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Research Article

Malondialdehyde Status, Trace Minerals and Hematologic Results of anemic-*T. equi* infected Egyptian Horses

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ABSTRACT

Equine piroplasmosis is a clinically significant widespread tick borne disease affecting equine population. Thus, this study aims to describe the clinical signs, correlation between the developed anemias and mineral status along with the lipid peroxidation product Malondialdehyde (MDA) in naturally occurring *T. equi* infected horses in Egypt. Twenty-five horses of different age and sex were involved in this study; horses have signs compatible with babesial infection were examined. Fever, hemoglobinuria and icterus were the most consistent clinical signs recorded in this study. The hematology picture showed decrease in red cell parameters along with leucocytosis. Macrocytic hypochromic anemia was found in infected horses alongside relative increase in copper and relative decrease in ferrous and zinc. MDA showed very statistically significant difference when compared to control data. In conclusion, high level of MDA along with reduction in PCV, HB, and RBCs count is indicating the presence of oxidative stress and implicating the process as a cause of anemia in *T. equi* infection. The mineral status (Cu, Zn, Fe) appeared to be directly affected by the parasite and the mineral status influenced by the anemic syndrome associated with *T. equi*. Inversely correlation between zinc and MDA level might be used in planning the treatment strategy of *T. equi*. Including these minerals in treatment regimen of *T. equi* will help to counter the anemic nature of the disease.

Key words: *T. equi*, Trace Minerals, Malondialdehyde, Hematology, Equine

INTRODUCTION

In equine practice, equine piroplasmosis is a widespread tick borne malady affecting horses all over the world (Kuttler 1988; DeWaal 1992; Rothschild and Knowles 2007). The clinical presentation of the disease varies; nevertheless, it mainly consists of fever, anemia, hemoglobinuria and icterus (Camacho *et al*., 2005; Deger *et al*., 2009; Abedi *et al*., 2014; Hussain *et al*., 2014). Two agents, namely *T. equi, T. caballi*, have been incriminating; however, *T. equi* is known to be the cause of the virulent illness while the infection with *B. caballi* tends to be mild to unapparent (Friedhoff *et al*., 1990).

Anemia defined as decrease the ability of blood to carry oxygen, one of the most common causes of anemia in horses is the hemo-protozoan parasite called "*Babesia"* (Morris 2006). Anemia can develop due to blood loss, destruction or decrease bone marrow production of RBCs (Merck publishing and Merial 2007).

A great portion of horses infected with babesia regardless the clinical presentation of disease suffering from varying degree of anemia manifested as decrease in RBCs, PCV, and hemoglobin (Allen *et al*., 1977; Zobba *et al*., 2008; Wise *et al*.,, 2013), acute infection are also characterized by decrease serum iron levels (DeWaal *et al*., 1987).

Trace minerals are essential for wellbeing and growth of animals, Zinc is involved in plentiful biological operations; zinc plays an integral role in antioxidant enzyme "superoxide dismutase" (Mates 2000; Classen *et al*., 2011). Copper is an essential component required to prevent anemia development (Sullivan and Ochs 1987), copper can operate as antioxidant by neutralizing the free radicals, as well (Araya *et al*., 2006).

The wide-known function of iron is the contribution in heme molecule synthesis, copper is mandatory to iron uptake in hemoglobin synthesis operation (Deger *et al*., 2005). Iron deficiency may lead to hypochromic microcytic anemia (Bainton and Finch 1964; Bian *et al*., 2003).

In recent years, several researches pinpoint the role of highly reactive oxygen radicals in pathogenesis of equine

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babesiosis (Deger *et al*., 2009), but still scarce papers discussing this topic.

Free oxygen radicals result in lipid peroxidation, which can be assessed by estimation of Malondialdehyde (MDA) levels (Esterbauer 1996). MDA is widely acceptable biomarker for lipid peroxidation estimation (Moore and Roberts 1998). RBCs membrane lysis causing RBCs fragility could be attributed to lipid peroxidation process takes place throughout *T. equi* infection (Ambawat *et al*., 1999).

