0.37	4.9 ± 0.15	5.02 ± 0.56
	**	2.38	0		1.72	1.08	0.71	1.1	2.62	1.05	3.98
	***	5.95	0		2.95	1.17	0.5	1.22	6.88	1.11	15.86
19	*	9.48 ± 0.1	8.12 ± 0.26	0	7.09 ± 0.44	9.67 ± 0.12	0.5 ± 0.24	4.24 ± 0.1	2.48 ± 0.36	3.89 ± 0.3	6.52 ± 0.55
	**	0.68	1.3		2.97	0.82	1.5	0.72	2.45	2.01	3.76
	***	0.46	1.69		8.84	0.67	2.26	0.51	5.99	4.05	14.12
20	*	8.43 ±0.21	6.4 ± 0.25	0	8.3 ± 0.3	9.27 ± 0.17	0.11 ± 0.11	7.67 ± 0.22	3.39 ± 0.35	4.98 ± 0.16	4.96 ± 0.51
	**	1.85	0.55		2.27	1.26	0.8	1.85	2.63	1.2	3.85
	***	3.44	0.30		5.16	1.58	0.64	3.44	6.93	1.44	14.8
21	*	8.89 ±0.13	4 ± 22	0	7.95 ± 0.17	9.73 ± 0.07	0.1 ± 0.1	6.5 ± 0.15	1.95 ± 0.41	5.63 ± 0.09	4.2 ± 0.48
	**	1.1	0.58		1.08	0.62	0.63	0.52	2.59	0.59	3.01
	***	1.2	0.33		1.18	0.39	0.4	0.27	6.72	0.34	9.09
22	*	8.78 ±0.17	7.9 ± 0.23	0	0	6.76 ± 0.16	0	4.92 ± 0.04	0	0	0
	**	1.22	0.74			1.41		0.27			
	***	1.48	0.54			2		0.08			
23	*	9.1 ± 0.51	1.17 ± 0.43	7.5 ± 0.46	0	9.83 ± 0.1	0	8.87 ± 0.32	0	0	0
	**	1.6	2.1	1.31		0.48		1.25			
	***	2.54	4.41	1.71		0.23		1.55			
24	*	9.53 ± 0.2	5.1 ± 0.48	0	7.56 ± 0.52	9.75 ± 0.15	0	4.41 ± 0.45	2.63 ± 0.46	5.47 ± 0.11	5.38 ± 0.62
	**	1.24	3.05		2.96	0.84		2.55	2.59	0.62	3.5
	***	1.54	9.32		8.77	0.71		6.51	6.69	0.39	12.24
25	*	9 ± 0.16	3.4 ± 0.7	0	9 ± 0.34	7.05 ± 0.21	0	7.2 ± 0.29	2.69 ± 0.71	4.06 ± 0.49	9.08 ± 0.43
	**	0.73	3.12		1.37	0.94		0.92	2.85	1.95	2.12

