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PREVALENCE OF TUMOURS AMONG POULTRY FLOCKS IN EGYPT (With 4 Tables)

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مدى إنتشار السرطانات فى قطعان الدواجن فى مصر

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وجد أن السرطانات هى المسئولة عن النفوق بنسبة ١١%، ٥%، ١,٢%، ٣% فى السلالات المحلية، الدجاج البياض، كتاكيت التسمين وبدواري أمهات اللحم على التوالي. تم فحص المصل وبياض البيض لوجود الأنتيجن العام (P-27) بواسطة إستخدام إختبار الإليزا. تم تحديد مستوى الفيروس الداخلى (Endogenous virus) فى معظم مزارع الدواجن بدرجات مختلفة، حيث كان أعلى معدل لهذا الفيروس فى قطعان كتاكيت اللحم (٢٥%) وأيضا فى كتاكيت السلالات المحلية (٣٣%). وجد أن معدل الفيروس الداخلى أعلى من معدل الفيروس الخارجى. أمكن تحديد الأجسام المضادة لمرض مارك فى المصل وصفار البيض للأمهات وكذلك فى مصل الكتاكيت الناتجة عنها ووجد أن هناك علاقة بينهما. وجد أن الأجسام المناعية الأمية لمرض مارك إستمرت حتى ٣-٤ أسابيع من عمر الكتاكيت.

SUMMARY

Tumours were found to be responsible for deaths at rates of 11%, 5%, 1.2%, and 3% in native breed chicks, layers, broilers, and broiler breeders respectively. Sera and egg-albumen were monitored for presence of P-27 common antigen of avian leukosis virus (ALV) by ELISA test. Titer of endogenous virus was detected in most of poultry farms at various degrees. The highest percentages were detected in meat type chicken flocks (25%) and native breed chicks (33%) and the titer of endogenous virus was higher than the titer of exogenous virus. Marek's disease antibodies were detected in yolk and sera of dams as well as in serum samples of the offspring and the ratio was found to be

correlated. Maternal antibodies of Marek's disease virus were detected till 3-4 weeks of age.

Key Words: Prevalence of tumours among poultry flocks in Egypt.

INTRODUCTION

Tumours still a cause of severe economic losses confronting the poultry production all over the world. Losses are mainly due to mortalities, high rate of condemnation and immune suppression (Calnek, 1992). Avian neoplasms are either infectious or noninfectious in nature according to the etiologic agents.

Both DNA and RNA viruses are responsible for avian tumours which are infectious in nature producing lymphoproliferative diseases affecting the peripheral nerves and other tissues and visceral organs as in case of Marek's disease (MD), affecting bursa of Fabricius and visceral organs as in case of lymphoid leukosis or affecting hematopoietic system as in case of erythro-blastosis and myelocytomatosis (Ellermann, 1921).

Avian leukosis (AL) and avian sarcoma (AS) viruses are the cause of numerous transmissible neoplasms like lymphoid leukosis (LL), erythro-blastosis, myelocytomatosis, osteopetrosis, endothelioma, nephroblastoma, hepatocarcinoma and connective tissue tumours (Burmester and Witter, 1971). Most losses are due to lymphoid leukosis which reached 23.2% (Purchase *et al.*, 1972-a,b). Avian leukosis virus may be found as exogenous virus which can be transmitted either horizontally through saliva and feces or vertically through eggs or found as endogenous virus which can be transmitted genetically in the germ cells of both male and female as DNA provirus (Cottral *et al.*, 1954, Rubin *et al.*, 1962 and Motta *et al.*, 1975). Testing of egg albumen is very efficient for detection of congenitally transmitting hens (Tsukamoto *et al.*, 1992).

ELISA test is an efficient and specific tool for detection of avian leukosis virus (ALV) in both egg albumen and blood serum, and no difference was found between biological assay for endogenous virus and ELISA test (Smith *et al.*, 1980; Todd *et al.*, 1993; Cadman *et al.*, 1994).

Marek's disease was described firstly in Hungary as polyneuritis by Marek (1907). Similar disease was reported in the United States (Kaupp, 1920). Agar gel precipitation (AGP) test is of great importance

in diagnosis of MD infections. MD virus has 6 precipitating antigens consistently three antigens are present which have been designated as A, B and C (Churchill *et al.*, 1969-a,b). The precipitating antigen A is closely related to virus-induced membrane antigen (Yachida *et. al.*, 1983; Ikuta *et. al.*, 1984).

Maternal antibodies to MD delay onset, reduce tumour formation, suppress the acute destruction of the lymphoid and haematopoietic tissues and prevent bursal atrophy leading to lengthen the latent period for cell-free virus as well as the antigen production in the feather follicles (Calnek, 1972- a,b; Burgoyne and Witter, 1973).

