Kafrelsheikh Vet. Med. J. Vol. 6 No. 2 (2008) (196-208)

SEROLOGICAL STUDIES ON CHICKEN INFECTIOUS ANEMIA VIRUS IN GHARBEIA PROVINCE

Moshira, A. El-abasy[*]*; Gamal, R. Hassab El-nabby*[**]*, Ahmed, S. Hegasy*[**] *and Salwa, M. Helmy*[*]

[*]Poultry Diseases and Microbiology Dept., Faculty of Vet. Med., Kafrelshiekh University.

[**] Microbiology Dept., Animal Health Research Institute, Tanta Lab.

ABSTRACT

A serological survey on sera of chickens had revealed detection of antibodies to chicken infectious anemia virus (CIAV) by using ELISA test. Blood samples were collected from different chicken farms in Gharbeia Province at different age groups (1-21-day-old, 6-week-old and up), different breeds and different purposes of breeding. The results showed that 71.9% of collected samples were positive to CIAV (antibody titers) whereas 28.1% were negative.

Seroconversion against CIAV especially non vaccinated flocks and the progenies which delivered from non vaccinated parents revealed 80.6% were positive to CIAV (antibody titers). While 47.8% of vaccinated flocks and the progenies which delivered from vaccinated parents were positive to CIAV (antibody titers). These results indicate wide spread of the infection in Gharbeia chicken farms.

All adult flocks may become subclinicaly CIAV infected and immuno-supressed due to the subclinical effect of CIAV infection in chicken flocks.

The haematological tests were in normal range and show no evidence of aplastic anemia.

INTRODUCTION

Chicken infectious anemia (CIA) is a viral disease of chickens characterized by aplastic anemia and generalized lymphoid atrophy with concomitant immunosuppression and frequently complicated by secondary viral bacterial or fungal infection ***(yassa et al., 1979)***. CIA appears to play a major role in the etiology of number of multifactorial diseases associated with hemorrhagic syndrome and aplastic anemia ***(yassa et al., 1979)***.

The disease constitutes a serious economic threat on 1-day-old till 3-week-old chicks in which it causes retarding of growth and mortality between 10-20%, occasionally it may reach up to 60%. Up till now CIA has no public health significance ***(Bulow, 1991)***.

The aim of the study was to screen poultry farms of different purposes of breeding for detection of CIAV antibodies in chicken sera in Gharbeia Province by using ELISA test.

MATERIALS AND METHODS

1. Materials:

1. Chicken sera samples of different breeds, different ages and different purposes of breeding in Gharbeia Province.
2. ELISA test system (IDEXX-USA)
3. Hemoglobin count and haematocrit value analysis system
4. Internal organs from chickens preserved in formaline 10% for histopathological examination.
5. The history of tested chickens including breeds, type of breeding, age, health status and CIAV vaccination of the parent breeders are described in Table (1).

Table (1): History of surveyed chicken flocks

No.	Breed	Type of breeding	Age	Case history	CIAV(vaccinated/non vaccinated)
1	Mixed Native	Broiler breeders	59 weeks	No healthy or productive problems during sample collection	Non vaccinated
2	Mixed Native	Progenies of group (1)	One day	Gumboro disease at 25-day-old with 8% mortality and coccidiosis with about 2% losses.	Delivered from non vaccinated parents
3	Arber-Acres	Broiler breeders	70 weeks	No healthy or productive problems during sample collection	Non vaccinated
4	Arber-Acres	Progenies of group (3)	One day	No. of the flock was 4000 with 1.6% mortality	Delivered from non vaccinated parents
5	Hubbard	Broilers	One day	Newcastle disease at age of 3 weeks in the flock vaccinated with Newcastle vaccine. Bacterial septicemia at the age of 40 days. Mortality was 10%.	Delivered from non vaccinated parents
6	Native	Yard chicken	21-70 days 50 weeks 17 weeks	No problem was recorded	Delivered from non vaccinated parents Non vaccinated Non vaccinated
7	Cobb	Broiler breeders Progenies	35 weeks One day	No healthy or productive problems during sample collection	Vaccinated Delivered from vaccinated parents
8	Brown-Bovans	Layers	75 weeks 26 weeks 6 weeks	Egg production was 73% Egg production was 91% No problem was recorded	Non vaccinated Non vaccinated Delivered from vaccinated parents
9	High Sex	Layer breeders	21 days	No problem was recorded	Delivered from vaccinated parents
10	Avian	Broiler breeders Progenies	75 weeks one day	No problem was recorded	Non vaccinated Delivered from non vaccinated parents
11	Hubbard Saso	Broilers Broilers	18 days 21 days	Mean body weight was 450g.	Delivered from non vaccinated parents

2. Methods:

2.1. Samples collection:

0.5 ml of non heparinized blood collected from wing vein of chicken or heart puncture in 1-day-old chicks.

