

Short Communication

Hemato-biochemical and Serum Iron Profile Study in Dogs with Hemoprotozoal Infection

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Abstract | Iron is an essential mineral for all living organisms and is integral to multiple metabolic functions. The most important function being oxygen transport in haemoglobin. The use of serum iron profile as a routine parameter is limited for assessment of anaemia in dogs. The present study was carried out in anaemic dogs with hemoprotozoal infection (n=6) to observe the type of iron metabolism disturbance that occur.. The mean \pm standard error (SE) of serum iron analytsts [serum iron, serum total iron binding capacity (TIBC) and percent transferrin saturation (%TSAT)] were measured by calorimetric analyser to estimate the iron profile status in anaemic dogs. Statistical significant difference ($P \leq 0.05$) was found between the serum iron parameter and non-significant difference between serum TIBC and %TSAT. The alteration in iron profile observed was similar to anaemia of inflammatory disease which also provided a conclusion regarding the therapeutic approach to be followed for the occurred iron metabolism disturbance.

Keywords | Anaemia, Serum iron, Serum total iron binding capacity, Percent transferrin saturation, Dogs

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Iron is an essential element for all living organisms and about 60-70% of total body iron is present in hemoglobin (McCown and Specht, 2011; Harvey, 2008). Imbalance of iron in the body will result in number of clinical manifestations and which may lead to severe complications or even be life threatening (McCown and Specht, 2011). Dietary iron is absorbed from duodenal enterocytes by the apical surface (Edison et al., 2008) into the plasma where it is bounded to transferrin for transport to cells within the body (Harvey, 2008). Disruption in iron intake, loss or regulation can lead to clinical manifestations such as iron deficiency anaemia, anaemia of inflam-

matory disease, and iron overload (Lieu et al., 2001; McCown and Specht, 2011). Iron deficiency caused by inadequate intake is rare in dogs because most foods have adequate iron content and major cause of iron deficiency anaemia in dogs is chronic blood loss, usually in the gastrointestinal tract (e. g. tumours, gastric ulcers, inflammatory bowel disease, and parasites) (Zaldivar-Lopez et al., 2014). Monitoring the iron status of an animal is done by evaluation of serum analytes (serum iron, serum total iron binding capacity (TIBC), serum ferritin and percent transferrin saturation (%TSAT) (Wians et al., 2001) but serum ferritin concentration has limited use in veterinary medicine

because reagents are species-specific and the assay is not widely available (Schaefer and Stokol, 2015). Depending on the magnitude of iron loss, anaemia may be regenerative or non-regenerative characterized by microcytic normochromic to microcytic hypochromic indices (Mitchell and Kruth, 2010). Hence alteration in serum analytes of iron is observed in anaemic patients thus helping in classification of iron disturbance and monitoring the therapy.

Causes of anaemia include a number of factors and classification of anaemia is an important step in determining the cause (Aird, 2000; Morrison, 2005).

The present study is directed to observe the type of iron metabolism disturbance occurring in anaemic dogs with hemoprotozoal parasite infection as limited data is available regarding iron metabolism disturbance in dogs with hemoprotozoal infection.

The present study was carried out in dogs of any age group and breed affected with hemoprotozoal infection with clinical signs suggestive of anaemia with alteration in complete blood count (CBC) profile value. Clinical examination of the dogs was performed as described by Ettinger and Feldman (2005). The serum iron profile analytes (serum iron, serum TIBC, %TSAT) was also measured. The study included 6 apparently healthy dogs as control groups which had a proper history of vaccination, deworming and were negative for any presence of diseases.

Out of 1247 cases examined at Department of TVCC Bombay Veterinary College, Parel, 20 clinically anaemic cases showed haemoglobin less than 10g% and PCV less than 37 percent. The overall prevalence of anaemia was 1.60%. Out of which 6 cases showed positive for hemoprotozoal infection.