FLOCK SAMPLE		Laboratory Works									
		NDV	AIV					IBV			EDS_{76}
			H5N1	H5N2	H5N3	H9	H7N1	Mass-41	4/91	D-274	
	***	0.53	9.73		1.87	0.89		0.84	8.1	3.8	4.51
26	*	9.8 ± 0.11	0	6 ±0.5	7.75 ± 0.37	10 ±0	0	3 ±0	4.63 ± 0.71	5.5 ± 0.27	9.63 ± 0.37
	**	0.41		1.93	1.04	0		0	2	0.76	1.06
	***	0.17		3.71	1.07	0		0	3.98	0.57	1.13
27	*	10 ±0	3.88 ± 0.41	0	8.19 ± 0.67	10 ±0	0	9.2 ± 0.25	1.94 ± 0.66	5.44 ± 0.18	9.94 ± 0.06
	**	0	2.03		2.66	0		0.79	2.62	0.73	0.25
	***	0	4.11		7.1	0		0.62	6.86	0.53	0.06
28	*	8.93 ±0.33	5.8 ± 0.24	0	9.92 ± 0.08	10 ±0	4.54 ± 0.32	4.93 ± 0.07	1.67 ± 0.54	0	8.88 ± 0.32
	**	1.28	0.94		0.41	0	1.56	0.26	2.66		1.57
	***	1.64	0.89		0.17	0	2.43	0.07	7.1		2.46
29	*	10 ±0	9.09 ± 0.2	0	10 ±0	10 ±0	0.25 ± 0.25	4.64 ± 0.19	3.44 ± 0.63	4.88 ± 0.16	9 ± 0.26
	**	0	1.35		0	0	1	1.26	2.53	0.62	1.03
	***	0	1.81		0	0	1	1.6	6.4	0.38	1.07
30	*	10 ±0	10 ±0	10 ±0	9.69 ± 0.22	9.99 ± 0.01	1.75 ± 0.32	3.77 ± 0.11	3.06 ± 0.38	4.33 ± 0.2	5.13 ± 0.37
	**	0	0	0	1.49	0.12	2.24	0.94	2.6	1.39	2.56
	***	0	0	0	2.22	0.01	5	0.88	6.74	1.93	6.54
31	*	8.08 ± 0.4	1.46 ± 0.67	0	7.62 ± 0.33	10 ±0	0	6.88 ± 0.3	3.54 ± 0.81	4.69 ± 0.24	6.62 ± 0.9
	**	1.44	2.4		1.19	0		1.32	2.93	0.85	3.23
	***	2.08	5.77		1.42	0		1.74	8.6	0.73	10.42
32	*	9.97 ±0.03	7 ±0.3	3.21 ± 0.59	6.27 ± 0.27	9.73 ± 0.12	0	6.74 ± 0.27	1.93 ± 0.65	5.67 ± 0.13	6.8 ± 0.8
	**	0.18	1.11	2.19	1.03	0.46		1.01	2.52	0.49	3.1
	***	0.03	1.23	4.8	1.07	0.21		1.02	6.35	0.24	9.6
33	*	3.88 ±1.34	10 ±0	0	5.5 ± 0.38	8.13 ± 0.12	0	0	1.5 ± 0.73	3.88 ± 0.23	7.5 ± 0.46
	**	3.8	0		1.07	0.35			2.07	0.64	1.31
	***	14.41	0		1.14	0.13			4.29	0.41	1.71
34	*	5.38 ±0.15	3.33 ± 0.87	0	8.8 ± 0.57	9.3 ± 0.34	3.2 ± 0.71	8.85 ± 0.3	2.1 ± 0.71	4.2 ± 0.59	6.1 ± 1.08
	**	0.83	2.6		1.81	1.06	2.25	1.07	2.23	1.87	3.41
	***	0.69	6.75		3.29	1.12	5.07	1.14	4.99	3.51	11.66
35	*	9.46 ±0.28	4.75 ± 0.66	0	10 ±0	9.65 ± 0.21	1.35 ± 0.48	3 ± 0.52	1.25 ± 0.51	5.4 ± 0.18	5.3 ± 0.51
	**	1.38	2.65		0	0.93	2.13	1.81	2.27	0.82	2.27
	***	1.91	7		0	0.87	4.56	3.27	5.14	0.67	5.17
36	*	6.92 ±0.22	7.38 ± 0.18	0	10 ±0	9.83 ± 0.17	1.5 ± 0.96	9.1 ± 0.28	1 ±1	5.83 ± 0.17	4 ±0
	**	1.08	0.52		0	0.41	2.35	0.88	2.45	0.41	0
	***	1.16	0.27		0	0.17	5.5	0.77	6	0.17	0

*** Average** **** Standard Deviation** ***** Variance** **0 = Not Done**

Sample referred to as zero had individual values that is totally below the cutoff

Table 3: Simultaneous viral affections in the examined samples

No.	Predicted viral affections	No.	Predicted viral affections	No.	Predicted viral affections	No.	Predicted viral affections
1	IBV_{D-274}	10	H9 + IBV_{D-274}	19	NDV+H7+H9 + IBV_{D-274}	28	H7+H9 $EDS_{76,+}$ H_5N_3
2	H9+EDS_{76} + IBV_{D-274}	11	H9+EDS_{76}+ IBV_{D-274} + IBV_{D-274}	20	H7+H9	29	NDV+H7+H9 $EDS_{76,+}$ H_5N_3
3	H9 + IBV_{D-274} +H_5N_3	12	H9	21	H7+H9 + IBV_{D-274}	30	NDV+H7+H9 + IBV_{D-274}+ H_5N_3
4	H9	13	H9+EDS_{76+} H_5N_3	22	H9	31	H9, EDS_{76}
5	H9+ H_5N_3	14	NDV+H7+H9 + IBV_{D-274}	23	NDV+H9	32	NDV+H9
6	NDV+H9	15	H9	24	NDV+H9 + IBV_{D-274}	33	H9
7	NDV+H9	16	H9	25	H9,EDS_{76}	34	H7+H9
8	H9 + IBV_{D-274} + H_5N_3	17	H7+H9 + IBV_{D-274}+ H_5N_3	26	H9, EDS_{76} + IBV_{D-274}	35	NDV+H7+H9+ H_5N_3
9	H9	18	H7+H9 + IBV_{D-274}+ H_5N_3	27	NDV+H9, EDS_{76} + IBV_{D-274}	36	H7+H9+ H_5N_3

Table 4: Viral Concurrency in relation to El- Sharkia localities

NO.	Localities of sharkia	Designation	Predicted viral affections
1	10th of Ramadan	●	H9,H7, EDS_{76}, IBV_{D-274}+ H_5N_3
2	Abo Hammad	■	H9,H7,NDV, EDS_{76}, IBV_{D-274}
3	Bilbees	▲	H9,NDV,EDS_{76}+ H_5N_3
4	Zagazig	★	H9,EDS_{76} , IBV_{D-274}
5	El-Salhia	⬢	H9,H7,NDV,EDS_{76}, IBV_{D-274}+ H_5N_3
6	El-Ibrahemia	▙	H9, EDS_{76}, IBV_{D-274}