2.2. Preparation of sera:

Collected blood samples were transferred into centrifugation at 3,000 rpm for 5 minutes. Separated sera were collected and transferred into Ependorf's tubes then kept in refregrator at -15ºC till used.

2.3. Enzyme Linked Immunosorbent Assay (ELISA):

The test was made by using ELISA plates coated with CIAV antigen for detection of antibody titers in sera samples of chickens. Anti-CIAV monoclonal antibody enzyme conjugate is added to the micro wells. In the presence of enzyme, the substrate is converted to a product which reacts with the chromophore to generate a blue colour. The absorbance at 650 nm, A (650), is measured using Spectrophotometer.

Results are calculated by dividing A (650) of sample by the A (650) of the negative control, resulting in S/N value.

The quantity of antibodies to CIAV is inversely proportional to the S/N value, and the presence of CIAV antibodies is indicator to a previous exposure to chicken anemia virus either by infection or by vaccination.

The presence or absence of antibody of CIAV is determined by S/N ratio. Samples with S/N greater than 0.6 are considered negative, while samples with S/N less than 0.6 are considered positive.

2.4 Hemtological examination:

Heparinized blood samples were collected from chickens (Hubburd and Saso) which its sera were tested by ELISA for detection of CIAV antibodies.Haemoglobin count detection and Haematocrit value detection were tested.

RESULTS

Table (2): CIAV-antibody titers of sera samples of 1-day-old chicks.

No.	Mixed native			Arbar-Acres			Hubberd Chicken		
	O.D	S/N	+/-	O.D	S/N	+/-	O.D	S/N	+/-
1	0.098	0.123	+	0.095	0.119	+	0.593	0.477	-
2	0.209	0.262	+	0.240	0.301	+	0.455	0.573	+
3	0.468	0.587	+	0.159	0.200	+	0.390	0.491	+
4	0.207	0.260	+	0.093	0.117	+	0.889	0.057	-
5	0.193	0.242	+	0.119	0.149	+	0.939	1.057	-
6	0.221	0.277	+	0.338	0.424	+	0.933	1.183	-
7	0.052	0.065	+	0.087	0.109	+	0.084	1.175	+
8	0.121	0.152	+	0.085	0.107	+	0.896	0.106	-
9	0.176	0.065	+	0.244	0.307	+	0.955	1.128	-
10	0.310	0.221	+	0.140	0.176	+	0.113	1.203	-
11	0.151	0.189	+	0.169	0212	+	0.106	1.435	+
12	0.044	0.055	+	0.162	0.203	+	0.720	0.134	-
13	0.300	0.376	+	0.150	0.188	+	0.896	0.907	-
14	0.431	0.541	+	0.194	0.150	+	0.719	1.128	-
15	0.015	0.082	+	0.117	0.147	+	0.831	0.906	-
16	0.076	0.095	+	0.129	0.162	+	0.693	1.047	-
17	0.060	0.079	+				0.138	0.873	+
18	0.084	0.105	+				0.945	0.174	-
19	0.345	0.433	+				0.182	1.190	+
20	0.198	0.319	+						
21	0.147	0.184	+						
22	0.057	0.072	+						
23	0.087	0.109	+						
24	0.214	0.269	+						
25	0.158	0.198	+						

Mean of – ve control = 0.796. Mean of + ve control = 0.314

Table (3): CIAV-antibody titers of sera samples of Cobb chickens.

