Research Article

Clinical and Hemato-biochemical Studies on Canine Anemia

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Abstract | The study was performed to assess the common etiopathologies, clinical and haematobiochemical alterations in anemic dogs that were admitted to the teaching veterinary hospital at faculty of veterinary medicine, Cairo University and private clinics in Cairo and Giza governorates, Egypt. A total number (71 dogs) was included in the present study. This number comprises 16 apparently healthy dogs and 55 anemic cases. Historical data, clinical and hematobiochemical investigations were applied on all animals for detection of the definitive common causes of anemia. Results revealed four main definitive causes of anemia included parvoviral infection (sub-group 1): 20 cases (36% of diseased cases), ectoparasitic infestation (sub-group 2): 18 cases (32%), malnutrition (sub-group 3): 7 cases (12%) and hepatic or renal diseases (sub-group 4): 10 cases (18%). The most recorded clinical manifestations in diseased dogs were pale mucous membranes, tachycardia, tachypnea and low performance. Haematological parameters included haemoglobin (Hb), total erythrocytic count (TEC) and haematocrit (HCT) revealed significant (P≤0.001) decrease in all diseased sub-groups compared with apparently healthy group. Serum biochemical parameters showed significant increase in alanine amino transferase (ALT) level in sub-groups 1and 4 (P≤0.001), sub-group 2 (P≤0.01), and sub-group 3 (P≤0.05). Aspartate amino transferase (AST) level showed significant increase in sub-group 4 (P≤0.001), sub-group 1 (P≤0.01) and sub-group 3 (P≤0.05). Significant decrease among glucose level was recorded in sub-group 1 (P≤0.001) and sub-groups 3 and 4 (P≤0.01). Iron level showed significant decrease in sub-group 1(P≤0.001), sub-group 3 (P≤0.01) and sub-group 4 (P≤0.05). Copper level decreased significantly in sub-groups 1 and 3 (P≤0.01). Total protein decreased significantly in sub-groups 1and 3(P≤0.001), sub-group 4 (P≤0.01) and sub-group 2 (P≤0.05). All results were in comparison with apparently healthy group records. Etiopathological identification of canine anemia in the light of hematobiochemical status is helpful for further investigations and therapeutic protocol decisions.

Keywords | Anemia, Dogs, Etiopathologies, Hematology, Biochemistry.

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INTRODUCTION

Anemia is considered as a one of the most important hidden affection among dogs. Its clinical manifestations drive the owner to visit a clinician to show what is behind and then manage. Clinical manifestations depend on its severity and duration and included pallor mucous membranes, tachypnea, weakness and decreased stamina. Other accompanied clinical signs included icterus and haemoglobinuria in other cases. Many anemic cases considered

subclinical and can't be recognized unless a complete blood picture was performed. Classification of anemia as regenerative or non-regenerative is helpful in determination of prognosis and monitoring of treatment response. Blood loss anemia is considered as regenerative type although no elevation in reticulocytes was recorded for the first two days during which it was named as pre-regenerative anemia. Anemia may be primary as seen in case of blood loss due to external or internal bleeding. It was characteristically moderate to severe regenerative anaemia. Secondary anaemia occurred mainly due to underlying inflammatory condition as in case of anaemia of inflammatory disease (AID) or chronic disease (ACD). It was characteristically mild to moderate non-regenerative type and was associated with chronic inflammatory, neoplastic and immune-mediated diseases (Waner and Harrus, 2001) and (Fry, 2010). The most recorded causes of anemia in dogs were blood sucking ectoparasites such as heavy infestation with fleas and ticks, endo parasites as hookworms, tumours, liver and kidney diseases and malnutrition (Dhuria et al., 2013).

Aim Of Work

The main aim of the present work is to identify the main causes of anemia in the light of confirmatory hematobiochemical picture among dogs that admitted to faculty of veterinary medicine hospital, Cairo University and other clinics in Cairo and Giza governorates from June 2019 to June 2021.

