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BIOCHEMICAL AND HAEMATOLOGICAL CHARACTERIZATION OF CHICKEN ANAEMIA VIRUS (CAV)

(With 3 Tables and 3 Figures)

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التوصيف الدموى والبيوكيميائى لفيروس أنيميا الدواجن

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أجريت هذه الدراسة كمحاولة لإيجاد بعض الإختبارات الدموية والبيوكيميائية المعملية التى تجرى على دم ومصل الكتاكيت والتى يمكن الإستعانة بها فى تشخيص الإصابة بالفيروس المسبب للأنيميا فى الدجاج CAV. وقد أجريت هذه الدراسة على تسعون كتكوتا من الكتاكيت الصغيرة والتى تبلغ يوم واحد من العمر والتى تتبع السلالة البلدية المصرية . تم تقسيم هذه الكتاكيت إلى ثلاثة مجموعات متساوية تحتوى كل منها على عدد ثلاثين كتكوتا والتى تم ذبحها بعد أسبوع وأسبوعين وثلاثة بالنسبة للمجموعة الأولى والثانية والثالثة على التوالى. أعيد تقسيم الكتاكيت فى كل مجموعة إلى ثلاثة أقسام صغيرة (تحت مجموعة) تحتوى كل منها على عشر كتاكيت وهى تحت المجموعة الضابطة وتحت المجموعة التى حقنت بالفيروس المرجع (الأجنبى) والثالثة والتى تم حقنها بالفيروس الحقلى (المنتشر فى البيئة المصرية). وقد تبين من تحليل النتائج إحصائيا حدوث نقص معنوى فى عدد كرات الدم الحمراء وهيموجلوبين الدم (فى المجموعتين الثانية والثالثة) ونسبة الهيماتوكريت (فى المجموعات الثلاثة). وكذلك نسبة الحديد فى مصل المجموعات الثلاث ونسبة كل من البروتينات الكلية والألبيومين (فى المجموعة الثالثة فقط) بالإضافة إلى نسبة الجلوبيولين بعد الأسبوعين الأول والثانى من بداية الحقن. كما لوحظ نقص فى وزن بعض الأجهزة المناعية مثل Thymus & Bursa fabricious وذلك عند مقارنتها بوزن الجسم مما يوحى بالتأثير المثبط للفيروس على الجهاز المناعى. كما أظهر تحليل مصل الدم حدوث زيادة فى نسبة اليوريا بعد أسبوعين وثلاثة أسابيع كما حدثت زيادة أيضا فى نسبة الكرياتينين والألكالين فوسفاتيز من بداية الأسبوع الأول حتى نهاية الأسبوع الثالث فى كل من تحت المجموعتين المصابتين. حدثت زيادة فى نسبة البيلوروبين الكلى والغير مباشر فى كتاكيت تحت المجموعة المصابة بالفيروس الحقلى فى المجموعة الأولى بينما حدثت زيادة فى الكتاكيت المصابة بكلتا النوعين من الفيروسات فى المجموعات الثانية والثالثة. فى نفس الوقت لم يحدث

تغيير فى نسبة الفسفور الغير عضوى والفوسفوليبيدات وكذلك البيلوروبين المباشر. من هذه الدراسة يتضح أن صورة الدم والتى ظهرت فى شكل نقص فى عدد كرات الدم الحمراء ونسبة الهيموجلوبين ونقص فى نسبة الهيماتوكريت تحت ٢٥ وكذلك زيادة إنزيمات كفاءة الكبد يمكن الإستعانة بها فى كشف الإصابة بمرض CAV بالإضافة إلى أعراض المرض والصورة التشريحية والإختبارات الفيروسية والمناعية الأخرى.

