

Serum Zinc, Iron and Copper Concentrations in Dogs Infected with *Hepatozoon canis*

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Abstract

In Turkey, canine hepatozoonosis is an emerging infection with a large number of cases detected during the past five years. In the present study, serum zinc, copper and iron concentrations of dogs infected with *Hepatozoon canis* were measured for the first time. Compared to the controls (n = 10), serum zinc and iron concentrations in infected animals (n = 14) decreased significantly ($p < 0.05$ and $p < 0.01$, respectively), whereas the concentrations of copper in diseased animals increased significantly ($p < 0.05$). In conclusion, the results of the present study indicate that *Hepatozoon canis* infection may cause alterations in serum zinc iron and copper concentrations. Furthermore, in the treatment of infected animals addition of zinc and iron to the ration of infected animals should be taken into consideration.

Hepatozoonosis, dog, trace minerals

Canine hepatozoonosis afflicts dogs in Asia, Africa, Southern Europe, South America and North America (Forlano et al. 2005). The main vector of the disease is the brown dog tick, *Rhipicephalus sanguineus* (Baneth et al. 2001). In Turkey, canine hepatozoonosis was reported for the first time by Tüzdil (1933) and it is expanding on the Aegean coast. *H. canis* lives in several cell types (such as muscular, hepatic or haematic) of canids and felids. Infection has been reported to be associated with a variety of clinical signs ranging from sub-clinical infection to a severe life threatening disease with distinct haematological, biochemical and histopathological alterations (Baneth and Weigler 1997; Criado-Fornelio et al. 2007; Eiras et al. 2007; Gavazza et al. 2003). Fever, anorexia, weight loss, anaemia, ocular discharge and weakness of the limbs are some of the clinical signs (Baneth and Weigler 1997; Criado-Fornelio et al. 2007; Forlano et al. 2005). Haematological and biochemical abnormalities are anaemia, leukocytosis, thrombocytopenia, hypoalbuminaemia, hyperglobulinaemia and increased serum alkaline phosphatase activity (Baneth and Weigler 1997). Histopathologically parasite-containing lesions can be found in many tissues such as in striated muscle, liver, lymph nodes, spleen and in pancreas (Ewing and Panciera 2003).

Studies on most micronutrients are complicated by the fact that the nutrient is multi-functional. For example, zinc is required in over 200 enzymes and so deficiency is likely to affect a number of different systems (Evans and Halliwell 2001). Zinc contributes to structure and function of important metalloenzymes, including carbonic anhydrase, alkaline phosphatase, RNA and DNA polymerases, and alcohol dehydrogenase (Gabor 1989). T-cell mediated responses are critical for host protection against parasitic infections (Shi et al. 1998) and any impairment of T cells or the cells that present antigen to them, caused by zinc deficiency, is therefore expected to cause a decrease in host protection. Yet, surprisingly few studies have examined the effect of dietary zinc deficiency on host-parasite interactions (Shi et al. 1998).

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Iron is a component of a number of metabolic enzymes, so it is needed for basic metabolic activities of cells (Miller 1985). Even for intracellular survival of some parasites an adequate supply of iron is required (Marquis and Gros 2007). Iron is a co-factor for superoxide dismutase, which protects cells against toxic oxygen species (Marquis and Gros 2007). In mammalian cells, iron is mostly present as poorly soluble Fe^{3+} , which is tightly bound to transferrin for transport or to ferritin for storage (Marquis and Gros 2007). Iron is also essential as a component of haemoglobin in red blood cells to prevent anaemia.

Copper is an essential trace element for all organisms, due to its action as a co-factor in many enzymes, but it is also potentially toxic because, like iron, copper can donate or receive electrons (Rasolomon et al. 2004). Copper is required for caeruloplasmin, which loads iron onto transferrin (Evans and Halliwell 2001). Increased plasma copper concentrations have been found in infectious processes; thus, following serum Cu concentrations is a useful monitor for some diseases (Pasa et al. 2003; Schroeder et al. 1967).