Blood was collected in sterile vacutainers (LS ECOTAINER EDTA K3). Serum was separated by using the LS ECOTAINER Plain vacutainer tubes. Hematological parameters under study that included hemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV), reticulocyte count, platelet count, white blood corpuscular count (WBC), differential leucocyte count (DLC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) was evaluated by the method as described by Benjamin (2007). Serum biochemistry parameters

under study that included Alkaline phosphatase (ALP), serum glutamate pyruvate transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), total serum protein, albumin, globulin, Albumin/Globulin ratio (A/G ratio), Blood Urea Nitrogen (BUN), urea, creatinine, total bilirubin (TB), direct bilirubin (DB) and indirect bilirubin (IB) were estimated by spectrophotometry in robonik prietest touch auto biochemistry analyser using the supplied reagent (M/s Span diagnostics, Kolkata). SGPT and SGOT were determined by IFFC method (Reitman and Frankel, 1957), total protein by biuret method (Gornall et al., 1949), albumin by BCG method (Gustafsson, 1978), total and direct bilirubin by diazo method (Ertingshausen et al., 1973), urea by GLDH-urease method (Tietz, 1995) and creatinine by Jaffe's reaction (Vasiliades, 1976). Indirect bilirubin was calculated as [IB = TB - DB]. Albumin/Globulin ratio (A/G ratio) was calculated as [A/G ratio = Albumin/Globulin]. Serum iron profile study parameters that such as serum iron, serum total iron binding capacity (TIBC) and percent transferrin saturation (%TSAT) were estimated by I-lab 600 acute auto analyser using the supplied reagent (Sigma Diagnostics, St. Louis, USA). Serum iron was determined by Ferene method (Smith et al., 1981), serum TIBC by biochemical method (Henery, 1984). Transferrin saturation was calculated by the following formula:

$$\% \text{TSAT} = (\text{Serum iron} / \text{TIBC}) \times 100$$

Cases with *Ehrlichia canis* infection were confirmed by using SNAP 3Dx kit (M/s IDEXX Laboratories, New Delhi) that detects *Ehrlichia canis* antibodies. Blood smear for hemoprotozoal parasite identification was prepared on a clean and dry glass slide with a drop of blood collected from the ear tip and stained with Leishman's stain.

Normally distributed data are reported as mean \pm standard error. Student's t-test was used for statistical analysis. Statistical significance was set at $P \leq 0.05$. This was performed by using the analysis tool pack software of Microsoft Excel 2007.

Out of the 6 cases positive for hemoprotozoal infection, it included 4 cases of Ehrlichiosis detected by SNAP 3Dx kit (Figure 1) and 2 cases of trypanosomiasis (Figure 2). Prominent clinical signs observed was fever, anorexia, pale mucous membrane, lymphadenopathy, splenomegaly and epistaxis. The red blood



Figure 1: SNAP 3Dx kit showing positive reaction to *Ehrlichia canis* antibodies

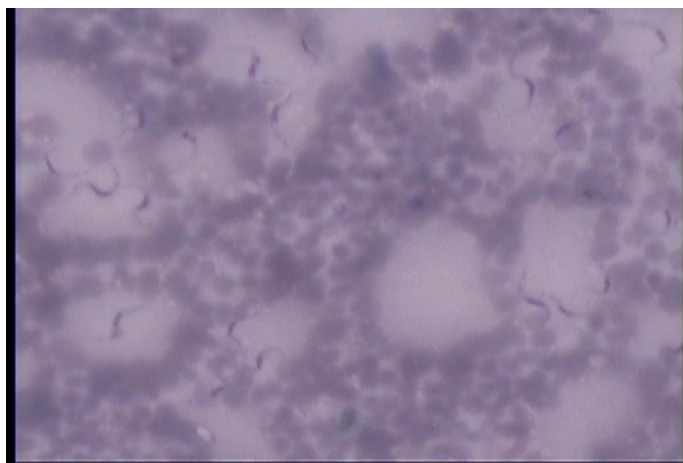


Figure 2: Blood smear showing presence of *Trypanosoma evansi* organisms

cell cellular morphology showed hypochromasia and microcytosis.