SUMMARY

In chickens, CAV appears to replicate primarily in haemopoietic precursor cells in the bone marrow and in thymic precursor cells in the thymus cortex where it has been shown to cause cytolytic infection. This work was carried out on ninety healthy one-day-old chicks of Egyptian breed. These chicks were classified into three equal groups (I , II , and III) Sacrificed after one, two and three weeks respectively. Each group was subdivided into three equal subgroups, representing the control, reference and field subgroups, inoculated intraperitoneally with saline, reference strain virus and field strain virus, respectively. The obtained results showed significant decrease in erythrocytic counts, haemoglobin concentration (in groups II and III) and PCV (in all groups) in both reference and field subgroups. It was noticed that there was a decrease in weight of some lymphatic organs like thymus and bursa fabricious in relation to body weight, and this means that the virus has immunosupressive effect. The biochemical tests on serum of both field and reference strain exhibited significant decrease, in serum iron (in all groups), serum total proteins and albumin (in group III only) and in serum globulins in both first and second groups. On the opposite side, both reference and field subgroups revealed significant increase in serum urea (in groups II and III), serum creatinine and serum alkaline phosphatase in all groups and serum alanine aminotransferase (ALT) in group III only. Furthermore, serum total and indirect bilirubin showed significant increase only in field subgroup of group I and in both reference and field subgroups of group II and III . Meanwhile , serum inorganic phosphorus, phospholipids and direct bilirubin revealed non sgnificant changes. From this study, It was concluded that the blood picture which appeared as a decrease in erythrocytic count & Hb concentration & decrease in haematocrit below 25 and the increase in liver function enzymes can be used as laboratory tests in CAV diagnosis in addition to symptoms of disease & histopathological examination and both virological and immunological tests.

Key words: *Chicken Anaemia Virus - Characterization*

INTRODUCTION

Chicken anaemia virus (CAV) was first isolated by Yuasa *et al.* (1979). The size of CAVwas estimated by electron microscope to be 2.17±0.043 (S.E) as recorded by Todd et al. (1990).

Economic losses due to chicken anaemia virus (CAV) stem from increased mortality, the cost of antibiotics used to control secondary bacterial infections and poor growth (Mc Nulty et al., 1991).

CAV produced anaemia or a plastic bone marrow in majority of intramuscularly inoculated one-day-old chicks. Moreover, the erythropioetic function of bone marrow is under the control of T-lymphocytes, so that the depletion of T-lymphocytes would cause a reduce proliferation of erythroblasts (Taniguchi et al., 1983 and Lucio et al., 1989). Furthermore, Mc Nulty et al (1991) stated that the haematological changes were first observed at about 8 days after inoculation in the form of decreases in haematocrit levels and erythrocytic count.

Regarding the effect of CAV on haematocrit Rosenberger and Cloud (1989) found that the mortality rate and number of birds with haematocrit 25 or less were consistently higher in susceptible group. In the same respect, Chettle et al. (1989) recorded that the haematocrit values of the affected birds were below normal . PCV of inoculated birds ranged from 4 % - 26 % and to be 30 % - 38 % in healthy birds (Smyth et al., 1993).

The lesions of CAV infection consisted of a plastic femoral bone marrow, diffuse subcutaneous and intramuscular haemorrhages, pale and swollen kidneys and liver and on occasion ecchymotic haemorrhage on the outer surface of the heart , typically bursae of Fibricious and spleen remained essentially normal in size and appearance (Rosenberger and Cloud, 1989).

Clinical biochemistry represents an important tool in the diagnosis and study of liver diseases. Activities of some enzymes in the plasma may be raised in response to acute liver injury by leaking from the liver into the circulation.

As a trial to find some laboratory tests which may help in diagnosis of CCA, The present work was planned to study the haematological and

biochemical pictures of blood and serum in chicks infected with field and reference strains of CAV.

MATERIALS and METHODS

Birds: Ninety healthy one-day-old chicks of Egyptian balady breed, fed starter meal and reared on wire net floor. These chicks were vaccinated against Newcastle disease by intraoccular route at 7th day of age simultaneously with inactivated oil emulsion Newcastle vaccine (Ivaz , Italy) at the same time by s\c injection in neck region .

These chicks were divided into three equal groups (of thirty birds in each group) resemble Group I, II and III. Each group was subdivided into three equal subgroups (of ten birds in each subgroup) resemble control, reference and field subgroups. The chicks of group I , II and III were Sacrificed after one, two and three weeks respectively.

Virus inoculation: (a) The reference strain subgroup: inoculated with CAV of Gifu-I strain, obtained from Dr. Yuasa (National institute of animal health, Japan) that was propagated on MDCC-MSB_1, cell line originated from Marek's disease T-cell spleenic lymphoma as described by McNulty et al. (1990). (b) The field stain subgroup: Inoculated with field virulent strain isolated from flocks showed characteristic symptoms of CAV which were mainly a plastic anaemia (pale a plastic bone marrow) & haematocrit value less than 25 in addition to clinical symptoms which were mainly Anorexia, pale comb and wattles, ruffled feathers , in addition to focal skin lesions specially on wings but may also be present on head around the rump, on the sides of thorax and abdomen, on the thighs, legs and feet. These lesions appear to be due to eccymotic skin haemorrhages. The skin turns blue and breaks, releasing serosenguinous exudate these lesions are prone to secondary bacterial infection leading to gangrenous dermatitis.