MATERIALS AND METHODS

Animals

The animals in this study from different canine breeds and age that included German Shephard, Pit bull, Husky, Golden retriever, Great Dane, Griffon and Rottweiler. In the present work 71 dogs (included 49 males and 22 females) were examined with ages ranged from 2 months to 10 years. Dogs were admitted to small animal clinic, faculty of veterinary medicine, Cairo University and small animal private clinics in Cairo and Giza governorates, Egypt from June 2019 to June 2021. These animals were divided into two groups: control group included 16 apparently healthy dogs (admitted for vaccination) and diseased group included 55 dogs suffered mainly from generalized pallor, pale mucus membrane and general weakness. Diseased group was sub-divided according to etiopathogencity into four subgroups included [sub-group 1: parvoviral infection (20 cases), sub-group 2: ectoparasitic infestation (18 cases), sub-group 3: malnutrition (7 cases) and sub-group 4: hepatic and renal disease (10 cases)].

Clinical Examination

In the present study, case historical data and general clinical examination were applied for all body regions and systems of all animals. Special clinical examination was applied in the light of the main owner's complaint of each case.

Blood And Serum Samples

From each admitted dog, about 4-6 ml blood sample was collected from cephalic or jugular vein and divided in to two parts. First part, whole blood sample (2 ml blood) was collected in vacum tube containing sodium ethylene diamine tetra acetate (EDTA) as an anticoagulant for haematological estimations of total erythrocyte count (TEC), haemoglobin (Hb) and packed cell volume PCV. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated through standard laboratory procedures. Second part of blood sample (4 ml) was collected in plain vacum tube without EDTA and centrifuged for separation of serum. The obtained serum was kept in deep freezer till the time (not more than three days) of parameters estimation included liver enzymes (ALT and AST), creatinine, glucose, iron, copper and total proteins according to specific kits instructions supported by spectrum® diagnostics, Egypt.

Fecal Samples

Stool samples were collected from each animal and examined macroscopically and microscopically for the presence of adult worms, parasitic ova and protozoal oocysts. Parvoviral rapid detection test was applied on fecal samples of suspected dogs with infection using immune-chromatographic assay (VDRG®- canine corona/canine parvovirus -MEDIAN Diagnostic Inc.).

Ultrasonographic Examination

Ultrasonographic examination was applied on cases suspected with hepatic and/or renal diseases using ultrasonographic device (Pie medical® scanner, Maastricht, Netherlands with macro-convex and micro convex transducers 3.5-5 and 5-7.5 MHz).

Statistical Analysis

All the records were added to Microsoft excel program and results were illustrated as mean ± Standard Error. Diseased dog's results were compared with control group data using student T- Test using SPSS program. P value less than or equals 0.05 were considered significant.

Ethics Statement

All research procedures were approved by the institutional animal care and use committee, faculty of veterinary medicine, Cairo University, Egypt with Certificate number (Vet cu12/10/2021/338).

Table 1: Hematological parameters in apparently healthy dogs compared with cases of anemia caused by common etiologies in Egypt.