DISCUSSION

Morrow (2008) stated that., demonstration of antibody through seroservillance simply shows what a bird has been in contact with at some time in the past., but this does not prove that a clinical syndrome is caused by a certain antigen., because vaccinated flocks will have antibody from vaccination., and natural infection could have occurred earlier and was not associated with this clinical syndrome. For these reasons a paired serum samples, taken at the time of clinical disease and in convalescence, provides the most convincing evidence of seroconversion and association of an agent to the clinical signs seen. He also mentioned that, testing the seroconversion in broilers is difficult because of their short life span, and for possessing maternally derived antibodies. For this reason sentinel birds should be grown on without neglicance., to allow clearer seroconversion demonstration.

During interpretation of serologic data It is usually impossible to differentiate between antibodies that are produced by vaccination or those resulting from field exposure to a given infectious agent. The only difference that may be observed is that the antibody titer following a field challenge may be higher than that observed following vaccination. A valid interpretation of serologic results requires a complete knowledge of the flock's vaccination history and disease situation. In the present study a detailed vaccination history is illustrated in table (1). Morrow (2008) stated that., the number of serum samples needed depends on the reason for the testing and test characteristics i.e. for diagnosis., 10–60 serum samples per group should be taken., smaller numbers can be taken from sentinel birds during paired sampling for demonstration of the seroconversion., more samples are required to show an overall decrease in the number of seronegative birds., and for confirmation of freedom from infection a maximum of 60 samples per group is needed to give 95% confidence that infection of 5% of the animals would be detected. In this case the observation of one positive result defines the group as infected. This sort of testing can be a part of an eradication programme. He also mentioned that for post vaccination response evaluation a 20–30 sera per group are enough.

Newcastle disease virus (NDV) is one of the highly contagious diseases contained in the list A of the Office International des Epizooties (OIE). The disease is caused by the avian paramyxovirus serotype-1 (APMV-1). It affects a variety of avian species but causes most severe disease and economic losses in domestic poultry Kaleta (1992). Infection with different strains of NDV may result in a broad variation in severity and spread of the disease, even in a single host species.

Beard and Hanson (1981). Grouped NDV strains into five pathotypes based on the induced clinical signs in infected chickens (i) viscerotropic velogenic (associated with high mortality and intestinal lesions). (ii) Neurotropic velogenic (associated with high mortality following central-nervous signs). (iii)

mesogenic (associated with low mortality, respiratory and nervous signs). (iv) lentogenic (associated with mild or clinical inapparent respiratory infections). (v) Asymptomatic enteritic (associated with inapparent intestinal infections).

ND is almost indistinguishable from HPAI Alexander (1997), Easterday *et al.*, 1997). The possibility of misdiagnosing HPAI as ND couldn't be ruled out in the field. When NDV infects chickens, antibody titers rise within 6 – 10 days and subsequently diminish slowly to zero Alexander (1997). Velogenic strains usually elicit a higher titer than mesogenic and lentogenic strains Alexander (1997), Alexander *et al.* (2004). In non-vaccinated chickens, HI titers specific to NDV can be taken as a sign of previous infection, and titers as high as 10 $\log_2$ suggests field exposure to pathogenic NDV Chrysostome *et al.* (1995), Alexander *et al.* (2004).

From another point of view Allan *et al.* (1978) were using ND- HI titer (using 4 HA units) as a predictive tools for the expected mortality and drop in egg production upon challenge as follow.

- When All individual HI values was 2^2 or less (100% mortality on challenge is expected).
- When all individual HI values was 2^2 to 2^5 log mean $2^{3.75}$ STDV 0.4 (10% mortality on challenge is expected).
- When all individual HI values was 2^4 to 2^6 log mean $2^{5.2}$ STDV 0.35(0% mortality on challenge is expected).
- When all individual HI values was 2^6 to 2^8 log mean $2^{6.5}$ STDV 1.2 (A serious drop in egg production, with no deaths could be expected and convalescent HI titer 2^{14} or greater could be reached).
- when All individual HI values was 2^9 to 2^{11} log mean $2^{10.5}$ STDV 1.4 (No drop in egg production, no deaths, and a convalescent titers 2^{11} to 2^{12} could be obtained),
- And finally if the range was 2^{11} to 2^{13} log mean $2^{11.2}$ STDV 1.3 (A flock that will remain free from any risk of NDV).

In the present study., the ND-HI titers were ranging from (0 up to 10) log 2 (table-2). The titer of sample (6,7,14,19,23,24,27,29,30,32,and 35) were ranging from (9.1 up to 10) $\log_2$ (table -2)., this could justify a NDV affection especially if we consider that the vaccines applied for these flocks is not sufficient to elect such titer.