One-day-old chicks were inoculated intraperitoneally with 0.1ml of CAV suspension containing 10^5 chicken infective dose (CID_{50}). The reference and field strains were propagated in MDCC-MSB_1, cells as described by McNulty et al. (1990) .

Immune reactions:

The direct and indirect immunoflourescence antibody technique (IFA) as described by Bulow et al. (1985), McNeilly et al. (1991) was used. In this test seven field strains were used and only one strain which produced

positive immunoflourescence reaction was selected for studying the biochemical effect.

Sampling and biochemical analysis: All chicks were Sacrificed at the end of one, two and three weeks for group I, II and III respectively for collection of (a) whole blood: in heparinized tubes and used for counting of erythrocytes according to Miller and Seward (1971), haemoglobin (Wintrobe, 1965) and PCV determination (Schalm et al., 1975).
(b) serum samples: the other part of the blood was incubated for 2 hours at 37°C, centrifuged at 3000 r.p.m. for separation of clear, nonhaemolyzed serum that was collected in dry tubes and used for determination of serum iron as Garcic (1979), serum inorganic phosphorus (Kuttner and Lichtenstein, 1942), serum phospholipids (Zilversmit and Davis, 1956), serum urea (Patton and Crouch, 1977), serum total proteins (Peter, 1968), serum albumin (Doumas and Watson, 1971), serum creatinine (Henery, 1974), Serum alkaline phosphatase (Jhon, 1982), serum ALT (King, 1965) and serum bilirubin (Jendrassik, 1938) using spectrophotometer model Shimadzu, Kyoto, Japan, cat. no. 204-000 10-02, serial no. 2344442. (c) Tissue samples : Bursa, liver and thymus were fixed with 10 % formalin, paraffin sectioned, stained with H & E and examined microscopically.

The obtained data were statistically analyzed using student "t" test (Snedecor and Cochran, 1967).

RESULTS

Table (1) shows haematological changes in blood of birds infected with CAV. It was found that birds infected with reference strain exhibited highly significant decrease in RBCs counts ($P<0.01$) after 2 and 3 weeks. Meanwhile, birds infected with field strain showed highly significant decrease after two weeks ($P<0.01$) and significant decrease after three weeks from the beginning of infection ($P<0.05$). Although, the haemoglobin concentration showed highly significant decrease ($P<0.01$) after two and three weeks in field strain subgroup, only significant decrease was recorded in haemoglobin of birds infected with reference strain ($P<0.05$) after two and three weeks. The concentration of PCV (%) was highly significantly decreased from the beginning of experimental infection with reference strain ($P<0.01$). In birds infected with field strain revealed highly significant decrease ($P<0.01$) after one week and only significant decrease recorded after second and third weeks from the beginning of infection ($P<0.05$) in PCV%.

produces pathological changes in intestinal mucosa which is necessary for absorption of ferrous ions and storage as ferritin.

Total protein exhibited significant decrease after three weeks from the beginning of infection. This drop in protein level was due to reduction in albumin level due to the effect of virus on liver the main site of albumin biosynthesis as recorded from histopathological examination (Fig. 1). The significant decrease in globulin was due to immunosupression produced by CAV that produced immune system damage (Yausa et al., 1980), this was clear from histopathological examination (Fig. 2 & 3). The level of the globulins returned to normal in third week which was in accordance with Yausa et al. (1985) who noticed that neutralizing antibody was not detected 14 days after inoculation but was present at 21 days after inoculation . The elevation in urea and creatinine levels were due to the effect of virus on kidneys (Taniguchi et al., 1983).

The chicken anaemia virus produced significant elevation in alkaline phosphatase, ALT enzymes and bilirubin as a result of effect of virus on hepatic tissues, in the form of small randomly scattered lymphoid aggregates and multifocal necrosis may be present (Smyth et al., 1993) which was confirmed by histopathological examination (Fig. 1). In the same respect, Ahmed et al. (1975) reported that in clinical cases of duck hepatitis virus type I there were lower serum levels of total proteins and albumin and elevated levels of alkaline phosphatase, alanine aminotransferase (ALT), bilirubin and creatinine. Moreover, serum ALT activities remained a test of choice to measure necrosis in small animals and primates and should be part of any liver profile (Kaneko et al., 1989). Furthermore, serum alkaline phosphatase continues to be useful marker for cholestasis in dog, however, modest increase also occur in hepatic necrosis.

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Table 1: Haematological changes in blood of birds infected with CAV .