Etiology		Control group Apparently healthy	Diseased dogs group			
			Sub-group 1	Sub-group 2	Sub-group 3	Sub-group 4
			Parvoviral infection	Ectoparasitic infestation	Mal-nutrition	Liver and kidney disease
Hb (g/L)		167 ± 3.4	112.2 ± 3.8***	116.3 ± 3.3***	85.7 ± 14.1***	100.6 ± 10.5***
TEC ($\times10^{12}$/L)		7.36 ± 0.16	5.12 ± 0.16***	4.93 ± 0.13***	3.78 ± 0.49***	4.63 ± 0.53***
HCT (Proportion of 1.0)		0.48 ± 0.01	0.34 ± 0.01***	0.33 ± 0.01***	0.28 ± 0.03***	0.30 ± 0.04***
MCV (fL)		65.4 ± 0.69	66.31 ± 0.95	67.10 ± 1.22	73.35 ± 0.96***	64.98 ± 2.98
MCH (pg/cell)		22.7 ± 0.20	21.92 ± 0.32*	23.59 ± 0.34*	22.27 ± 0.9	21.79 ± 0.69
MCHC (g/L)		345 ± 2.6	331.1 ± 4.3**	349.8 ± 5.0	304.2 ± 13.8***	337.2 ± 12.8
RDW (%)		13.6 ± 0.61	15.97 ± 0.24***	16.97 ± 0.26***	13.25 ± 0.14	13.54 ± 0.63
Platelets Count ($\times10^9$/L)		268.38 ± 23.88	232.75 ± 12.68	235.78 ± 23.76	263.5 ± 62	131.6 ± 36.69**
WBCs Count ($\times10^9$/L)		12.02 ± 0.51	6.068 ± 0.56***	14.67 ± 1.23	20.62 ± 4.32***	11.4 ± 3.46
Neutrophils	Staff (%)	0.27 ± 0.02	0.36 ± 0.05	0.40 ± 0.04**	0.41 ± 0.08*	0.45 ± 0.14*
	Segmented (%)	6.83 ± 0.33	3.21 ± 0.42***	8.81 ± 0.85*	9.79 ± 3.61	6.776 ± 2.09
Lymphocytes (%)		3.45 ± 0.32	1.65 ± 0.19 ***	3.57 ± 0.84	6.31 ± 1.23**	2.01 ± 0.78
Eosinophils (%)		0.55 ± 0.07	0.24 ± 0.03***	1.30 ± 0.11***	1.11 ± 0.48*	0.35 ± 0.08
Monocytes (%)		0.92 ± 0.10	0.57 ± 0.05**	0.69 ± 0.12	2.99 ± 0.27***	1.78 ± 0.79
Basophils (%)		0.00 ± 0.00	0.00	0.01 ± 0.00	0.00	0.01 ± 0.01

P-value: *** P ≤0.001, ** P ≤0.01, * P ≤0.05

Table 2: Serum biochemical constituents in apparently healthy dogs compared with cases of anemia caused by common etiologies in Egypt.

Etiology	Control group Apparently healthy	Diseased dogs group			
		Sub-group 1	Sub-group 2	Sub-group 3	Sub-group 4
	-	Parvoviral infection	Ectoparasitic infestation	Mal-nutrition	Liver and kidney disease
ALT (µkat/L)	0.77 ± 0.03	1.21 ± 0.07***	1.22 ± 0.14**	1.09 ± 0.23*	5.56 ± 1.60***
AST (µkat/L)	0.57 ± 0.03	0.73 ± 0.05**	0.84 ± 0.13	0.68 ± 0.04*	2.07 ± 0.30***
Creatinine (µmol/L)	95.5 ± 5.30	98.1 ± 7.96	85.7 ± 4.42	91 ± 8.84	100.8 ± 32.71
Glucose (mmol/L)	5.68 ± 0.15	4.37 ± 0.12***	5.39 ± 0.17	4.52 ± 0.12**	4.53 ± 0.33**
Iron (µmol/L)	18.9 ± 1.26	9.16 ± 0.47***	16.66 ± 1.32	10.87 ± 2.94**	12.7 ± 2.03*
Cupper (µmol/L)	16.8 ± 0.45	14.85 ± 0.36**	18.1 ± 0.65	13.77 ± 0.23**	15.17 ± 1.06
Total Protein (g/L)	63 ± 0.9	55 ± 1.5***	60 ± 0.80*	52 ± 2.00***	55 ± 3.60**

P-value: *** P ≤0.001, ** P ≤0.01, * P ≤0.05

RESULTS AND DISCUSSION

In the present work, four main common etiopathologies causing canine anemia were recorded. They included canine parvoviral infection, ectoparasites infestation especially by ticks and fleas, malnutrition, and hepatic and/or renal disease.

Parvoviral Infection Related Anemia

The parvo virus infected group included 20 dogs (36% of total diseased dogs) of different breeds and age range from 2 to 6 months. All cases were admitted with pale mucous membranes, bloody diarrhea, vomiting, dehydration and weight loss (Fig. 1A&B). Affected cases were positive with canine parvoviral rapid antigen test kits (Fig. 2A). Regarding haematological examination, mean values of HCT, TEC, Hb and TLC were significantly (p≤0.001) decreased in affected cases compared to healthy dogs (Table 1). These findings were agreed with (Harizan et al., 2021). The recorded anemia in the affected dogs was classified as macrocytic hypochromic regenerative type as there was a prominent anisocytosis presented as significant (P≤0.001)