The hemato-biochemical alterations when compared to the apparently healthy control dogs (Table 1) showed statistically significant difference regarding parameters Hb, TEC, reticulocyte count and platelet count. Statistical significant difference was also observed in the TP, albumin, A/G ratio and BUN parameters when compared with the apparently healthy control group. Apart from the above, the cases also

Table 1: Mean±SE values of CBC, LFT, KFT in apparently healthy and anaemic dogs

Parameters	Control group	Clinical cases
Hb (g %)	13.38 ± 0.37	7.10 ± 1.09*
T.E.C(×10 ⁶ /μl)	5.76 ± 0.13	2.92 ± 0.45*
P.C.V (%)	40.15 ± 1.12	22.4 ± 3.01*
Reticulocyte count (%)	1.67 ± 0.33	1.17 ± 0.17*
W.B.C(×10 ³ /μl)	11450 ± 1393.26	10250 ± 1061.68
Platelets(×10 ⁵ /μl)	283666.70 ± 25935.39	132000 ± 17970.3*
M.C.V (fL)	69.67 ± 1.09	79.92 ± 4.33
M.C.H (pg)	23.18 ± 0.37	24.77 ± 1.36
M.C.H.C (g/dl)	33.25 ± 0.07	31.12 ± 1.24
T.B(mg/dl)	0.32 ± 0.048	0.80 ± 0.11
D.B(mg/dl)	0.17 ± 0.03	0.40 ± 0.10
I.B(mg/dl)	0.15 ± 0.03	0.40 ± 0.06
S.G.P.T(IU/L)	81.33 ± 3.13	101.885 ± 60.316
S.G.O.T(IU/L)	53.52 ± 2.89	137.98 ± 60.32
A.L.P(IU/L)	94.87 ± 3.65	179.6 ± 71.07
T.P(g/dl)	8.10 ± 0.15	6.91 ± 0.46*
Albumin(g/dl)	3.90 ± 0.20	1.89 ± 0.31*
Globulin(g/dl)	4.37 ± 0.16	5.01 ± 0.32
A/G	0.89 ± 0.06	0.37 ± 0.16*
BUN(mg/dl)	8.99 ± 0.35	22.25 ± 5.95*
Urea(mg/dl)	19.21 ± 0.761	47.68 ± 12.73
Creatinine(mg/dl)	0.84 ± 0.07	1.26 ± 0.13

*Significant (P ≤ 0.05) at t-crit (2.06)

Table 2: Mean ±SE value of serum iron profile of apparently healthy and anaemic dogs

Parameters	Control group	Clinical cases
Serum iron (μg/dl)	141.83 ± 7.91	109.32 ± 22.96*
Serum TIBC (μg/dl)	319.33 ± 31.89	239.3 ± 34.99 NS
TSAT (%)	47.53 ± 6.54	50.47 ± 9.79 ^{NS}

*Significant (P ≤ 0.05) at t-crit (2.06); NS- Non Significant

Table 3: Mean ± SE values of CBC in clinical cases before and after treatment

Parameters	Before treatment	After Treatment	t-stat	t-crit
Hb (g %)	7.10 ± 1.09	9.15 ± 1.04	4.77*	2.57
T.E.C (×10 ⁶ /μl)	2.92 ± 0.45	4.25 ± 0.29	5.81*	
PCV (%)	22.40 ± 3.01	28.92 ± 3.53	4.07*	
WBC (×10 ³ /μl)	10250 ± 1061.68	12400 ± 3023.91	0.83	
Reticulocyte count (%)	1.17 ± 0.17	2.17 ± 0.28	4.47*	
Platelets (×10 ⁵ /μl)	132000 ± 17970.35	185333.3 ± 18895.62	3.69*	
MCV(fL)	79.92 ± 4.33	67.37 ± 6.14	1.70	
MCH(pg)	24.77 ± 1.36	20.86 ± 1.42	4.64*	
MCHC(g/dl)	31.12 ± 1.24	31.81 ± 0.71	0.38	

* Significant (P ≤ 0.05)