Haematological Parameters	Group I (First week post inoculation)			Group II (Two weeks post inoculation)			Group III (Three weeks post inoculation)		
	Control group	Reference strain group	Field strain group	Control group	Reference strain group	Field strain group	Control group	Reference strain group	Field strain group
RBCs count (10^6/µL)	2.7±0.1	2.8±0.2	2.12±0.3	2.8±0.1	1.4±0.1**	1.1±0.5**	3.1±0.1	1.1±0.3**	1.5±0.7*
Haemoglobin (gm/dL)	8.6±1.2	8.4±1.1	7.9±1.0	8.3±0.3	5.8±0.9*	4.3±0.3**	8.4±0.8	5.5±0.9*	5.7±0.3**
PCV (%)	29.3±0.6	17.6±0.8**	20.7±0.5**	27.4±1.0	20.0±0.5**	20.3±2.6*	30.0±1.8	20.0±2.4**	22.0±2.9*
MCHC %	29.4±3.3	50.3±2.3**	38.2±3.4	30.3±2.5	29.1±3.6	21.2±2.8*	28.1±1.4	42.5±2.5**	25.9±1.2

2: The effect of CAV on Spleen/body weight ratio (Sp./B.wt. ratio), Bursa/body weight ratio (B./B.wt. ratio) and Thymus/body weight ratio (Th./B.wt. ratio).

Organ index	Group I (One week post inoculation)			Group II (Two weeks post inoculation)			Group III (Three weeks post inoculation)		
	Control	Reference	Field	Control	Reference	Field	Control	Reference	Field
Sp./B.wt. ratio	0.068±0.009	0.059±0.008	0.037±0.03	0.092±0.007	0.102±0.008	0.119±0.006**	0.75±0.006	0.181±0.005**	0.118±0.008**
B./B.wt. ratio	0.211±0.01	0.122±0.06	0.124±0.08	0.334±0.01	0.222±0.02**	0.242±0.02**	0.347±0.09	0.307±0.01	0.304±0.08
Th./B.wt. ratio	0.331±0.01	0.230±0.08	0.106±0.1*	0.414±0.09	0.379±0.03	0.311±0.05	0.681±0.02	0.444±0.08**	0.414±0.02**

* Significant at P<0.05 .

** Significant at P<0.01 .

Table (3) : Biochemical changes in serum of birds infected with CAV .

Biochemical parameters	Group I (One week post inoculation)			Group II (Two weeks post inoculation)			Group III (Three weeks post inoculation)		
	Control	Reference	Field	Control	Reference	Field	Control	Reference	Field
Iron (U/g/dL)	96.1±3.1	75±2.2**	80.2±5.3*	95.6±6 4	40.5±4.3**	50.7±2.2**	112.4±5.8	58.6±8.6**	47.7±4.8**
Inorganic phosphorus (mg/dL)	6.7±1.2	8.9±2 5	6.9±1.5	6.2±0.5	5.1±0.6	5.0±0.4	5.6±0.6	4.8±0.5	5.3±0.5
Phospholipids (mg/dL)	126.1±5.3	136.2±7.3	154.1±17.2	160.0±9 4	174.5±7.1	203.0±19 2	137±10.3	160.8±9 8	158.6±15.9
Total proteins (gm/L)	4.9±0.2	48±1.0	49±0.2	45±0.4	47±0.2	41±0.2	48±0.1	35±0.5*	29±0.1**
Albumin (gm/L)	3.2±0.1	40±0.2	42±0.2	3.3±0.1	36±0.3	33±0.3	31-0.2	20±0.1**	19±0.5**
Globulins (gm/L)	17±0.2	08±0.2**	07±0.1**	18±0.2	11±0.1**	08±0.3*	17±0.4	15±0.1	10±0.1
A/G ratio	1.8	5.0	2..5	2.8	3.0	1..3	1.8	0.67	1..9
Urea (mg/dL)	24±1.7	21.3±1.2	17.3±3.8	28.4±3.6	69.8±11.1**	62.5±3.3**	25.1±7.8	58.1±3.6**	71.8±8.0**
Creatinine (mg/dL)	1.5±0.4	2.9±0.4*	10.8±0.8**	1.3±0.1	4.9±0.7**	4.2±0.4**	1.2±0.3	4.8±0.2**	4.7±0.5**
Alkaline phosphatase (U/L)	109.2±8	160.8±7.2**	281.2±11.2**	105.1±9.1	220.8±4.6**	139.2±9.9*	107.6±9.7	148.6±6.4**	247.6±5.9**
ALT (U/L)	45.2±3.2	42.1±5.1	45.2±1.6	40.3±7.1	40.6±3.9	32.3±3.1	43.2±3.2	52.4±2.1*	60.7±1.9**
Total bilirubin (mg/dL)	1.2±0.1	1.8±0.3	4.8±0 2**	1.4±0.3	3.1±0.2**	3.4±0.1**	1.8±0.6	3.5±0.1*	3.8±0.6*
Direct bilirubin (mg/dL)	0.5±0.1	0.4±0.01	0.5±0.1	0.6±0.2	0.3±0.02	0.6±0.01	0.8±0.2	0.4±0.1	0.4±0.01
Indirect bilirubin (mg/dL)	0.7±0.2	1.4±0.3	4.3±0.6**	0.8±0.1	2.8±0.2**	2.8±0.9*	1.0±0.1	3.1±0.4**	2.4±0.3**