No.	O.D	S/N	+/-	No.	O.D	S/N	+/-	No.	O.D	S/N	+/-
1	0.136	0.171	+	12	0.044	0.055	+	23	0.044	0.055	+
2	0.079	0.099	+	13	0.090	0.113	+	24	0.050	0.063	+
3	0.043	0.054	+	14	0.057	0.071	+	25	0.048	0.060	+
4	0.053	0.066	+	15	0.099	0.124	+	26	0.081	0.102	+
5	0.045	0.057	+	16	0.057	0.071	+	27	0.107	0.134	+
6	0.074	0.059	+	17	0.064	0.080	+	28	0.045	0.057	+
7	0.042	0.053	+	18	0.119	0.149	+	29	0.081	0.102	+
8	0.043	0.054	+	19	0.069	0.087	+	30	0.044	0.055	+
9	0.043	0.054	+	20	0.097	0.121	+	31	0.142	0.178	+
10	0.089	0.111	+	21	0.154	0.193	+	32	0.218	0.273	+
11	0.056	0.081	+	22	0.049	0.061	+	33	0.057	0.094	+

Mean of – ve control = 0.76 . Mean of + ve control = 0.312. Samples: 1-14 represent 1-day-old progenies, 15-28 represent parent females of 53-week-old and 29-33 represent parent males.

Table (4): CIAV-antibody titers of sera samples of yard native and breeder chickens.

NO	Yard native			High Sex layer breeders			Broiler breeders		
	O.D	S/N	+/-	O.D	S/N	+/-	O.D	S/N	+/-
1	0.092	0.116	+	1.434	1.371	-	0.101	0.131	+
2	0.285	0.107	+	0.962	1.210	-	0.370	0.470	+
3	0.101	0.127	+	1.122	1.411	-	0.122	0.147	+
4	0.212	0.266	+	0.726	0.913	-	0.691	0.870	-
5	0.111	0.140	+	1.112	0.998	-	0.143	0.175	+
6	0.143	0.180	+	0.775	1.399	-	0.094	0.108	+
7	0.178	0.224	+	0.887	0.975	-	0.211	0.261	+
8	0.188	0.236	+	1.012	1.116	-	0.134	0.168	+
9	0.157	0.197	+	0.718	1.273	-	0.082	0.122	+
10	0.174	0.219	+	0.696	0.903	-	0.142	0.178	+
11	0.252	0.317	+	0.731	0.875	-	0.512	0.642	-
12	0.221	0.278	+	0.946	0.920	-	0.990	0.124	+
13	0.224	0.281	+	1.040	1.190	-	0.589	0.738	-
14	0.291	0.366	+	0.898	1.308	-	0.136	0.171	+
15	0.253	0.318	+	0.964	1.130	-	0.120	0.151	+
16	0.176	0.221	+	0.723	1.212	-	0.456	0.527	+
17	0.128	0.161	+	1.037	0.909	-	0.589	0.738	-
18	0.145	0.182	+	0.715	1.304	-	0.110	0.138	+
19	0.088	0.115	+	0.752	0.899	-			
20	0.224	0.281	+	0.674	1.186	-			
21	0.197	0.247	+	0.752	0.946	-			
22	0.295	0.370	+	0.674	0.848	-			
23	0.124	0.156	+	0.672	0.797	-			
24	0.271	0.341	+	0.602	0.757	-			
25				0.766	0.963	-			
26				0.793	0.998	-			

Mean of – ve control = 0.797. Mean of + ve control = 0.312

Table (5): CIAV-antibody titers of sera samples of Bovans layer chickens.

NO	H1			H2			H3		
	O.D	S/N	+/-	O.D	S/N	+/-	O.D	S/N	+/-
1	0.385	0.483	+	0.593	0.743	-	0.716	0.897	-
2	0.314	0.393	+	0.846	1.060	-	0.761	0.954	-
3	0.684	0.857	-	0.651	0.816	-	0.873	1.094	-
4	0.607	0.761	-	0.788	0.987	-	0.725	0.909	-
5	0.237	0.297	+	0.153	0.192	-	0.808	1.013	-
6	0.432	0.541	+	0.673	0.844	-	0.732	0.917	-
7	0.117	0.147	+	0.261	0.327	-	0.652	0.817	-
8	0.207	0.260	+	0.642	0.805	-	0.646	0.809	-
9	0.151	0.189	+	0.729	0.914	-	0.846	1.060	-
10	0.198	0.248	+	0.946	1.186	-	0.863	1.081	-

Mean of – ve control = 0.789 . Mean of + ve control = 0.314

H1 represent chickens in late period of production (75-week-old), CIAV non vaccinated. H2 represent chickens in late period of production (26-week-old), CIAV non vaccinated. H3 represent chickens in Breeding period (6-week-old).

