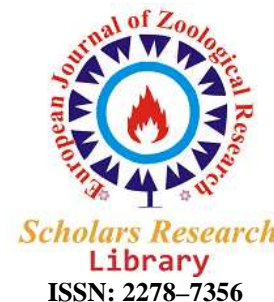




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Comparative study of serum zinc, copper and selenium in horses with strangles and healthy horses

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ABSTRACT

Strangles disease which was caused by *Streptococcus equi* is one of the common infectious diseases among horses that annually affect many horses in different regions. Role of elements such as zinc, copper and selenium in the immune system and antioxidant activity in horses has been distinctly determined. The objective of this study was to evaluate the serum profile of zinc, copper, ceruloplasmin and selenium in horses with Strangles and to compare these values with healthy horses. 21 horses with Strangles based on clinical signs and laboratory-based experiments (culture from respiratory secretions and fluids draining lymph nodes) was identified from horse clubs around Tabriz-Iran and after taking a complete history of each horse, blood samples were obtained from jugular vein and after clotting, serum was separated. Simultaneously, blood samples of 18 healthy horses were taken. Mean concentrations of zinc of serum in Strangles affected horses were significantly lesser than control group ($P=0.001$). mean concentrations of copper and ceruloplasmin of serum of Strangles affected horses were more than healthy horses, this increase was NOT significant in the case of copper but was significant in the case of ceruloplasmin ($P=0.547$ and $P=0.002$, respectively). Mean concentrations of selenium was similar to zinc and suggested significant decrease in horses with Strangles ($P=0.003$). In the group of sick horses we observed increase in serum copper with increase in ceruloplasmin and this correlation was significant ($P=0.002$ and $r=0.938$) but, in the group of healthy horses this increase was NOT significant. Mean of any of the measured parameters between the different age groups showed no significant difference in the patient group and there was not any correlation between age of sick horses and mean concentrations of above elements. The eventual result is that in horses with Strangles mean serum values of zinc and selenium is reduced and ceruloplasmin as a inflammatory factor is amplified and in treatment of these animals injection of supplemental components with zinc and selenium is recommended.

Key word: copper, zinc, selenium, strangles, horse

INTRODUCTION

Strangles is an acute illness caused by infectious agent, *Streptococcus equi*. Acute inflammation of upper respiratory tract and purulent lymph nodes are some of its characteristics. The distribution of this disease is universally, however, decrease in number of horses and advances in treatment methods made this disease less significant in most countries. Virulence of the disease had been observed in military stables and stables of draft horses that nowadays is reduced. Now, small outbreaks can be seen in bat horses, race horses and horse-riding schools [1-3]. Causative agent is present in nasal discharge and pus of boils. Only horses are susceptible to this disease and although they might be infected at any age but are more sensitive at 1 to 5 years. The disease may occur at any season of the year, but the cold weather and wet conditions are more likely. *Streptococcus equi* is relatively resistant in environmental conditions; in this regard indirect transfer in infectious stables even after one month can be anticipated. Infection of nasal and pharyngeal mucos, acute inflammation of nose and pharynx and infiltration of the microbe to lymph

nodes cause suppurate them and might create purulent foci in different organs such as kidneys, brain, liver, spleen and joints [4-6]. Infections of upper respiratory tract coincide with purulent nasal discharge and enlarged lymph nodes of the throat are the characteristic of strangles. In initial stages of the disease these symptoms can be confused with rhinitis, viral pneumonia, viral arthritis and tracheitis but in mentioned disorders lymph nodes enlargement is not as much as in strangles [7,8]. The role of selenium and vitamin E as an antioxidant is traditionally considered and supplemental selenium may have a protective role in animals. Zinc is a trace element for animals that must be supplied through diet. The role of this element in structure of several enzymes like lipase, superoxide demutase, alkaline phosphatase and etc, is very important [9,10]. Role of zinc in the conservation of the insulin molecule, Kreteogenesis, protection from effects of stress, prevent degeneration of the testis, strengthens the immune system, enhancement of fertility in animals and etc, is distinctly considered [11-15]. Also, the role of copper in superoxide desmutase and cytochrome oxidase activity, synthesis of myelin and cutaneous pigments and keratinization has been demonstrated [16-18]. These three elements have significant role in luster of the outer coating and strength of the hoof. It seems that in this condition (Strangles) because of inappetence, serum levels of this element will be faced with deficiency [11,13]. This study was carried out to evaluate serum levels of zinc, copper and selenium in Strangles affected horses and to compare these values with healthy horses.