AIV-(H5) Comin *et al.* (2013) found that HI test has a near perfect accuracy that might be considered as a gold standard test. The test enables processing large amounts of samples in a short time, if the reference viral antigen is close enough to the virus isolate to be tested when panels of different antigens are used. In the present study, we had used H5 with three distinct neuraminidases i.e. (H5N1, H5N2, and H5N3) these represent the AIV commercially available viral antigens in the Egyptian market.

Timm Harder (2012): In a personal communication mentioned that, the neuraminidases has a significant role in HI as well. He explained., that neuraminidases are enzyme that binds to its substrate, the sialic glycans that are also used as the cellular receptor for influenza A virus HA proteins. He also gave a further interpretation as follow. (Given the situation of a duck holding that had experienced an $H_{11}N_1$ infection. Serum taken from these birds three weeks later will react in HI with the H_5N_1 and the H_7N_1 antigens but none of them will react with the H_5N_3 or the H_7N_7 ones. Thus such sera are considered H_5/H_7 negative despite their reactivity with one H_5/H_7 antigen!). In addition, he mentioned that HI assay are difficult to perform and more difficult to interpret! This is because HA and NA are always linked to diagnostic antigens (inactivated viruses). On the other hand, HI assays are highly specific. Thus, even antibodies raised against one of the Egyptian H_5N_1 cluster 2.2.1.1 viruses will probably not react with (European) H_5N_1 antigen due to the large antigenic distance between these HA antigens. In the present study, samples were collected from vaccinated bird. Vaccines in Egypt represent these viral categories (H_5N_1, H_5N_2, H_5N_3) in a reassortant form., so it became very difficult for us to evaluate to obtained results and because poultry holders had stopped the inclusion of sentinel birds in the breaded poultry batches., we thought that the high HI titer for the H_5N_3antigen observed in samples (3,6,8,13,17, 18,28,29,30,35 and 35) was relevant to the vaccinal strain used as mentioned by Timm Harder (2012)., but on second thought we had found that flock sample (3,6,8 and 30) were applying vaccination against AIV using the H_5N_1 reasssortants and flock sample (13,28) were totally not vaccinated against AIV and that flock samples (17,18,29,35 and 36) had anonymous history., this may reflect H_5N_3 circulation in the fore mentioned flock samples.

AIV-(H7) Abdel whab *et al.* (2013) mentioned that Avian influenza viruses of H_5 and H_7 subtypes exhibit two different pathotypes in poultry: infection with low pathogenic (LP) strains results in minimal, if any, health disturbances, whereas highly pathogenic (HP) strains cause severe morbidity and mortality. LPAIV of H_5 and H_7 subtypes can spontaneously mutate into HPAIV. Ten outbreaks caused by HPAIV were preceded by circulation of a predecessor or LPAIV in poultry. Three of them were caused by H_5N_2 subtype and seven involved H_7 in combination with N_1, N_3, or N_7. Abdelwhab *et al.* (2014) mentioned that., H_7 subtype HA gene has been found in combination with all nine NA subtype genes. Most exhibit low pathogenicity and only rarely high pathogenicity in poultry (and humans). During the past few years

infections of poultry and humans with H_7 subtypes have increased markedly. In the present study., fortunately H_7 vaccines is not yet allowed to Egypt., this had facilitated our mission in interpreting the obtained results, which aim to ascertain its existance in Sharkia., although the GMT of the HI against $H_7N_1$was ranging from (.1±.01 – 4.54±.32) (table -2). It worth to mention., that examined samples revealed positive seroreactive values that exceeded the cut off values which could be interpreted surely as infection in samples (14, 17, 18,19,20, 21,28, 29,30,34,35 and 36) had a positive seroreactive that exceeded the cutoff values OIE (2010). This gives a sure incidence of AIV-H7 in different localities of sharkia governorate but this incidence was milder than the previously recorded incidence by Afifi *et al.* (2013). The incidence of the disease in sharkia based on a seropositive reactors could be speculated to start in (June, July, Sept., Oct., and November 2014) in the locations from which samples were collected such as (El-Salhia, 10th of Ramadan, El-Ibrahemia and Abo Hammad) (table 2 figure 1), it also worth to mention that the positive seroreactives were totally from commercial layers except for flock (sample 29) which were collected from a broiler breeder flock. It should be also noted that the total seropositive samples exceeding the cutoff values were (63/1317 with a percent of 4.78%).

Abdel whab *et al.* (2014) mentioned that wild birds are the natural reservoir of the H_7 virus. Geographically, the most prevalent subtype is H_7N_7, which is endemic in wild birds in Europe and was frequently reported in domestic poultry, whereas subtype H_7N_3 is mostly isolated from the Americas. In humans, mild to fatal infections were caused by subtypes H_7N_2, H_7N_3, H_7N_7 and H_7N_9.