The current study designed to investigate hematologic alterations, selected mineral status (Zn, Cu and Fe) along with MDA level in anemic horses naturally infected with babesiosis.

MATERIALS AND METHODS

Twenty-five horses (n=25) of different age and sex were involved in this study; each horse was subjected to comprehensive physical examination including pulse rate, respiratory rate, superficial lymph nodes, visible mucous membrane status, chest auscultation and fecal sample examination; the rectal temperature and clinical presentation were recorded, the existence of ticks on the coat was determined. Ticks were collected and identified based on morphological characteristics (Nuttal *et al*., 1908). All the infected cases were collected during August and September 2014 from Giza governorate, Egypt.

For hematology, blood was collected from jugular vein in EDTA-containing tubes; clinical hematology was done within 2 hours after the sample collection (Feldman *et al*., 2000). Giemsa-stained thin blood films were inspected under the microscope for direct recognition of intraerythrocytic stages of the hemoparasite (Brown 1993); only horses given positive results in the three blood films were involved in this study. Sera of infected animals were analyzed for Zn, Cu and Fe using specific test kits (Spectrum-Diagnostics, Egypt).

MDA was calorimetrically analyzed using respective chemical test kit (Biodiagnostic, Egypt) according to manufacturer's instructions.

The hematological, minerals and MDA data of infected horses were compared with control data collected from the same locality. Statistical analysis was performed using Student's t test (STATISTICA for Windows, version 5.1., StatSoft, Inc. 1984- 1996). P <0.05was considered of statistical significance.

RESULTS

Pertinent signalmen of the affected horses were recorded, the highest infection rate was found in age group of 6-9 years (11/25, 44%), followed by age group above 9 years old (8/25, 32%) and the least affected group was those between 1-5 years (6/25, 24%). The stallions appeared to be almost equally infected (14, 56%) as mares (11, 44%). The collected ticks were identified based on their morphological characteristics as ***Rhipicephalus*** *spp.*

The most recorded clinical sign was fever (39.5 ± 0.194) presented in all infected animals. The other clinical signs recorded were inappetence, hemoglobinuria, and icterus and congested mucous membrane as well.

Three stained blood film of each animal were examined under oil-immersion lens, the examination reveals the presence of small piriform, circular merozoite in RBCs, which is indicative for *T. equi*.

The mean hematologic values are shown in table 1. Significant decrease in RBCs, HB content and PCV percentage were recorded along with significant increase in WBCs count were observed. Red cell indices showed significant increase in MCV along with significant decrease in MCHC suggesting macrocytic hypochromic anemia. Significant decrease in platelet count was observed.

The mineral status of the infected animals are shown in table 2. A relatively high copper level along with relatively low zinc and ferrous levels were observed in infected horses compared to control data.

MDA levels are shown in table 2, result showed extremely statistically significant increase in MDA level in infected horses compared to the control data.

Table 1: Statistical analysis showing hematologic alterations in diseased horse compared to control data

Parameter	Patient data	Control data
RBCs ($\times 10^6/\mu l$)	4.324±0.212*	6.18±0.149
Hemoglobin (g/dl)	9.134±0.749*	15.420±1.320
PCV (%)	27.25±1.41*	34.40±0.93
MCV (fl)	64.019±1.744*	55.70±1.123
MCHC (%)	33.179±2.065*	44.800±1.125
WBCs ($\times 10^3/\mu l$)	13.504±0.980*	6.7200±0.333
Platelets ($\times 10^3/\mu l$)	113.45±10.65*	170.40±27.96

* Show statistically significant difference at $P<0.05$.

Table 2: Statistical analysis showing mineral status and MDA in diseased horse compared to control data

Parameter	Patient data	Control data
Copper	22.484± 1.823*	13.782±0.274
Zinc	14.951±1.002*	51.666±4.216
Iron	92.400±15.702*	177.981±18.242
MDA	6.1581± 0.6496*	2.5783±0.3123

* Show statistically significant difference at P<0.05.