On the Aegean coast, canine hepatozoonosis is an emerging infection with a large number of cases detected during the past recent years (Karagenc et al. 2006). To the best of our knowledge, the serum concentrations of trace elements have not been reported previously in dogs infected with *H. canis*. To this end, in the present study, we intended to measure serum zinc, copper and iron concentrations in dogs infected with *H. canis*.

Materials and Methods

Animals

Fourteen dogs infected with *H. canis* along with 10 healthy dogs were used in this study. All the animals, aged two to eight years, were mixed-breed dogs. Diagnosis of the infection was performed by microscopical examination of gametocytes in neutrophil-like cells in peripheral blood as well as using molecular techniques including the polymerase chain reaction (PCR) and sequence analyses in the first part of this study (Kiral et al. 2005) related information was given explicitly. Twelve of the diseased dogs were female and two were male. Seven of the control animals were female and three were male. All of the infected dogs showed some or all of the clinical signs of the disease, including fever, lethargy, weight loss, stiffness, and signs of pain, paralysis and ocular discharge.

The study was approved by the Adnan Menderes University Animal Use and Care Committee.

Biochemical analysis

Blood samples were taken from the cephalic vein and centrifuged at 2,400 g for 10 min. The serums were collected in plastic tubes and stored at -20 °C until analyses. Serum zinc, iron and copper concentrations were measured using commercial available kits (Randox Laboratories, UK) with a spectrophotometer (Shimadzu, UV-1601). The analyses were carried out according to the manufacturer's instructions.

Statistical Analysis

The data were analysed using Student's *t*-test and Pearson correlation coefficient (SPSS Version 12.0), and $P < 0.05$ was considered significant.

Results

As shown in Table 1, the mean serum concentrations of zinc in healthy animals were 62.05 ± 7.59 µg/dl. Serum concentrations of zinc in diseased animals, however, decreased significantly ($p < 0.05$) to 36.33 ± 8.02 µg/dl. Similarly, a significant ($p < 0.01$) decrease in iron concentrations of infected animals was observed. The mean serum iron levels of infected animals declined from 86.03 ± 19.88 µg/dl to 57.62 ± 7.46 µg/dl. Serum copper concentrations in diseased animals, however, increased significantly ($p < 0.05$) from 100.60 ± 1.18 µg/dl to 135.67 ± 8.67 µg/dl.

Table 1. Comparison of some serum trace elements in dogs with *Hepatozoon canis* and healthy animals

Indicators	N	Controls	n	<i>Hepatozoon canis</i>	P
Zinc (µg/dl)	10	62.05 ± 7.59	14	36.33 ± 8.02	< 0.05
Iron (µg/dl)	10	86.03 ± 19.88	14	57.62 ± 7.46	< 0.01
Copper (µg/dl)	10	100.60 ± 1.18	14	135.67 ± 8.67	< 0.05

* $P < 0.05$, ** $P < 0.01$

Discussion

There is considerable evidence for interactions between parasitic diseases and essential trace elements (Filteau and Tomkins 1990). Two general types of abnormality of trace element status can be identified: a specific deficiency arising from dietary inadequacies (Forlano et al. 2007) and imbalance resulting from the disease (Forlano et al. 2005). In both cases, trace element status can be adequately assessed by serum elemental analysis (Klasing 1988).

Infection with *H. canis* causes a distinct clinical syndrome characterised by fever, anorexia, weight loss, anaemia, ocular discharge and weakness of the limbs (Baneth et al. 2003; Vincent-Johnson et al. 1997). The ability of animals to cope with parasitic infection may be influenced by mineral nutrition, in particular Zn, Cu and Fe of the trace elements (Miller 1985). Deficiency of some trace minerals may weaken host protection and result in elevated parasite numbers (Shi et al. 1998). The extent of the impairment in host protection due to trace element deficiency can be sufficient to increase the risk of morbidity and mortality due to parasitic infections and reversal of trace element deficiency may restore impaired host protection. However, the serum concentrations of trace elements have not been reported previously in dogs infected with *H. canis*. This might be in part due to the relatively low incidence of hepatozoonosis.