While, infections of humans have been associated mostly with exposure to domestic poultry, infections of poultry have been linked to wild birds or live bird markets. Fred Leung (2012) in a personal communication commented on the role of wild migratory bird in AIV disease transfer as follow (although the virus movements were recorded in the known migratory bird routes but in the opposite direction for the migratory bird flow route). Generally, depopulation of infected poultry was the main control tool. In contrast to recent cases caused by subtype H_7N_9, human infections were usually self-limiting and rarely required antiviral medication. Close genetic and antigenic relatedness of H_7 viruses of different origins may be helpful in development of universal vaccines and diagnostics for both animals and humans. Due to the wide spread of H_7 viruses and their zoonotic importance more research is required for better understanding of the epidemiology, pathobiology and virulence determinants of these viruses.

AIV-(H9) Positive seroreactive samples were recorded in the submitted samples from different localities of Sharkia governorate starting from (Jan 2014). The GMT of HI titers was ranging from (.32 up to 10). Even. In (sample15) 2 / 24 sample exceeded the cutoff values. Observing these results and considering that only flock sample (1 and 9) had received vaccines against H_9N_2 lead us to conclude., a wide distribution of H_9 in Sharkia governorate ,it also should be pointed for the fact that the distance between vaccination in flocks (1) and sample collection exceeded 100 day this could justify infection and with flock (9) we can observe that birds were simultaneously vaccinated with (AIV H_9+NDV) and that the serologic response for NDV is very low compared to the serologic response for AIV-H9 which suggests superinfection with H9. The total seropositive sample for H9 were (736 out of 1317 examined) i.e.(55.8%).

Afifi *et al.* (2013). Examined the potential existence of H_7 and H_9 AIV circulating among chicken flocks in Egypt. Serum samples were collected from chicken flocks that experienced respiratory distresses and/or variable mortality rates. H_7 and H_9 virus infections were screened by HI assay. Concerning Sharkia governorate. A 133 serum samples were collected from one broiler, one breeder and two layer flocks. 52 out of 133 examined sera, seropositve i.e. (39 %) were recorded for H_7 .As for H_9 A 113 out of 133 seropositive sample were recorded i.e. (84 %). Prevalence of both H_7 and H_9 antibodies were higher in layer followed by breeder then broiler flocks. Afifi *et al.* (2013) concluded that special consideration should be paid to control influenza viruses in Egypt, as pandemic influenza strains may develop unnoticed given the presence of subclinical infections, and the possibility of re-assortment with the prevailing endemic H_5N_1 virus strains exciting in Egypt.

Afifi *et al.* (2013) mentioned that., they were able to isolate H_9N_2 from broiler flock from Alexandria Governorate in the northern part of Egypt (unpublished data) however, only a broiler flock from Beni-Suef was found seropositive to H_9. Interestingly, another recent report for the isolation of H_9N_2 from quail in Egypt was also reported El-Zoghby *et al.* (2012). Meanwhile, the isolation of AIV subtype H_7 from Egypt was only recorded from migratory birds Aly *et al.* (2010) and Soliman *et al.* (2012) as follow., on 2004 (H_7N_1). On 2004, 2005, and 2006 (H_7N_7). On 2006 (H_7N_9), and on 2007 (H_7N_3). Interestingly, H_7 serological results on backyard from 11 villages in the nearby areas were negative Aly *et al.* (2008). To the best of our knowledge, no recent report revealed isolation of H_7 from commercial or backyard poultry populations. But historically, LPAIV A/turkey/ Egypt/88 (H_7N_1) was isolated Khafagy *et al.* (1992); however, they reported the absence of H_7 seropositive sera when testing 6124 chicken and 92 turkey sera

Khafagy *et al.* (1995). However, the presences of antibodies against AIV H7 were reported Afifi *et al.* (1999). Egypt is located in the pathway of migratory birds and represents a hinge zone of wild bird migration, where the East Africa–West Asia and Black Sea–Mediterranean's flyways overlap and large diversity of species migrating to and from South Africa, Europe, and Central Asia were detected in Egypt Soliman *et al.* (2012). It was recorded that the migratory birds plays a role in the introduction but not the spread of AIV to other wild and domestic species that are present in their migratory pathways Feare (2010), Soliman *et al.* (2012). Increased numbers of seropositive were observed in farms located within the migratory route of wild birds Al-Natour and Abo-Shehada (2005).