DISCUSSION

Equine babesiosis is one of the most significant tick-borne sickness, with an economic global impact on the equine business (Zobba *et al*., 2008). The highest occurrence of the disease was at 6-9 years, previous reports dealt with the seropositivity showed higher affinity of *T. equi* to exist in older equids (Kouam *et al*., 2010; Salib *et al*., 2013); however, `Grandi *et al*., 2011 found no statistical association between the infection status and age.

The stallions appeared to be almost equally infected as mares; the gender appeared not to play a role in the infection rate (Asgarali *et al*., 2006; Karatepe *et al*., 2009; Kouam *et al*., 2010), however other contradicted reports showed higher infection rate in males than females (Salib *et al*., 2013), males were significantly less affected than mares (Shkap *et al*., 1998).

Higher infection rate was found in summer, the infection rate is strongly correlated with the tick population, climatic aspects, such as temperature and humidity have great influence on the habitat and dynamics of ticks' population (Young and Leitch 1981; Chilton and Bull 1994; Leschnick *et al*., 2008). *Rhipicephalus spp.* was identified as the vector, the genus *Rhipicephalus* is

considered an African type tick with 63 *spp* confined to Afro-tropical territory only and one was thought to be restricted to African territory has recently gained access to Arabian peninsula (Walker *et al*., 2000; Olwogh *et al*., 2007).

Equine piroplasmosis causes different clinical picture depending on the severity of the infection. Fever, inappetence, hemoglobinuria, and icterus were the most consistent clinical signs recorded in this study; this clinical presentations agreed with previous reports (Camacho *et al*., 2005; Deger *et al*., 2009; Abedi *et al*., 2014; Hussain *et al*., 2014). However, the clinical picture has been described as changeable and often nonspecific (Bashiruddin *et al*., 1999). Marked hemolysis and consequential anemia were responsible for the clinical picture (Wise *et al*., 2013). In Mediterranean region in which piroplasmosis is an endemic disease, symptomatic illness can occur year round with specific or vague signs (Friedhoff *et al*., 1990, Zobba *et al* 2008).

Small piriform, circular merozoite in RBCs, which is indicative for *T. equi* was found via microscopic examination, direct diagnosis includes demonstration of intraerythrocytic merozites in Giemsa stained blood by molecular detection (Nagore *et al*., 2004; Alhassan *et al*., 2007).

Reduction in RBCs, HB content and PCV percentage along with significant increase in WBCs count were recorded in this study, piroplasmosis is known to induce anemia in most horses in spite of the clinical picture (Wise *et al*., 2013). The excessive intravascular hemolysis along with phagocytic role of neutrophils and monocytes in response to parasite antigen adhered to RBCs surface and toxic hemolytic factor released by the parasite itself might be contributed to the anemia (Ambawat *et al*., 1999; De Gopegui *et al*., 2007; Zygner *et al*., 2007). The increased leukocyte count was observed, the elevation in infected RBCs along with stimulation of lymphoid system and bone marrow are directly correlated to elevated WBCs count (Sandhu 1996; Ike *et al*., 2005; Ibrahim *et al*., 2011).

The red cell indices showed increase in MCV (macrocytic) along with reduction in MCHC (hypochromic) anemia, red cell indices (MCV, MCH, and MCHC) have been reported to be variable (Ambawat *et al*., 1999). However, macrocytic hypochromic anemic pattern has been recorded in previous reports dealt with *T. equi* (Al-Saad 2009; Ibrahim *et al*., 2011). In recently published research, anemia was attributed to consequence of oxidative damage in RBCs (Nazifi *et al*., 2008) principally those correlated with SOD and GPx (Nazifi *et al*., 2009). Zinc-containing enzyme "Carbonic anhydrase" converts CO_2 and without this enzyme, the conversion rate will be slower (Tripp *et al*., 2001; Kohen and Limbach 2001).

The mineral status of examined horses showed relatively high copper level accompanied by relatively low zinc and ferrous level, significant elevation in Cu along with significant reduction in Zn have been reported in anemic patients (Ece *et al*., 1997; Gürgöze *et al*., 2006; Turgut *et al*., 2007; Dede *et al*., 2008).