elevation in red cell distribution width (RDW). These findings are in accordance with the studies conducted by (Andrea et al., 2017). This reduction may be due to gastro-intestinal haemorrhage and blood loss via vomiting and diarrhea (Sharma et al., 2005). In the same context, virus possesses a cytotoxic effect on bone marrow hematopoietic cells leading to myeloid and erythroid hypoplasia especially at the acute phase of infection (Macintire and Smith, 1997). This explanation was agreed with the study conducted by (Agnihotri et al., 2017). The mean value of total leukocytic, segmented neutrophilic, lymphocytic and eosinophilic counts were significantly decreased (P≤0.001) compared with healthy dogs (Table 1). Decrease in the total leukocytic count that associated with higher mortality rate considered as sentient prognostic indicator for the condition. This is coincides with the study of (Bhargavi et al., 2017). Another significant (P≤0.01) decrease in mean value of monocytes in affected dogs is recorded. This result may be due to migration of monocytes from circulation to the affected sites encountered the inflammatory process. Similar findings were also recorded by (Terzungwe, 2018) and (Kataria et al., 2020).

Regarding serum biochemical constituents (Table 2), the mean values of serum glucose, iron and total proteins were significantly low (P<0.001) in infected cases while this decrease was significant (P≤0.01) among copper level. Hypoglycemia in the affected dogs may be due to inappitance and/or anorexia (Shinde et al., 2000; Bhargavi et al., 2017; Kumar and Kumar, 2017). Serum iron and copper decrease may be due to excessive loss by bloody diarrhea. Total proteins decrease may be due to leakage of serum protein through damaged and inflamed intestinal villi (Biswas et al., 2005). Significant increase in enzymes activity of ALT (P≤0.001) and AST (P≤0.01) was recorded. These results were in accordance with (Khare et al., 2020) and (Dash et al., 2019).

Ectoparasitic Infestation Related Anemia

This group composed of 18 dogs suffering from ticks and fleas infestation mainly (32 % from diseased dogs). The most common recorded ectoparasites were ticks (*Rhipicephalus sanguineus*) and fleas (*Ctenocephlides canis*). These results were in agreement with those of (Abuzeid, 2015; Saleh et al., 2019; Nguyen et al., 2020). All group cases were admitted with pale mucous membranes, itching, alopecia, weight loss and presence of ticks or fleas on the examined animals (Fig. 1C). In the term of haematological examination (Table 1), results revealed significant (p≤0.001) reduction in HCT value, TEC and Hb level in affected cases compared with healthy dogs. These results were in agreement with (Tkacheva and Glazunova, 2018). Anemia was classified as macrocytic normochromic regenerative type with prominent anisocytosis. Mean values of staff, segmented neutrophils and eosinophils were significantly increased in affected cases (P≤0.01), (P≤0.05) and (P≤0.001) respectively compared with healthy dogs. These results were in accordance with (Katariya et al., 2018). Lymphocytes, monocytes and basophils do not show any significant difference. Regarding serum biochemistry (Table 2), the mean value of ALT was significantly increased in affected dogs compared with healthy dogs while AST was increased without significant changes. These findings were in accordance with (Bilwal et al., 2017; Bilwal et al., 2018; Tandel et al., 2019). The mean value of total protein was significantly decreased (p≤0.05) in affected cases compared with healthy dogs. This was in agreement with results of (Bilwal et al., 2017; Tandel et al., 2019; Salem et al., 2020). Hypoproteinaemia due to hypo-albuminemia was caused by decreased intake and chronic inflammation (Tandel et al., 2019).