The objectives of this work were directed for studying the prevalence of tumours among poultry farms, detection of exogenous and endogenous avian leukosis virus antigen in egg albumen and blood serum using ELISA test as well as for detection of antibodies against Marek's disease virus in egg yolk and blood serum. Also for determination of the waning of maternal antibodies to MD in one- day-old chicks.

MATERIALS and METHODS

Reference MD virus antigen and antiserum:

Reference MD precipitating antigen and antiserum were kindly supplied by Dr. S. H. Kleven, Poultry Dis. Research Center, Athens, Georgia, USA.

ELISA Kits:

Avian leukosis virus antigen ELISA test Kits (ProFLOK®) produced by Kirkegaard & Perry Laboratories Inc. (KPL), Maryland, USA were used in ELISA test.

Chicken flocks:

10 layer (20-60 weeks), 3 broiler breeder (6-7 weeks), 5 native breed (15-50 weeks) and 15 broiler (4-7 weeks) flocks raised in Upper Egypt were put under periodical observation for 6 months. Mortalities were recorded and cases showing solid tumours in post-mortem examination were subjected to histopathological examination.

Serum samples:

Fifty serum samples were collected from each flock of either layers, meat type breeder flocks, and native breed chicks. Sera were subjected to ELISA test for detection of ALV.

Yolk extraction:

5 ml yolk were aspirated and mixed with the same volume of phosphate buffer saline for 30 seconds in an agitator for 4 times. Emulsions were centrifuged for 30 minutes at 6000 rpm in angle-head centrifuge at 4 C. The supernatants were collected and tested on the same day by AGP test.

Albumen:

Fresh eggs were collected from different chicken flocks, albumen was harvested by puncturing a small hole at the midway between the middle and small end of the eggs. Albumen was withdrawn carefully to avoid obtaining yolk materials and was assayed for ALV by ELISA test.

ELISA test:

Sera or albumen were added to the coated plates and incubated for 30 minutes. Plates were washed three times, enzyme conjugate was added, then washed. Substrate was added, then stop solution. Absorbance at 405 nm was estimated using an automatic Biotek EL 311S reader. Titers were calculated using soft ware program of Flockchek.

Agar gel precipitation test:

The medium used for AGP test was prepared according to Lanconescu and Samberg (1970). Slides were covered by 1% Noble agar in 8% NaCl solution. Samples of sera and yolk extract as well as known positive control serum were added and incubated at 37 C for 24-48 hours then were read.

Histopathological examination

Specimens were collected from different organs, sectioned and fixed in 10% neutral buffered formalin, then stained with Haematoxylin and Eosin (H&E) and examined microscopically, (Neumann and Witter, 1979).

RESULTS

Mortalities due to tumours:

Records of the rate of mortalities due to tumours are showed in Table (1). Tumours were responsible for mortalities at rates of 11%, 5%, 1.2%, and 3% in native breed chicks, layers, broilers, and broiler breeders respectively.

Histopathological findings:

Tumours were confirmed histopathologically and were found to be responsible. The most common histopathological findings were

infiltration of neoplastic cells of examined smears especially from liver, spleen and kidneys.

Detection of exogenous and endogenous ALV:

Table (2) shows the titers and the rate of detection of exogenous and endogenous ALV in sera and albumen. The highest percentage of positive cases with endogenous virus was detected in broiler breeders (25%- 15 out of 60 samples) and native breed chicks (33.2%- 83 out of 250 samples). The titer of endogenous virus was higher than the titer of exogenous virus.

Detection of MD precipitating antibodies:

MD precipitating antibodies in yolk and sera of dams as well as in sera of offspring are shown in Table (3). It was clear that MD precipitating antibodies were lower in offspring originating from dams vaccinated only with turkey herpes virus (THV) vaccine than those originated from dams vaccinated with THV and Rispens vaccines.

Waning of MD precipitating antibodies:

The waning of MD precipitating antibodies is shown in Table (4).

DISCUSSION

A six-month study showed that mortalities due to tumours in internal organs represented 11%, 5%, 1.2%, and 3% in native breed chicks, layers, broilers, and broiler breeders respectively. Diagnosis depended on post-mortem examination and was confirmed by histopathological examination. Higher rates of losses were reported in Egypt by Mousa (1974), which could be attributed to the application of restricted vaccination programs as well as breeding from genetically tumour resistant breeders specially in layers and broiler breeders.