The protozoal agents is considered to increase the cytokines and effecter cells, zinc is believed to play a role in macrophage uptake and destroying the insulting agent (Wirth *et al*., 1989; Scott 2000). Wellinghausen *et al*., (1999) assumed the decrease in zinc level in patients with alveolar echinococcosis is correlated with increase the zinc utilization by the parasite, this assumption could be applied in equine piroplasmosis. Copper is directly proportional with humoral response to the infection (Dede *et al*., 2008); during inflammatory processes, increase the levels of ceruloplasmin, resulting in elevation of the copper levels (Healy and Tipton 2007). The significant decrease in serum iron can be contributed to anemia and excess utilization of iron in regulating hematopoietic process (Hussein *et al*., 1997).

MDA level showed extremely statistically significant increase in infected horses compared to control horses, MDA is the product of lipid peroxidation (Esterbauer, 1996). Nitric oxide production increased during equine babesiosis (Deger *et al*., 2009; Saleh 2009). There were several reports demonstrating the rise in MDA with other babesial agents (Kumar *et al*., 2006; Crongaj *et al*., 2010; Esmaeilnejad *et al*., 2012). This augment in lipid peroxidation in equine may be marker of oxidative stress and of erythrocyte injury caused by babesiosis (Deger *et al*., 2009). `Murase *et al*., (1996) attributed the damage in non-parasitized erythrocytes to oxidative stress exposure during *B. gibsoni* infection. The inversely proportional between erythrocyte MDA and PCV percentages suggesting the augment in lipid peroxidation may be one of the factors implicated in the decrease of PCV values and consequently the anemia (Esmaeilnejad *et al*., 2012).

In conclusion, high level of MDA along with reduction in PCV, HB, and RBCs count is indicating the presence of oxidative stress and implicating the process as a cause of anemia in *T. equi* infection. The mineral status (Cu, Zn, Fe) appeared to be directly affected by the parasite and the mineral status influenced by the anemic syndrome associated with *T. equi.* Inversely correlation between zinc and MDA level might be used in planning the treatment strategy of *T. equi*.

REFERENCES

Abedi V, G Razmi, H Seifi and A Naghibi, 2014. Molecular and serological detection of Theileria equi and Babesia caballi infection in horses and Ixodid ticks in Iran. Ticks and tick-borne diseases, 5: 239-244.

Alhassan A, H Iseki, C Kim, N Yokoyama and I Igarashi, 2007. Comparison of polymerase chain reaction methods for the detection of Theileria equi infection using whole blood compared with pre-extracted DNA samples as PCR templates. Tropical Animal Health and Production, 39: 369-374.

Allen PC, WM Frerichs and AA Holbrook, 1977. Experimental acute babesia caballi infections. I. Red Blood cell dynamics. Experim Parasitol, 37: 67-77

Al-Saad KM 2009. Acute babesiosis in foals. Journal of Animal and Veterinary Advances, 114: 173-194.

Ambawat HK, DV Malhotra, S Kumar and S Dhar, 1999. Erythrocyte associated haemato-biochemical changes in Babesia equi infection experimentally produced in donkeys. Veterinary Parasitology, 85: 319-324.

Araya M, F Pizzarro, M Oliveres, M Arredondo, M Gonzalez and M Mendez, 2006. Understanding

copper homeostasis in humans and copper effects on health. Biological Research, 39:183-197.

Asgarali Z, DK Coombs, F Mohammed, MD Campbell and E Caesar, 2006. A serological study of Babesia caballi and Theileria equi in Thoroughbreds in Trinidad. Veterinary Parasitology, 144: 167-171.

Bainton DF and C Finch, 1964. The diagnosis of iron deficiency anemia. The Amer J Med, 37: 62-70.

Bashiruddin JB, C Cammà and E Rebêlo, 1999. Molecular detection of Babesia equi and Babesia caballi in horse blood by PCR amplification of part of the 16S rRNA gene. Vet Parasitol, 84: 75-83.