Table (6): CIAV-antibody titers of sera samples of Mixed Native, Arbar-Acres broiler breeders and Saso broiler chickens

No	Mixed native					Arbar-Acres					Saso broiler				
	O.D	S/N	+/-	No	O.D	S/N	+/-	No	O.D	S/N	+/-	No	O.D	S/N	+/-
1	0.356	0.448	+	21	0.248	0.312	+	1	0.082	0.103	+	1	0.498	0.626	-
2	0.162	0.204	+	22	0.234	0.294	+	2	0.108	0.136	+	2	0.581	0.730	-
3	0.096	0.121	+	23	0.162	0.203	+	3	0.118	0.148	+	3	0.581	0.730	-
4	0.268	0.337	+	24	0.127	0.159	+	4	0.108	0.136	+	4	0.126	0.159	+
5	0.293	0.368	+	25	0.181	0.228	+	5	0.102	0.128	+	5	0.502	0.631	-
6	0.321	0.403	+	26	0.048	0.217	+	6	0.165	0.208	+	6	0.566	0.823	-
7	0.116	0.146	+	27	0.173	0.186	+	7	0.141	0.177	+	7	0.742	0.933	-
8	0.254	0.319	+	28	0.158	0.198	+	8	0.104	0.131	+	8	0.495	0.622	-
9	0.409	0.514	+	29	0.197	0.247	+	9	0.124	0.156	+	9	0.148	0.186	+
10	0.304	0.382	+	30	0.240	0.301	+	10	0.102	0.128	+	10	0.726	0.913	-
11	0.260	0.327	+					11	0.137	0.172	+	11	0.398	0.501	+
12	0.238	0.299	+					12	0.087	0.109	+	12	0.737	0.801	-
13	0.280	0.352	+					13	0.114	0.144	+	13	0.573	0.720	-
14	0.286	0.360	+					14	0.093	0.117	+	14	0.431	0.501	+
15	0.111	0.140	+					15	0.104	0.131	+	15	0.315	0.396	+
16	0.213	0.267	+					16	0.123	0.155	+	16	0.133	0.168	+
17	0.170	0.214	+					17	0.111	0. 140	+	17	0.287	0.361	+
18	0.309	0.388	+					18	0.089	0.142	+	18	0.375	0.472	+
19	0.323	0.406	+					19	0.081	0.102	+	19	0.285	0.358	+
20	0.295	0.370	+									20	0.405	0.509	+

Mean of – ve control = 0.797 . Mean of + ve control = 0.314

Table (7): Summary of ELISA test results.

No	Breed	CIAV vaccination						Total	+ve		-ve	
		Vaccinated			Non vaccinated							
		No	+ve	+ve%	No	+ve	+ve%		No	%	No	%
1	Native	0	0		79	79		79	79	100	0	0
2	Broilers	14	14		0	0		14	14	100	0	0
3	Broilers	0	0		65	39		65	39	60	26	40
4	Broiler breeders	19	19		0	0		19	19	100	0	0
5	Broiler breeders	0	0		27	26		27	26	96	1	4
6	Layer breeders	26	0		0	0		26	0	0	26	100
7	Layers	0	0		20	10		20	10	50	10	50
8	Layers	10	0		0	0		10	0	0	10	100
Total		**69**	**33**	**47.8**	**191**	**154**	**80.6**	**260**	**187**	**71.9**	**73**	**28.1**

DISCUSSION

In this study, the serological survey for antibodies in chicken farms in Gharbeia Provence cleared that 71.9% of collected samples were positive to CIAV (antibody titers) and 28.1% were negative. Similar results were obtained by ***Zaki and El-Sunousi (1994)*** who reported that 70% of sera samples collected from chickens were CIAV positive.