Table 4: Mean ± SE values of LFT in clinical cases before and after treatment

Parameters	Before treatment	After treatment	t-stat	t-crit
T.B.(mg/dl)	0.80 ± 0.11	0.45 ± 0.03	2.99*	2.57
D.B.(mg/dl)	0.40 ± 0.10	0.2 ± 0.05	1.88	
I.B.(mg/dl)	0.40 ± 0.06	0.23 ± 0.03	3.96*	
SGPT(IU/L)	101.89 ± 25.91	72.17 ± 6.14	1.31	
SGOT(IU/L)	137.98 ± 60.32	102.59 ± 16.79	0.78	
ALP(IU/L)	179.6 ± 71.07	209.27 ± 29.59	0.60	
TP(g/dl)	6.91 ± 0.46	7.10 ± 0.53	0.65	
Albumin(g/dl)	1.89 ± 0.31	2.37 ± 0.27	4.25*	
Globulin(g/dl)	5.01 ± 0.32	4.57 ± 0.28	1.82	
A/G ratio	0.37 ± 0.07	0.52 ± 0.06	5.13*	

*Significant (P ≤ 0.05)

showed leucopenia with neutropenia and lymphopenia, hypoalbuminemia, hyperglobulinemia, hyperbilirubinemia, increase in ALT, AST and ALP levels.

Table 2 depicts the serum iron profile between the control group and clinical cases and it showed statistical significant difference between the serum iron parameter and non-significant difference between serum TIBC and %TSAT. There was also a decrease in serum TIBC levels compared to the control group.

The affected cases were therapeutically managed as per the standard line of treatment. Cases with *E. canis* were treated with Doxycycline @ 5mg/kg B.W. for 15 days and cases with *Trypanosoma sp. evansi* infection were treated with Triquin (Quinapyramine Sulphate and chloride) at 0.025ml/kg B.W. once. No adverse outcomes were observed from the cases during the

therapy. The hemato-biochemical and serum iron profile parameters were re-evaluated in the affected cases after clinically recovery and is summarized in Table 3, 4, 5 and 6. Statistical significant difference was observed in Hb, TEC, PCV, reticulocyte count, platelet count, MCH, TB, IB, Albumin, A/G ratio, BUN, urea and creatinine parameters after therapeutic intervention. Among the serum iron profile, statistical significant difference was observed in serum iron parameters.

The clinical findings were in accordance as observed by Harrus et al. (1997), Harrus et al. (1998) and Warner and Harrus (2000). The results showed alterations in the hemato-biochemical and serum iron profile parameters when compared to the control group suggesting the presence of anemia with disturbance in the iron metabolism.

Giger (2005) came up with the opinion that low values of Hb, TEC and PCV in the cases may be attributed to deficiency of iron, destruction of red blood corpuscles and/or excess blood loss and reduced erythropoietic activity. Waner (2008) also stated that anaemia in *Ehrlichia sp.* infection is attributed to direct effect of the organism on the red blood cells. Aquino et al. (2002) and Abenga et al. (2005) also reported low PCV, Hb, and RBC in *Trypanosoma* infections. There was also leucopenia, neutropenia and lymphopenia in the affected animals which coincided with the findings of Warner and Harrus (2000). The alterations in the T.P., Albumin, A/G ratio was reported by Supriya et al. (2011) in clinical case with ehrlichiosis. There was also alteration in AST, ALT, ALP and hyperbilirubinemia and it was either due to secondary to hyperglobulinemia as a compensatory mechanism for the maintenance of normal blood viscosity increased by high globulin levels or due to increase in the immunoglobulin levels as a result of the hemoprotozoal infection (Aquino et al., 2001).