Bian K, Z Gao, N Weisbrodt and F Murad, 2003. The nature of heme/Iron-induced protein tyrosine nitration. Proc NaTL Acad Sci USA, 100: 5712-5717.

Brown AB, 1993. Hematology: principles and procedures, 6th edn, Lea and Feibiger, Philadelphia, PA, USA.

Camacho AT, FJ Guitian, E Pallas, JJ Gestal, AS Olmeda, MA Habela, SR Teiford III and MA Spielman, 2005. Theileria (Babesia) equi and Babesia caballi infections in horse in Galicia, Spain. Trop Anim Health Prod, 37: 293-302.

Chilton NB and CM Bull, 1994. Influence of environmental factors on oviposition and egg development in Amblyomma limbatum and Aponomma hydrosauri (Acari: Ixodidae). Inter J Parasitol, 24: 83-90.

Classen HG, U Gröber, D Löw, J Schmidt and H Stroke, 2011. Zinc deficiency symptoms, causes, diagnosis and therapy. Med Monatsscher Pharm, 34: 87-95.

Crnogaj M, R Petlevski, V Mrljk, I Kis, M Torti, N Kucer, V Matijatko, I Sacer and I Stokoic, 2010. Malondialdehyde levels in serum of dogs infected with Babesia canis. Vet Med, 55: 163-171.

Dede S, Y Değer, S Değer and P Tanrıtanır, 2008. Plasma Levels of Zinc, Copper, Copper/Zinc Ratio, and Activity of Carbonic Anhydrase in Equine Piroplasmosis. Biolog Trace Elem Res, 125: 41-45.

Deger S, K Biçek and Y Deger, 2005. The changes in some of biochemical parameters (Iron, Copper, Vitamin C and Vitamin E) in infected cattle with Theileriosis. The Journal of the Faculty of Veterinary Medicine University Of Yuzuncu Yil 16: 49-50.

Deger S, Y Deger, K Bicek, N Ozdal and A Gul, 2009. Status of lipid peroxidation, antioxidant and oxidation products of nitric oxide in equine babesiosis: Status of antioxidant and oxidant in equine babesiosis. Journal of Equine Veterinary Science, 29: 743-747.

De Gopegui RR, B Penalba, A Goicoa, Y Espada, LE Fidalgo and L Espino, 2007. Clinico-pathological findings and coagulation disorders in 45 cases of canine babesiosis in Spain. Vet J, 174: 129-132.

De Waal DT 1992. Equine Piroplasmosis: A review. Brit Vet J, 148: 6-14.

De Waal DT, J Van Heerden and FT Potgieter, 1987. An investigation into the clinical pathological changes and serological response in horses experimentally infected with Babesia equi and babesia caballi. Onderstepoort J Vet Res, 54: 561-568.

Ece A, BS Uyamik, A Iscan, P Ertan and MR Yigitoglu, 1997. Increased serum copper and decreased serum zinc levels in children with iron deficiency anemia. Biolog Trace Elem Res, 59: 31-39.

Esmaeilnejad B, M Tavassoli, S Asri-Rezaei, B Dalir-Naghadeh and H Malekinejad, 2012. Status of lipid peroxidation and antioxidant enzymes in goats naturally infected with Babesia ovis. Acta Parasitologica, 57: 228-234.

Esterbauer H, 1996. Estimation of peroxidative damage: a critical review. Pathologie Biologie (Paris), 44: 25-28.

Feldman BF, JG Zinkl, C Jain and PE Gasper, 2000. Schalm's veterinary Hematology, 5th edn., Wiley-Blackwell Publishing, Ames, Iowa, USA.