Avian adenovirus (AAV) associated with clinical disease was isolated from an outbreak of respiratory disease in quail Olson (1950). Since that time, AAVs were seen in all types and breeds of chickens and from a variety of other avian species. AAV were frequently isolated from the respiratory and/or intestinal tracts of the apparently healthy chickens, and their role in the etiology of clinical disease was regarded as insignificant Yates *et al.* (1976) and Winterfield (1984). Sometimes., AAVs were associated with a variety of specific disease such as (quail bronchitis, inclusion body hepatitis (IBH), egg drop syndrome (EDS), turkey hemorrhagic enteritis (THE), marble spleen disease and respiratory manifestation) Du Bose *et al.* (1958), Du Boes and Grumbles (1959), Ismail (1966), Ahmed and El-Sisi (1969), Fadly and WinterfieId (1973), Rosenberger *et al.* (1974), Hoffmann *et al.* (1975), McFerran (1981), McFerran (1989), McFerran (1991) and McFerran and Stuart (1990). Papanikoloau *et al.* (1985) and McFerran and Stuart 1990). Specific pathogenicity for poultry and vertical transmission had led AAVs to receive more attention McFerran and Stuart (1990). AAV should not be neglected as complicating factors in the course of some poultry diseases e.g Mycoplasmosis and IBV Monereal and Ahmed (1963), Monereal (1966), Monereal (1968), Awad *et al.* (1973), Dhillon and Kibenge (1987)., and in birds vaccinated with spray IBV at day-old or immune-suppressed by IBDV Mousa *et al.* (1984), McFerran and Stuart (1990).

Egg drop syndrome 1976 (EDS_{76}) is an economically important viral disease characterized by a severe drop in egg production as well as the production of shell less, thin-shelled, discolored or misshapen eggs Van Ecz (1982). Although, the occurrence of EDS_{76} in layers has been reported worldwide but there is limited information on its occurrence in broiler chickens Meulemans *et al.* (1979).

Hemagglutinating AAV is represented by $EDS_{76.}$ There are three evident patterns of EDS_{76}. Classical EDS_{76} following the introduction of virus into primary breeding stock, probably through a vaccine grown in duck cells. Spread was through the embryo and the resulting reactivation of virus at peak of egg production gave an apparent breed and age susceptibility. The second pattern is the endemic form where lateral spread between flocks via contaminated eggs occurs at any age of laying hens. The third (sporadic) form is again seen in any age or breed of birds resulting from infection from ducks and geese or any infected wild bird McFerran and Stuart (1990).

To our knowledge, reports on seroprevalence of EDS_{76} in chickens in commercial farms in Sharkia are scanty. Although killed vaccines against EDS_{76} such as (IBV+NDV+EDS_{76}) or (IBV+NDV+EDS_{76} +AIV) are used in commercial layer flocks. In the present study three broiler flocks (sample 2), (sample 11) and (sample 13) were serologically positive and had HI titre of (6.75 ± 0.16, 5 and 7) respectively after neglecting the cutoff values., these flocks were located in (zagazig, Belbees and El-salhia). On the other hand., layer flocks in (sample 6), (sample 7) and sample 8) had a seropositive HI titer at age of 20wk, 26wk, and 26wk of age that was {7.98 ± 0.17, 7.09 ± 0.3 and 3.25 ±0.45} respectively., these flocks had received the triple killed vaccine at age around 11 WK of age during the rearing period in integrated plants but the layer flocks in small holders such as flock (sample 25), (sample 26), (sample 27), (sample 28), (sample 29) and (sample 31) had HI titer ranging from (6.62±0.9 up to 9.94±.06) which could be referred to as an infection. These flock were located in (Belbees, 10th of Ramadan, El-Ibrahemia and Abo-hammad).

Singh *et al.* (1995) screened 22 broiler flocks using 347 serum samples examined with the (HI) test., they found that 114 sample i.e. (32.9%) were positive for antibodies to (EDS_{76}).,the HI titres of these serum samples ranged from 2 to 9 log2 and the overall geometric mean titre was 3.9 log2. All of the examined flocks in the present study were positive for antibodies suggesting widespread EDS'$_{76}$ infection in this region of Egypt. The presence of high HI antibody titres suggests that infection was acquired at an early age and this could not be due to maternal antibodies as the present study was limited to birds 5 to l0 weeks old and maternally derived antibodies are known to persist only up to 3 weeks of age McFerran (1981), McFerran and Stuart (1990). Vertical transmission is common in EDS'$_{76}$ virus infection but congenitally infected birds may not become serologically positive until 25-28 weeks of age VanEcz (1982). Because it has been observed that congenital infection remains dormant until sexual maturity or in response to stress of production Nawathe and Abegunde (1980). Contrary to this the present observation of HI antibody in broiler birds provides evidence for horizontal spread of the virus. This is supported by other workers who have

confirmed the transmission of the virus at different stages throughout the rearing of chickens Cook and Darbyshire (1981). McFerran (1979) also reported the lateral spread of the virus because small farmers raise broilers and laying chickens on the same premises. An infected flock of broilers may be a potential risk to laying chickens and hence regular monitoring of broiler flocks for infection should be an essential component in the control of the disease.

Infectious Bronchitis Virus (IBV) is a highly contagious acute viral disease of the upper respiratory tract of chickens, it can also replicate in epithelial tissues of kidneys, gonads and oviduct of chickens causing their pathology and affecting the performance Lee *et al.* (2004).