Low serum zinc levels have been reported in parasitic diseases (Tasci et al. 1995). Decreased zinc concentrations in diseased animals found in the present study are in line with this finding. According to previous reports, parasites in the host are dependent on zinc for many metabolic and biochemical pathways (Scott and Koski 2000). For example, some parasitic nematodes have exceptionally high concentrations of zinc-dependent superoxide dismutase (Clark et al. 1986), and schistosome worms have been shown to have particularly high uptakes of zinc relative to host tissue, suggesting that they may have a particularly high need for this molecule, so the reason of low levels of zinc in infected dog may be due to the high uptake of zinc by *H. canis*. However, on the other hand, dogs infected with *H. canis* are at risk of protein-energy malnutrition, as well as deficiencies in micronutrients including zinc. Therefore, it is not clear whether depression in serum zinc concentrations in infected animals originated from malnutrition or from the high uptake of zinc by *H. canis*. This question needs to be pursued.

The trace element concentrations in serum samples change during infection (Pasa et al. 2003; Wagner et al. 1991). These changes are part of the defence strategies of the organism, induced by IL-1, IL-6, and TNF- α (Klasing 1988; Wagner et al. 1991). The removal of iron from circulation results from the production of apolactoferrin removing Fe-transferrin and sequestering iron in compartments that are nutritionally unavailable to parasites. On the other hand, it is reported that iron is needed for basic metabolic activity but is also required as a co-factor for superoxide dismutase, which protects certain intracellular parasites against toxic oxygen species generated by the phagocyte NADPH oxidase (Marquis and Gros 2007). However, from this statement it cannot be concluded whether the reason of the decreased serum iron level in infected animals is the use of iron by the intracellular parasite or its diversion to other parts of the body. We suggest that studies be conducted to investigate in more depth the mechanisms by which iron concentrations declined.

Contrary to the decreased levels of zinc and iron, a significant increase in serum copper concentration of sick animals was observed. Although plasma copper concentrations are commonly used as a surrogate marker of copper status in many clinical trials, plasma copper represents a very small amount of total body copper stores, and there is a poor correlation between plasma copper concentrations and body stores (Jackson 1989). To this end, increased levels of serum copper may be explained with a relocation of copper, decrease in stores and reciprocal increase in circulating copper. Furthermore, increased

serum copper concentration in infected animals observed in the present study is consistent with the previous findings that host leukocyte-induced metabolic changes resulting in an increase of serum copper (Delacruz et al. 1993; Pasa et al. 2003).

In conclusion, alterations in serum zinc, iron and copper concentrations in diseased animals may depend on *H. canis* infection. However, studies concerning the alterations in concentrations of trace elements in parasitic diseases are complicated by the fact that the trace elements are multi-functional. To this end, the monitoring of trace element concentrations in infected animals especially with treatment opportunity may be helpful to clarify the reason of alterations in trace elements in dogs with *H. canis*.

Sérové koncentrace zinku, železa a mědi u psů s onemocněním *Hepatozoon canis*

Hepatozoonóza psů je v Turecku novým onemocněním, jehož výskyt byl v posledních pěti letech detekován u značného počtu jedinců. V této studii byla poprvé sledována sérová koncentrace zinku, mědi a železa v souvislosti s infestací psů druhem *Hepatozoon canis*. V porovnání s kontrolní skupinou psů ($n = 10$), byly u nakažených jedinců ($n = 14$) sníženy sérové koncentrace zinku ($p < 0.05$) a železa ($p < 0.01$), zatímco koncentrace mědi v séru se výrazně zvýšila ($p < 0.05$).

Výsledky naší studie dokazují, že nákaza *Hepatozoon canis* může vyvolat změny sérových koncentrací zinku, železa a mědi. Zároveň je vhodné vzít v úvahu možnost přidávat železo a zinek do krmné dávky během terapie psů nakažených hepatozoonózou.

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