Table 5: Mean ± SE values of KFT in clinical cases before and after treatment

Parameters	Before Treatment	After Treatment	t-stat	t-crit
BUN (mg/dl)	22.25 ± 5.95	10.56 ± 1.76	2.72*	2.57
Creatinine (mg/dl)	1.26 ± 0.13	0.89 ± 0.10	2.74*	
Urea (mg/dl)	47.68 ± 12.73	22.62 ± 3.77	2.72*	

*Significant (P ≤ 0.05)

Table 6: Mean ± SE values of serum iron profile in anemic cases before and after treatment

Parameters	Before treatment	After treatment	t-stat	t-crit
S. iron (µg/dl)	109.32 ± 22.96	124.18 ± 25.47	3.33*	2.57
S. TIBC (µg/dl)	293.30 ± 34.99	253.20 ± 31.54	1.78	
TSAT (%)	50.47 ± 9.79	52.69 ± 9.792	1.44	

*Significant (P ≤ 0.05)

Serum iron and TIBC were lower than the control group, suggesting that iron metabolism disturbance

does occur in hemoprotozoal infection and the disturbance is of the order seen in anemia due to inflammatory disease (Lillihook et al., 1998 and Furnanello et al., 2005). These lower levels are due to iron retention within macrophages resulting from increased uptake of iron by different pathways as well as inhibition of iron recycling by blockage of iron export (Stijlemans et al., 2008) and can be accredited as defence mechanism of body against infection to withhold iron utilization by the invading microbes for their growth (Fry, 2011 and McCown and Specht, 2011). Kelly et al. (2013) observed microcytic anaemia as the cellular changes in dogs affected with ehrlichiosis. Silva et al. (1995) and Gunaseelan et al. (2009) reported the presence of microcytic hypochromic cellular changes in dogs affected with *Trypanosoma evansi*. The prominent cellular changes observed in our study showed microcytosis and hypochromasia and is in accordance with the opinion of the above mentioned authors. The reason for the microcytosis in some dogs has been proposed to be associated with chronic inflammatory disease as a result of prolonged sequestration of storage iron and microcytosis has been reported in many different types of inflammatory disease in dogs, including gastrointestinal, infectious, respiratory and skin disease (Gavazza et al., 2012).

Use of Doxycycline and Triquin (quinapyramine sulphate and chloride) for ehrlichiosis and trypanosomiasis, respectively, for therapy was followed by Mylonakis et al. (2011) and Juyal (2011). The significant changes on the post treatment levels on the parameters can be attributed due to the etiological specific therapy this also resulted in restoration of levels of serum iron analytes to normal without any exogenous use of iron supplementation.

In conclusion the results from this study, hemoprotozoal diseases will result in disturbance of iron metabolism subsequently leading to anaemia. The characteristics of the iron metabolism disturbance in present study were analogous to anaemia of inflammatory diseases. From the above iron disturbances observed in the study it can also be inferred that therapy should not involve exogenous iron administration since it may lead to aggravation of the existing infection through microbial utilization of iron. Hence serum iron profile studies indeed are a valuable aid in the diagnosis of iron metabolism disturbances and should be included as a routine diagnostic parameter in the anaemic dogs.

CONFLICT OF INTEREST

There is no conflict of interest among all or any of the authors.