* Significant at P<0.05 .
**Significant at P<0.01 .
Data are represented as mean ± S.E.

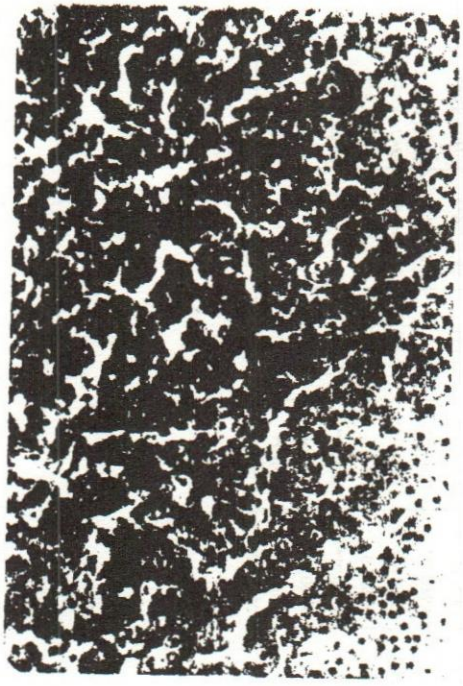

Fig.(1) Liver of chiken infected by field strain of CAV, showing focal necrosis of hepatocytes (H & E stain X 250).

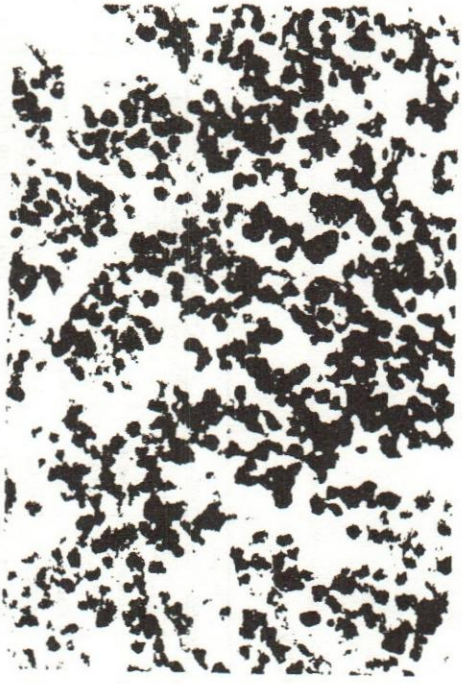

Fig.(2) Thymus of chiken infected by field strain of CAV, showing edema, lymphoid depletion and intranuclear inclusion bodies.(H & E stain X 250).

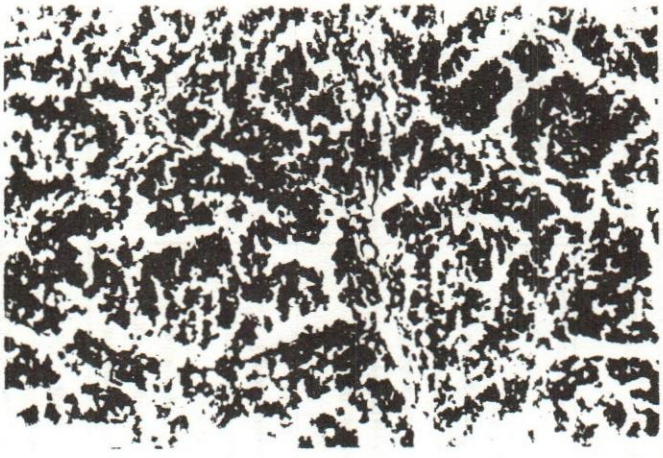

Fig.(3) Bursa of Fabricious of chiken infected by field strain of CAV, showing follicular edema and depletion. (H & E stain X 100)