Friedhoff KT, AM Tenter and I Muller, 1990. Haemoparasites of equines: impact on international trade of horses. Revue scientifique et technique de l'OIE, 9: 1187-1194

Grandi G, G Molinari, M Tittarelli, D Sassera and LH Kramer, 2011. Prevalence of Theileria equi and Babesia caballi infection in horses from northern Italy. Vector borne and zoonotic diseases (Larchmont, N.Y.), 11: 955-956

Gürgöze MK, A Olçücü, AD Aygün, E Taskin and M Kiliç, 2006. Serum and hair levels of zinc, selenium, iron, and copper in children with iron-deficiency anemia. Biolog Trace Elem Res, 111: 23-29.

Healy J and K Tipton, 2007. Ceruloplasmin and what it might do. J Neural Transm, 114: 777-781.

Hussain MH, M Saqib, F Raza, G Muhammad, MH Asi, MK Mansoor, M Saleem and A Jabber, 2014. Seroprevalence of Babesia Caballi and Theileria equi in five draught equine populated metropolis of Punjab, Pakistan. Vet Parasitol, 202: 248-256.

Hussein AH, NAES Mohammed and HK Mohammed, 1997. Theileriosis and babesiosis in cattle: haemogram and some biochemical parameters. IXth International Congress on Animal Hygiene, Helsinki, Finland

Ibrahim AK, IS Gamil, AA Abd-El baky, MM Hussein, AA Tohamy, 2011. Comparative Molecular and Conventional Detection Methods of Babesia equi (B. Equi) in Egyptian Equine. Global Veterinaria, 7: 201-210

Ike K, K Takeuchi, Y Uchida and S Imai, 2005. Hematological findings and antibody responses in Syrian hamster (Mesocricetus auratus) infected with Babesia microti. Journal of Veterinary Med Science, 67: 457-460.

Leschnik M, G Kirtz, A Tichy and E Leidinger, 2008. Seasonal occurrence canine babesiosis is influenced by local climate conditions. Inter J Med Microbiol, 298: 243-248.

Karatepe B, M Karatepe, A Cakmak, Z Karaer and G Ergün, 2009. Investigation of seroprevalence of Theileria equi and Babesia caballi in Nigde province, Turkey. Trop Anim Health Prod, 41: 109-113.

Kohen A and HH Limbach, 2005. Isotope effects in chemistry and biology, CRC press, Boca Raton, Florida.

Kouam MK, V Kantzoura, AA Gajadhar, JH Theis, E Papadopoulos and G Theodoropoulos, 2010. Seroprevalence of equine piroplasms and host-related factors associated with infection in Greece. Vet Parasitol, 169: 273-278.

Kumar A, JP Varshney and RC Patra, 2006. A comparative study on oxidative stress in dogs

infected with Ehrlichia canis with or without concurrent infection with Babesia gibsoni. Vet Res Commun, 30: 917-920.

Kuttler KL, 1988. World-wide impact of Babesiosis. In: M. Ristic (eds.), Babesiosis of domestic animals and man, CRC press, Boca Raton, Florida, USA, pp: 1-22.

Mates JM, 2000. Effects of antioxidant enzymes in the molecular control or reactive oxygen species toxicology. Toxicology, 153: 83-104.

Merck publishing and Merial 2007. Blood disorder of horses: anemia in horses, Merck manual for pet health.

Moore K and LJ Roberts, 1998. Measurement of lipid peroxidation. Free Radical Research, 28: 659-671.

Morris DD, 2006. The hemolymphatic sytem. In: AJ Higgens and JR Snyder (eds), The equine Manual.

Murase T, T Ueda, O Yamato, M Tajima and Y Maede, 1996. Oxidative damage and enhanced erythrophagocytosis in canine erythrocytes infected with Babesia gibsoni. J Vet Med Sci, 58: 259-261.

Nagore D, J Garcia-Sanmartin, AL Garcia-Perez, RA Juste and A Hurtado, 2004. Detection and identification of equine Theileria and Babesia species by reverse line blotting: Epidemiological survey and phylogenetic analysis. Vet Parasitol, 123: 41-54.

Nazifi S, SM Razavi, M Mansourian, B Nikahval and M Moghaddam, 2008. Studies on correlation among parasitaemia and some hemolytic indices in two tropical diseases (Theileriosis and Anaplasmosis) in Fars province of Iran. Trop Anim Health Prod, 40: 47-53.