Prevention of IB is achieved mainly through vaccination. Although in most cases IBV strains within a geographic region are distinct as mentioned by Callison *et al.* (2001), Gelb *et al.* (2005), Ignjatovic *et al.* (2006). Because IBV undergoes frequent changes in the viral genome, mainly in the S1 gene which result in point mutations promoting the emergence of new antigenic variants Bochkov *et al.* (2007), Ammayappan *et al.* (2008) and Lee *et al.* (2008). The multiple IBV serotypes and its antigenic variation adds complexity to the proper selection of vaccination protocol and proper selection of serologic method to analyses the test results Jackwood and De Wit (2013). Vaccine strains should be selected to represent the antigenic spectrum of isolates in a particular region, because attenuated vaccines are known to have a limited range of protection, confined in many cases to homologous strains, rendering vaccination partially successful Lin *et al.* (2005). The use of heterologous vaccine strains, either simultaneous or sequentially, has broadened the protection spectrum in some cases Cook *et al.* (1999), but it is difficult to predict which combinations may confer the best protection. On the other hand, more virulent vaccine strains may have a broader range of protection, but their use is not recommended to avoid the risk of a disease outbreak Darbyshire (1985).

In the present study HI for IBV using the D-274 antigen of the German variant led us to conclude that the majority of the examined flocks were seropositive after neglecting the cutoff values and considering that., these flocks didn't receive such protective vaccines as seen with sample (2, 3, 8, 10, 11, 14, 17, 18,19, 21,24, 26, 27and 30). On the other hand Infection with Mass type IBV was noticed in (sample 8)., the history of this flock shows a drop in egg production and deformed egg quality. This flock was re-examined in (sample 20) at 51 week of age where a high immune titers was still detectable.

Abdelwhab and Abdel-Moneim (2015) had wondered! Will Egypt be the epicenter of the next influenza pandemic? We think yes especially if we consider viral Concurrency as seen in (table 3-4) beside neiglecance in poultry operation.

During investigation of the increased morality or dropped egg production in the examined flocks bacteriological work was performed parallel to the serosurvillance surprisingly a very resistant bacteria was detected, these resistant bacteria was initially identified as klebsilla spp. further examination is ongoing.