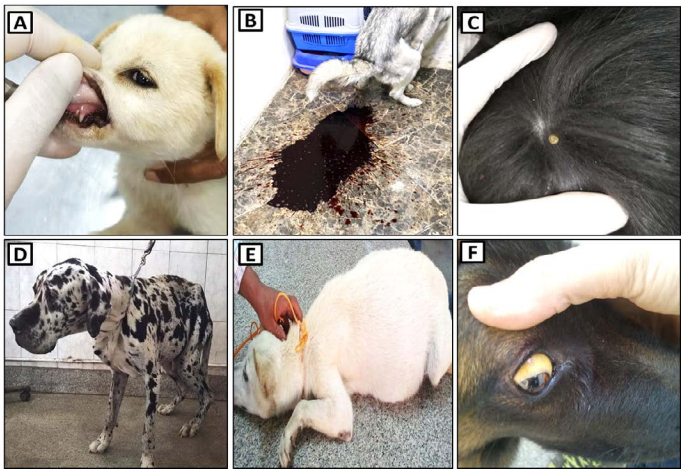

Figure 1: Clinical pictures of common causes of anemia in dogs. A: severe pale oral mucous membrane in mixed breed anemic puppy. B: severe bloody diarrhea associated with parvoviral infection in six months old Husky puppy. C: adult tick infestation in black coat German shepherd dog. D: progressive weight loss with prominent decrease in body condition scoring in two years old Great Dane with malnutrition. E: abdominal distention (Ascites) due to chronic hepatitis in three years old mixed breed mongrel dog. F: yellowish discoloration of episclera and mucous membranes due to hepatic jaundice that confirmed later with laboratory work.

Malnutrition Related Anemia

Malnutrition is a metabolic status which occurs when the supply of available nutrients does not match the demands; its severity depends on the extent and duration of the dietary deficit which can lead to adverse metabolic consequences. In this group, 7 cases (12%) were scrutinized for malnutrition. The most common recorded clinical manifestations were pale mucous membranes and progressive weight loss (Fig. 1D). Hematological parameters (Table 1) showed significant (p≤0.001) reduction in HCT value,

TEC and Hb level in comparison with the healthy dogs. Significant (P≤0.001) increase in MCV and decrease of MCHC suggested that anemia is of macrocytic hypochromic type. Etiology of the anemia in case of malnutrition may be due to vitamin B12, protein, copper and iron deficiencies. So, anemia patterns may show great diversity. The link between protein deficiency and anemia is secondary due to impaired hemoglobin synthesis (Felder et al., 2016; Tamimi and Wali, 2019). Significant (P≤0.001) leucocytosis associated with eosinophilia was recorded in affected dogs compared with healthy ones. There were four cases diagnosed with internal parasites (Fig. 2B) included *Toxocara canis* (2 cases) and mixed infection with *Toxocara canis* and *Toxascaris leonina* (2 cases) that explain the elevation of both parameters. Significant elevation of lymphocytic count wasn't conclusive as there is no correlation between lymphocytic count and nutrition (Fabretti et al., 2015; Keller, 2019; Fabretti et al., 2021). Significant (P≤0.001) elevation of monocytes in affected dogs reflects stress monocytosis that depends mainly on severity and duration of deficiencies. By looking closer to serum biochemistry (Table 2), significant (P≤0.01) reduction in the levels of glucose, iron and copper was recorded in affected dogs as a result of decreased offered nutrients, starvation and malabsorption associated with parasitic infection. The mean level of total proteins was significantly decreased (P≤0.001) in affected dogs. This coincides with (Ruaux et al., 2017) results that malnutrition may lead to depletion of essential amino acids in plasma. Many researchers considered total protein as a nonspecific marker for nutritional status (Nakajima et al., 2014).