Nazifi S, SM Razavi, F Hasanhani and Z Esmailnezad, 2009. Effect of the severity of Theileria annulata infection on some hematological parameters and antioxidant enzymes in naturally infected cattle. Bulg J Vet Med, 12: 63-71.

Nuttal GHF, C Waraburton C, WF Cooper and IE Robinson, 1908. Ticks, a monograph of Ixodoidea, - part I, Cambridge university press.

Olwoch JM, AS Van Jaarsveld, CH Scholtz and IG Horak, 2007. Climate change and the genus Rhipicephalus (Acari: Ixodidae) in Africa. Onderstepoort J Vet Res, 74: 45-72.

Rothschild CM and DP Knowles, 2007. Equine Piroplasmosis. In: DC Sellon, MT Long (eds.), Equine infectious diseases Saunders, Elseiver, St. Louis, Mo, p: 465-473.

Saleh MA, 2009. Erythrocytic oxidative damage in crossbred cattle naturally infected with Babesia bigemina. Res Vet Sci, 86: 43-48.

Salib FA, RR Youssef, LG Rizk and SF Said, 2013. Epidemiology, diagnosis and therapy of Theileria equi infection in Giza, Egypt. Vet World 6: 76-82

Sandhu GS, 1996. Histopathological, biochemical and hematological studies in cross-bred calves and hematological studies in cross bred calves, M.V.Sc. Thesis, Punjab Agricultural University, Ludhiana.

Scott ME and KG Koski, 2000. Zinc deficiency impairs immune responses against parasitic nematode infections at intestinal and systemic sites. J Nutr, 130:1412S-1420S.

Shkap V, I Cohen, B Leibovittz, E Savitsky Pipano, G Avni, U Giger, L Kappmeyer and D Knowles, 1998. Seroprevalence of Babesia equi in Israel using competitive inhibition Elisa and IFA assays. Veterinary Parasitology, 76: 251-259.

Sullivan JL and HD Ochs, 1978. Copper deficiency and immune system. Lancet, 312: 686.

Tripp BC, K Smith and JG Ferry, 2001. Carbonic anhydrase: new insights for an ancient enzyme. J Biolog Chem, 276: 4861-4868.

Turgut S, A Polat, M Inan, G Turgut, G Emmungil, M Bican, TY Karakus and O Genc, 2007. Interaction between anemia and blood levels of iron, zinc, copper, cadmium and lead in children. The Indian J Pediatrics, 74: 827-830.

Walker JB, JE Keirans and IG Horak, 2000. The Genus Rhipicephalus (Acari, Ixodidae), A Guide to the Brown Ticks of the World, Cambridge Univ Press, p: 643.

Wellinghausen N, W Jöchle, S Reuter, WA Flegel, A Grünert and P Kern, 1999. Zinc status in patients with alveolar echinococcosis is related to disease progression. Parasite Immunol, 21: 237-241.

Wirth JJ, PJ Fraker, F Kierszenbaum, 1989. Zinc requirement for macrophage function: effect of zinc deficiency on uptake and killing of protozoan parasite. Immunology, 68: 114-119.

Wise LN, AM Pelzel-McClusky, RH Mealey and Knowles, 2013. Review of Equine Piroplasmosis. J Vet Inter Med, 27: 1334-1346.

Young AS and BL Leitch, 1981. Production of Rhipicephalus appendiculatus with high infections of Theileria parva. J Parasitol, 67: 751-752.

Zobba R, M Ardu, S Niccolini, B Chessa, L Manna, R Cocco, M-L P Parpaglia, 2008. Clinical and Laboratory Findings in Equine Piroplasmosis. J Equine Vet Sci, 28: 301-308.

Zygner W, O Gojska, G Rapacka, D Jaros and H Wedrychowicz, 2007. Hematological changes during the course of canine babesiosis caused by large Babesia in domestic dogs in Warsaw (Poland). Vet Parasitol, 145: 146-151.