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فـي هـذه الدراسـة تـم فحـص ١٣١٧ عينـة سـيرم تـم تجميعهـا مـن ٦ قطعـان دواجـن تسـمين وقطيعـين دواجـن أمهـات تسـمين و ٢٨ قطيـع دواجـن بيـاض تجـاري وتـم فحصـهم باختبـار مـانع الـتلازن الـدموي ضـد الانتجينـات الملزنـه لكـرات الـدم الحمـراء للفيروسـات الآتيـه} فيـروس النيوكاسـل عتـرة اللاسـوتا وفيـروس الانفلـونزا العتـرة H_5N_1,H_5N_2,H_5N_3,H_9,H_7 وفيـروس الالتهـاب الشـعبي العتـرة IBV-$_{4/91}$,IBV-$_{D274}$,IBV-$_{MASS41}$ و فيـروس الادينـو العتـرة EDS_{76} }. باسـتعمال التخفيـف الثنـائي للوغـاريتم الثنـائي وبالضـوابط اللازمـة للاختبـار. عنـد الفحـص للأجسـام المناعيـة الملزنـة لكـرات الـدم الحمـراء ضـد أنتيجـين الإنفلـوانزا H_7 تـم رصـد ٦٣ عينـة إيجابيـة مـن ١٣١٧ عينـة ايجابيـة بنسـبة ٤.٧٨% وذلـك عنـد فحـص العينـات أرقـام [١٤، ١٧، ١٨، ١٩، ٢٠، ٢١، ٢٩، ٣٠، ٣٧، ٣٥) وأمكـن رصـد تـاريخ حـدوث ذلـك فـي شـهر يونيـو ويوليـو وسـبتمبر وأكتـوبر ونـوفمبر ٢٠١٤ فـي منطقـة الصـالحية والعاشـر مـن رمضـان والإبراهيميـة وأبـو حمـاد، الجـدير بالـذكر أن هـذه الأجسـام المناعيـة تـم رصـدها فـي قطعـان الإنتـاج التجـاري للبـيض فقـط دون غيرهـا مـن بـاقي القطعـان محـل الدراسـة. عنـد الفحـص للأجسـام المناعيـة الملزنـة لكـرات الـدم الحمـراء ضـد أنتيجـين الإنفلـوانزا H_9 وجـدت تتـرات مناعيـة مرتفعـة فـي كـل العينـات المفحوصـة علـي الـرغم مـن اهمـال كثيـر مـن المـربين التحصـين ضـد هـذه العتـرة الفيروسـية. وعنـد الفحـص للأجسـام المناعيـة الملزنـة لكـرات الـدم الحمـراء ضـد أنتيجـين فيـروس H_5 مـع تنـوع النيورأمنيـديز المصـاحب حيـث تـم الإختبـار ضـد (H_5N_1, H_5N_2, H_5N_3). حتـى تكـون نموذجـاً ممـثلاً لأنتيجينـات اللقاحـات المسـتعملة فـي مصـر، وكـان تفسـير النتـائج مـن الصـعوبة بمكـان فـي ظـل توقـف المـربيين دمـج الطيـور الكاشـفة مـع الطيـور المحصـنة. فـي بـادئ الأمـر وجـدنا تتـرات مناعيـة عاليـة ضـد العتـرة الفيروسـية H_5N_3 فـي القطعـان [٣، ٦، ٨، ١٣، ١٧، ١٨، ٢٨، ٢٩، ٣٠]، وكـان القطيـع رقـم [٣، ٦، ٨، ٣٠] محصـنين ضـد العتـرة H_5N_1 والقطعـان (١٣، ٢٨) غيـر محصـنين وبـاقي القطعـان لـم يكـن لهـا تـاريخ تحصـين ضـد الأنفلـونزا، وهـذا قـد يعكـس تـدوير العتـرة H_5N_3 فـي قطعـان الـدواجن السـابق ذكرهـا. وعنـد الفحـص للأجسـام المناعيـة الملزنـة لكـرات الـدم الحمـراء ضـد أنتيجـين النيوكاسـل عتـرة اللاسـوتا تـم رصـد ارتفـاع التتـر المنـاعي الـذي تـراوح مـن (٩.١ إلـى ١٠) أثنـاء فحـص عينـات القطعـان (٦، ٧، ١٤، ١٩، ٢٣، ٢٤، ٢٧، ٢٩، ٣٠، ٣٢، ٣٥) وهـذه النتـائج يمكـن تأويلهـا علـى أنهـا اصـابات قـد حـدثت فـي الصـالحية وبلبـيس وأبـو حمـاد والعاشـر مـن رمضـان. وعنـد الفحـص للأجسـام المناعيـة الملزنـة لكـرات الـدم الحمـراء ضـد أنتيجـين فيـروس الالتهـاب الشـعبي لاحظنـا عنـد اسـتعمال انتيجـين الإلتهـاب الشـعبي D-274 ضـد العتـرة الألمانيـة المغـايرة وجـود اسـتجابة مناعيـة فـي قطعـان دواجـن غيـر محصـنة ضـد هـذه العتـرات كمـا فـي العينـة رقـم (٢، ٣، ٨، ١٠، ١١، ١٤، ١٧، ١٨، ١٩، ٢١، ٢٤، ٢٦، ٢٧، ٣٠) وكـذلك لاحظنـا اصـابة واحـدة بـالعترة الكلاسـيكية لفيـروس الإلتهـاب الشـعبي فـي العينـة رقـم (٨). وعنـد الفحـص للأجسـام المناعيـة الملزنـة لكـرات الـدم الحمـراء ضـد أنتيجـين فيـروس الادينـو (متلازمـة انخفـاض البـيض) فـي هـذه الدراسـة أمكـن رصـد ثلاثـة قطعـان بهـا اسـتجابة مناعيـة إيجابيـة ضـد أنتيجـين فيـروس EDS_{76} فـي ثلاثـة قطعـان تسـمين فـي العينـة (٢، ١١، ١٣) والتـي تـم جمعهـا مـن مدينـة الزقـازيق، بلبـيس ، الصـالحية. ومـن ناحيـة أخـرى فقـد تـم رصـد اسـتجابة مناعيـة ايجابيـة عنـد فحـص قطعـان دواجـن البيـاض التجـاري وكانـت تتـراوح مـن (٦.٦٢ الـي ٩.٩٤) فـي العينـات (٢٥، ٢٦، ٢٧، ٢٨، ٢٩، ٣١) والتـي تـم جمعهـا مـن مـدن بلبـيس والعاشـر مـن رمضـان والإبراهيميـة وأبـو حمـاد. ومـن خـلال هـذه الدراسـة وضـح لنـا أيضـاً أن مـن الأسـباب المباشـرة للمشـاكل المرضـية أن أصـحاب المـزارع يتبعـون أنظمـة تحصـين مكتوبـة مسـبقاً فـي دليـل التربيـة دون النظـر الـى دراسـة الـدور الـذي تلعبـه المناعـة الاميـة فـي الاسـتجابة المناعيـة أو فحـص المسـتويات المناعيـة قبـل اتخـاذ قـرار التحصـين. تجـدر الإشـارة الـي انـه اثنـاء فحـص المشـاكل الحقليـة سـواء كانـت ارتفـاع فـي نسـب النفـوق او انخفـاض فـي معـدلات انتـاج البـيض. كانـت الفحـوص البكتريولوجيـة تجـري بـالتوازي مـع الفحـوص السـيرولوجية وقـد تمكنـا مـن عـزل معزولـة مقاومـة للمضـادات الحيويـة مـن ميكـروب الكلبسـيلا. وهـذا محـل مزيـد مـن الفحـوص وقـد اشـرنا الـي وجـوده مـع التداخلات الفيروسية التي اشرنا اليها لمحاولة تبرير مسببات النفوق المرتفع في محافظة الشرقية.