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REFERENCE

- Abenga JN, Ezebuio CO, David K, Fajinmi AO, Samdi S (2005). Studies on anaemia in Nigerian local puppies infected with *Trypanosoma congolense*. *Vet. Arhiv.* 75: 165-174.
- Aird B (2000). Clinical and haematological Manifestations of anaemia In: Schalm's Veterinary Hematology, 5th ed. Lippincott Williams & Wilkins, Philadelphia. Pp. 140-142.
- Aquino LPCT, Machado RZ, Alessi AC, Santana AE, Castro MB, Marques LC, Malheiros EB (2002). Haematological, biochemical and anatomopathological aspects of the experimental infection with *Trypanosoma evansi* in dogs. *Med. Vet. Zootec.* 54: 8-18.
- Benjamin MM (2007). Outline of Veterinary Clinical Pathology. 3rd ed. Kalyani Publishers, Ludhiana. Pp. 351-360.
- Edison ES, Bajel A, Chandy M (2008). Iron homeostasis: new players, newer insights. *Eur. J. Haematol.* 81(6): 411-424. <http://dx.doi.org/10.1111/j.1600-0609.2008.01143.x>
- Ertingshausen G, Digne L, Fabiny B, Tiffany O, Lassy SJ (1973). Single reagent method for rapid determination of total bilirubin with centri chem analyser. *Clin. Chem.* 19: 1366-1369.
- Ettinger SJ, Feldman EC (2005). Textbook of Veterinary Internal Medicine diseases of dogs and cats. 6th Ed. Elsevier Saunders, Philadelphia. Pp. 1422-1492.
- Furlanello T, Fiorio F, Caldin M, Lubas G, Solano-Gallego L (2005). Clinicopathological findings in naturally occurring cases of babesiosis caused by large form *Babesia* from dogs of northeastern Italy. *Vet. Parasitol.* 134: 77-85. <http://dx.doi.org/10.1016/j.vetpar.2005.07.016>
- Fry MM (2011). Nonregenerative Anaemia: Recent Advances in Understanding Mechanisms of Disease. Proceeding of the ACVP/ASVCP Concurrent Annual Meetings Dec.3-7, 2011 Nashville, Tennessee, USA (<http://www.ivia.org>)
- Gavazza A, Rispoli D, Bernabo N, Lubas G (2012). Retrospective and observational investigation of canine microcytosis in relationship to sex, breed, diseases, and other complete blood count parameters. *Comp. Clin. Pathol.* 21: 545-553. <http://dx.doi.org/10.1007/s00580-010-1127-x>
- Giger URS (2005). Regenerative anemias caused by blood loss or hemolysis. In: Textbook of Veterinary Internal Medicine. 6th ed. Elsevier Saunders, Philadelphia. Pp. 1716-1730.
- Gornall AG, Bardawill CJ, David MM (1949). Determination of serum proteins by means of biuret reaction. *J. Biol. Chem.* 177: 751-756.
- Gunaseelan L, Senthilkumar K, Selvaraj P, Kathiresan D (2009). Haemato biochemical changes in a case of canine trypanosomiasis. *Tamilnadu J. Vet. Anim. Sci.* 5: 122-123.
- Gustaffson JEC (1978). Automated serum albumin determination by use of immediate reaction with Brom. Crysol green reagent. *Clin. Chem.* 24: 369-373.
- Harrus S, Aroch I, Lavy E, Bark H (1997). Clinical manifestations of infectious canine cyclic thrombocytopenia. *Vet. Rec.* 141: 247-250. <http://dx.doi.org/10.1136/vr.141.10.247>
- Harrus S, Waner T, Kysary A, Aroch I, Voet H, Bark H (1998). Investigation of splenic functions in canine monocytic ehrlichiosis. *Vet. Rec.* 62: 15-27.
- Harvey JW (2008). Iron metabolism and its disorders in: clinical biochemistry of domestic animals, 6th ed. Academic Press, UK. Pp. 259-288. <http://dx.doi.org/10.1016/B978-0-12-370491-7.00009-X>
- Henery JB (1984). Clinical diagnosis and management by laboratory methods. W.B. Saunders, Philadelphia. Pp. 1434.
- Juyal PD (2011). Newer perspectives in the diagnosis and control of trypanosomiasis (surra) in domestic livestock in India. *Internationale Wissenschaftliche Publikationen:* 1-13.
- Kelly PJ, Xu C, Lucas H, Loftis A, Abete J, Zeoli F, Stevens A, Jaegersen K, Ackerson K, Gessner A, Kaltenboeck B, Wang C (2013). Ehrlichiosis, Babesiosis, Anaplasmosis and Hepatozoonosis in Dogs from St. Kitts, West Indies. *PLoS ONE* 8(1): e53450. <http://dx.doi.org/10.1371/journal.pone.0053450>
- Lieu PT, Heiskala M, Peterson PA, Yang Y (2001). The roles of iron in health and disease. *Mol. Aspects Med.* 22(1-2): 1-87. [http://dx.doi.org/10.1016/S0098-2997\(00\)00006-6](http://dx.doi.org/10.1016/S0098-2997(00)00006-6)
- Lilliehook I, Egenvall A, Harold WT (1998). Hematopathology in Dogs Experimentally Infected with a Swedish Granulocytic *Ehrlichia* Species. *Vet. Clin. Pathol.* 27: 116-122. <http://dx.doi.org>