In order to study the prevalence of ALV among different chicken flocks, the P-27 antigen was monitored by ELISA test. Endogenous virus was detected in chicken sera and exogenous virus was detected in egg albumin. The highest incidence of both endogenous and exogenous virus was found in native breed chicks and to lesser extent in broiler breeders, while the lowest values were recorded in layers. This variation may be due to differences in susceptibility (Jungheer and Hughes, 1965; Biggs, 1967) or due to the restricted biosecurity measurements adopted in the organized poultry industry. Titers of endogenous virus were found

to be higher than the titers of exogenous virus. Similar results were reported by Payne and Howes (1991).

The precipitating antibodies of MD were detected in sera and egg yolk of breeders at nearly equal values. Antibodies were also detected in sera of offspring at lower rate (about 80%) and were correlated to the rate of positivity in their dams. Antibodies were higher in breeders with history of double vaccination (Rispens and HVT) than those vaccinated only with HVT (Witter and Lee, 1984).

To study the waning of maternal antibody to MD, serum samples were subjected to AGP test at weekly intervals. Results showed that antibodies decreased gradually and disappeared at fourth week of age. Similar results were reported by Edison *et al.* (1978) who reported that baby chicks originated from vaccinated dams possess variable levels of antibodies till 3-4 weeks of age.

It could be concluded that tumours are responsible for high deaths in all types of chickens. The titers of endogenous virus of AL were higher than the titers of exogenous virus. The levels of MDV antibodies in yolk and sera of dams and in sera of offspring were correlated.

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Table 1: Mortalities due to tumours in different chicken flocks

A- Layers

Flok No.	Total mortalities	Mortalities due to tumours	%
1	81	5	6.1
2	69	4	5.7
3	90	8	8.9
4	65	0	0
5	76	2	2.6
6	78	6	7.6
7	59	0	0
8	95	7	7.3
9	81	0	0
10	105	8	7.6
Total	799	40	5%

B- Broiler breeders

1	56	1	1.9
2	78	3	3.8
3	35	1	2.9
Total	169	5	3%

C- Native breed chicks

1	150	19	12.7
2	146	15	10.3
3	139	16	11.5
4	165	18	10.9
5	80	10	12.6
Total	680	75	11%

D- Broilers

1	59	1	1.7
2	65	0	0
3	46	0	0
4	52	1	1.9
5	57	1	1.8
6	65	2	3.1
7	42	0	0
8	46	1	2.2
9	51	1	1.9
10	57	0	0
11	41	0	0
12	36	0	0
13	59	2	3.4
14	35	0	0
15	39	0	0
Total	750	9	1.20%

Table 2: Results of ELISA test for presence of ALV antigen in serum and albumen in different chicken flocks

A- Layers

Flok	Serum			Albumen		
	No. of samples	+ve	Mean titer	No. of samples	+ve	Mean titer
1	50	0	29	50	0	14
2	50	9	213	50	3	126
3	50	0	16	50	0	23
4	50	4	156	50	0	13
5	50	6	198	50	3	146
6	50	0	8	50	0	28
7	50	4	132	50	2	135
8	50	6	167	50	2	142
9	50	7	182	50	3	156
10	50	8	204	50	2	125
Total	500	44		500	15	

B- Broiler breeders

1	20	4	159	20	2	142
2	20	3	143	20	1	124
3	20	8	197	20	3	167
Total	60	15		60	6	

C- Native breed chicks

1	50	19	351	50	5	132
2	50	14	153	50	3	159
3	50	11	195	50	2	197
4	50	18	285	50	6	146
5	50	21	397	50	2	136
Total	250	83		250	18	

Table 3: Incidence of MD precipitating antibodies in egg yolk and sera of dams and offspring.

Chicken type	Flock No.	Dams						Offspring		
		Egg yolk						Serum		
		sample	+ ve	%	samples	+ ve	%	samples	+ve	%
Breeders* (Meat type)	1	30	28	93	30	30	100	30	24	80
	2	30	28	93	30	30	100	30	25	83
	3	30	30	100	30	30	100	30	26	86.6
	4	30	26	86.6	30	30	100	30	22	73
	5	30	26	86.6	30	30	100	30	20	66.6
Native breed** _s	1	30	21	70	30	24	80	30	18	60
	2	30	19	63	30	23	76.6	30	16	53
	3	30	19	63	30	20	66.6	30	16	53
	4	30	20	70	30	22	73	30	17	56.6
	5	30	16	53	30	20	66.6	30	14	46.6

* vacc. by Rispens & HVT.

** Vacc. by HVT.

Table 4: Results of waning of MD maternal antibodies in one day old chicks.

Age in Weeks	Samples	+ ve	%
One day	15	15	100
1 Week	15	15	100
2 Week	15	13	86.6
3 Week	15	5	33.3
4 Week	15	0	0