The study cleared that presence of seroconversion against CIAV especially in non vaccinated flocks and their progenies which delivered from non vaccinated parents as shown in Tables 2, 3, 4, 5 and 6 in which 80.6%were positive CIAV antibody titers, that indicate wide spreading of the virus in chicken farms. The prevalence of CIAV antibodies in chicken serum largely depended on the age of the flock which was higher in the older pullet and layer flocks than the younger broiler flocks. The results indicated that all flocks older than 6-8 weeks become infected. Similar findings reported by ***Owaad et al. (2004)***.

The seroconversion against CIAV in vaccinated flocks and progenies delivered from vaccinated parents were presented in Tables 3, 4 and 5 in which 47.8% were positive to CIAV (antibody titers). Although, progenies delivered from vaccinated parents showed negative antibody titers may be due to depletion of maternal antibodies which of 10 days half life as reported by ***Pages et al. (1997).***

Chicks from parents of positive CIAV antibodies (either vaccinated or non vaccinated) showed positive CIAV antibody titers (Tables 2, 3 and 4). These results were confirmed by the results of ***Yassa et al. (1983)*** and ***Goryo et al. (1987)*** who proved the congenital transfer of CIAV antibodies from naturally infected or vaccinated parents to progenies.

The survey cleared that all adult flocks may become subclinically CIAV infected because of the presence of seroconversion against CIAV in most of non vaccinated flocks as shown in Tables 4, 5 and 6. Similar results obtained by ***McNulty et al. (1991)*** who cleared that all chicken flocks older than 6-8 weeks of age were CIAV infected without typical clinical signs. The seroconversion with high titers against CIAV in non vaccinated parent flocks which delivered maternal antibodies with high titers to their progenies as shown in Tables 2, 4 and 6 may be due to repeated CIAV natural infections of these parents which lead to boosting of CIAV antibody level in their sera as reported by ***McNulty et al. (1991)***.

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دراسات سيرولوجية على عدوى فيروس انيميا الدجاج بمنطقة وسط الدلتا

د./ مشيرة عباس محمد العباسى [*] ، ***د./ جمال رجب حسب النبى*** [**]

ط.ب/ أحمد صالح حجازى [**] ، ***ا.د/ سلوى محمود حلمى*** [*]

[*] **قسم امراض الدواجن ورئيس قسم الميكروبيولوجى – كلية الطب البيطرى – جامعة كفر الشيخ0**

[**] **معهد بحوث صحة الحيوان – طنطا**

استهدفت الدراسة عمل مسح سيرولوجى للبحث عن الاجسام المضادة لفيروس انيميا الدجاج المعدى فى 260 عينة سيرم مجمعة من مزارع مختلفة الأعمار(1–21 يوم و 6 أسابيع وأكبر) ومختلف السلالات(بلدى واجنبى) ومختلف أغراض التربية (بدارى وأمهات بياض) فى محافظة الغربية باستخدام اختبار الاليزا.

وقد أسفرت نتائج هذا المسح عن 71.9 % من العينات إيجابية لفيروس أنيميا الدجاج المعدي ، 28.1 % منه سلبية لهذا الفيروس. ولم يكن هناك أي أعراض يشتبه في أنها تخص مرض أنيميا الدجاج الفيروسي المعدي خلال تفقد مزارع الدواجن .

كما اتضح من هذا المسح مدى انتشار العدوى بفيروس انيميا الدجاج والذى يظهر فى إيجابية عينات السيرم بالأجسام المضادة للفيروس خاصة فى القطعان التى لم يتم تحصينها ضد هذا الفيروس او القطعان التى نتجت من أمهات غير محصنة حيث كانت نسبة إيجابية العينات بالأجسام المضادة للفيروس 80.6% بينما بلغت نسبة إيجابية عينات السيرم 47.8% فى القطعان المحصنة ضد هذا الفيروس أو القطعان الناتجة من أمهات محصنة ويمكن أن يعزى ذلك الى نفاذ الأجسام المضادة المتوارثة.

أوضحت الدراسة أن انتقال الأجسام المضادة للفيروس من الأمهات الإيجابية إلى الكتاكيت الناتجة يمكن ان تحميها من أخطار هذا المرض خلال الفترة الحرجة من العمر(1–20 يوم)0 كما يمكن لقطعان الدجاج كبيرة العمر أن تصاب بفيروس أنيميا الدجاج ولكن دون ظهور أية أعراض.