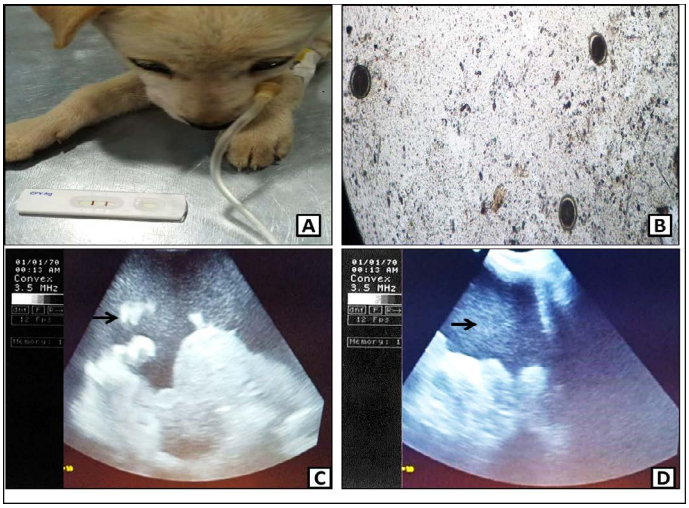

Figure 2: Advanced diagnostic tools used for determination of the definite cause of anemia in dogs. A: in-clinic rapid antigen test kit showing positive infection with parvovirus in two months old Golden Retriever puppy. B: *Toxocara canis* infection (ova) in four months old mixed breed puppy (4 X). C: ultrasonographic view showing hyperechogenic hepatic mass (lymphosarcoma) in five years old German shepherd dog (arrow). D: ultrasonographic view showing hyperechoic hepatic tissue (arrow) due to chronic hepatitis in three years old Golden Retriever bitch.

Liver And Kidney Disease Related Anemia

In the present study, 10 (18%) anemic dogs were diagnosed with hepatic diseases included [8 cases: chronic hepatitis (4 cases), acute hepatitis (2 cases) and hepatic tumour (2 cases)] and renal disease (2 cases with renal failure). The most recorded clinical signs were pale mucous membranes, halitosis, ascites, icterus, and weight loss (Fig. 1E&F). Hematological investigation (Table 1) revealed significant (p≤0.001) reduction in HCT value, TEC and Hb level in diseased dogs. The type of recorded anemia was non-significant microcytic hypochromic, non-regenerative type which matched with the finding of (Elhiblu et al., 2015). Significant reduction of haemoglobin level may be ascribing to increased degradation of erythrocytes via increased transit time through spleen and reduction of portal blood flow. Another explanation recorded by (Prebavathy et al., 2020) that reduction was concomitant to decrease the availability of micronutrients by hepatic cells necessary for synthesis of haemoglobin. Significant (P≤0.01) reduction in platelets count in diseased dogs was recorded in the present work and come in agreement with (Kozat et al., 2017). This may attributed to decreased production of thrombopoietin by liver. Also, disseminated intravascular coagulopathy that occurred with liver diseases may result in thrombocytopenia. Regarding serum biochemical constituents (Table 2), mean values of ALT and AST were significantly (P≤0.001) increased in affected dogs compared with control group. These results may be due to increased damage of hepatic cells that come in agreement with (Assawarachan et al., 2020). Glucose level showed significant (P≤0.01) decrease in diseased dogs as there was severe alteration in metabolic pathways by hepatic cells. Significant reduction (P≤0.05) in serum iron level was detected in diseased dogs that agreed by (Lakshmi et al., 2021). Total proteins level was significantly (P≤0.01) decreased in affected dogs compared with healthy group. This may be due to decrease synthesis of albumin by liver or increase albumin excretion by damaged kidney that leads to protein losing nephropathy. Regarding ultrasonographic examination, a hyperechogenic hepatic mass was recorded in two cases and diagnosed later as lymphosarcoma (Fig. 2C). Another finding included hyperechogenic hepatic tissue that appeared in cases with anemia due to chronic hepatitis (Fig. 2D).

CONCLUSION

From all hereinbefore results, it could be concluded that clinical examination which applied routinely in veterinary clinics is of great importance, but it can in most cases give only preliminary diagnosis without conclusive answer to

establish a confirmatory and managing strategies. A step forward is hematobiochemical investigation which affords a better chance for identification of the type of anemia. Ultrasonography was helpful in diagnostic process but to some extent not all cases necessitate examination through this tool. The corner stone is establishment of a link between the type of anemia and its common etiopathologies that will be helpful in prognosis and therapeutic decisions.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest for the present study.

NOVELTY STATEMENTS

Establishment of a link between each type of anemia and its common cause. that will be helpful for clinicians in prognosis and therapeutic decisions among dogs in Egypt.

AUTHORS CONTRIBUTION

Prof. Dr. Ossama Mohamed Abdu, Dr. Hitham Abdel-Saeed and Prof. Dr. Kawkab Abd El Aziz Ahmed designed the study idea. Dr. Mohamed Saeed M. Hassan performed all clinical, laboratory and statistical analysis procedures of the study under the supervision of Dr. Hitham Abdel-Saeed.

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