- [org/10.1111/j.1939-165X.1998.tb01030.x](http://dx.doi.org/10.1111/j.1939-165X.1998.tb01030.x)
- McCown JL, Specht AJ (2011). Iron homeostasis and disorders in dogs and cats: A review. *J. Am. Ani. Hosp. Assoc.* 47: 151-160. <http://dx.doi.org/10.5326/JAAHA-MS-5553>
 - Mitchell K, Kruth S (2010). Immune mediated hemolytic anemia and other regenerative anemias in: textbook of veterinary internal medicine diseases of dog and cat, 7th ed. W.B Saunders Publications, Philadelphia. Pp. 772.
 - Morrison WB (2005). Textbook of Veterinary Internal Medicine diseases of dogs and cats. 6th Ed. Elsevier Saunders, Philadelphia. Pp. 2-9, 211-215.
 - Mylonakis ME, Siarkou VI, Koutinas AF (2011). Myelosuppressive Canine Monocytic Ehrlichiosis (*Ehrlichia canis*): An update on the pathogenesis, diagnosis and management. *Israel J. Vet. Med.* 65: 129-135.
 - Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* 28: 56.
 - Silva RAMS, Herrera HM, Domingos LBS, Ximenes FA, Davila AMR (1995). Pathogenesis of *Trypanosoma evansi* infection in dogs and horses: hematological and clinical aspects. *Ciencia Rural, Santa Maria.* 25(2): 233-238. <http://dx.doi.org/10.1590/S0103-84781995000200010>
 - Schaefer DMW, Stokol T (2015). The utility of reticulocyte indices in distinguishing iron deficiency anemia from anemia of inflammatory disease, portosystemic shunting, and breed-associated microcytosis in dogs. *Vet. Clin. Pathol.* 44: 1 109-119.
 - Stijlemans B, Vankrunkelsven A, Brys L, Magez S, De Baetselier P (2008). Role of iron homeostasis in trypanosomiasis-associated anemia. *Immunobiology.* 213: 823-835. <http://dx.doi.org/10.1016/j.imbio.2008.07.023>
 - Smith JE, Moore K, Schoneweis D (1981). Coulometric technique for iron determination. *Am. J. Vet. Res.* 42: 1084-1087.
 - Supriya S, Shradha P, Subhash B, Anupam C (2011). A Clinico-Pathological Report of Canine Ehrlichiosis in a Doberman pinscher. *Vet. World.* 4(8): 374-375.
 - Tietz NW (1995). Clinical Guide to Laboratory Tests, 3rd Ed. Philadelphia; W.B. Saunders.
 - Vasiliades J (1976). Reaction of alkaline picrate with creatinine. 1. Kinetics and mechanism of formation of the mono-creatinine picric acid complex. *Clin. Chem.* 22: 1664.
 - Warner T, Harrus S (2000). Canine Monocytic Ehrlichiosis, Recent Advances in Canine Infectious diseases, International Veterinary Information Services.
 - Waner T (2008). Hematopathological changes in dogs infected with *Ehrlichia Canis*. *Israel J. Vet. Med.* 63: 21-29.
 - Wians FH Jr, Urban JE, Keffer JH and Kroft SH (2001). Discriminating Between Iron Deficiency Anemia and Anemia of Chronic Disease Using Traditional Indices of Iron Status vs. Transferrin Receptor Concentration. *Am. J. Clin. Pathol.* 115(1): 112-118.
 - Zaldivar-Lopez, Iazbik MC, Marín LM, Couto CG (2014). Iron Status in Blood Donor Dogs *J. Vet. Int. Med.* 28: 211-214. <http://dx.doi.org/10.1111/jvim.12254>