MATERIALS AND METHODS

This study was on 21 horses with Strangles in equestrian clubs around Tabriz in one year (December 1390 to December 1391). Sickness of horses was confirmed by clinical and laboratory signs (culture from respiratory secretions and fluids draining lymph nodes). After taking a complete history of each horse, blood samples were obtained from jugular vein and after clotting, serum was separated. Simultaneously, blood samples of 18 healthy horses with exactly the same age, ration and management were taken. In serum samples, levels of selenium, copper and zinc were measured by atomic absorption and ceruloplasmin levels were measured by spectrophotometer and Sunderman and Nomoto biochemical method.

Methods of statistical analysis

For data analysis we used SPSS software and for the comparison of means between two groups Ttest was used. In order to compare age groups ANOVA statistical method was used and Correlation method was used to determine relationships between parameters.

RESULTS

Mean serum zinc levels in the group of horses with Strangles (21 horses) were 84.98 ± 1.15 $\mu\text{g/dl}$ and in control group (18 horses) were 93.00 ± 1.27 $\mu\text{g/dl}$ and the difference of means between the two groups was significant ($P=0.001$) (table 1 and figure 1).

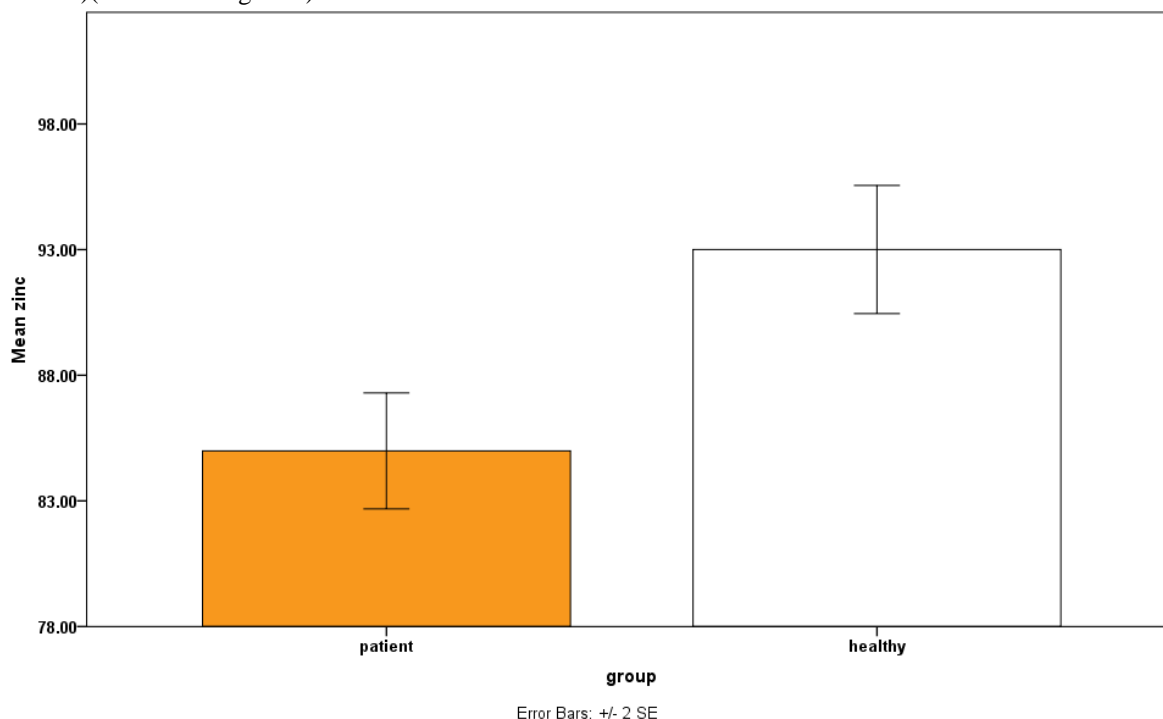


Figure 1: Mean serum zinc levels in Strangles affected horses and healthy horses

Mean serum copper levels in the group of horses with Strangles was significantly more than control group ($P=0.547$). These values were $74.45 \pm 1.76 \mu\text{g/dl}$ and $73.13 \pm 1.26 \mu\text{g/dl}$, respectively (table 1 and figure 2).

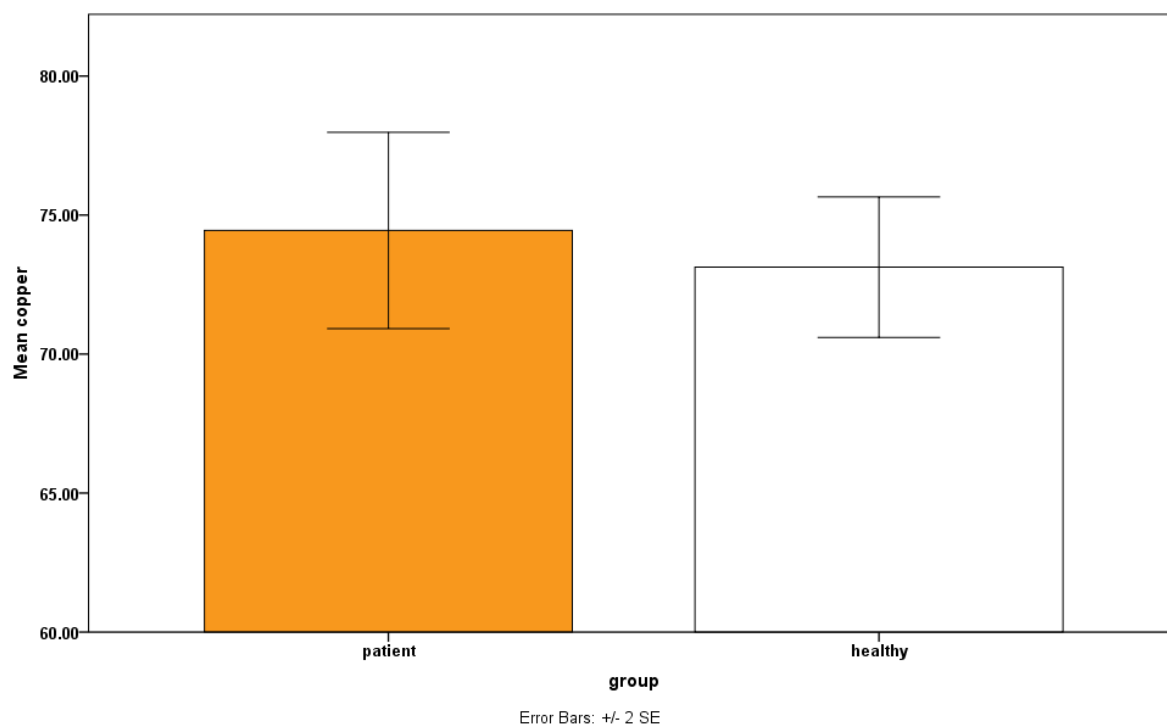


Figure 2: Mean serum copper levels in Strangles affected horses and healthy horses

Mean serum ceruloplasmin levels increased in horses with Strangles, so that the value in horses with Strangles was $66.21 \pm 2.34 \text{ mg/dl}$ and in control group was $49.37 \pm 2.93 \text{ mg/dl}$. This increase was Not statistically significant ($P=0.002$) (table 1 and figure 3).

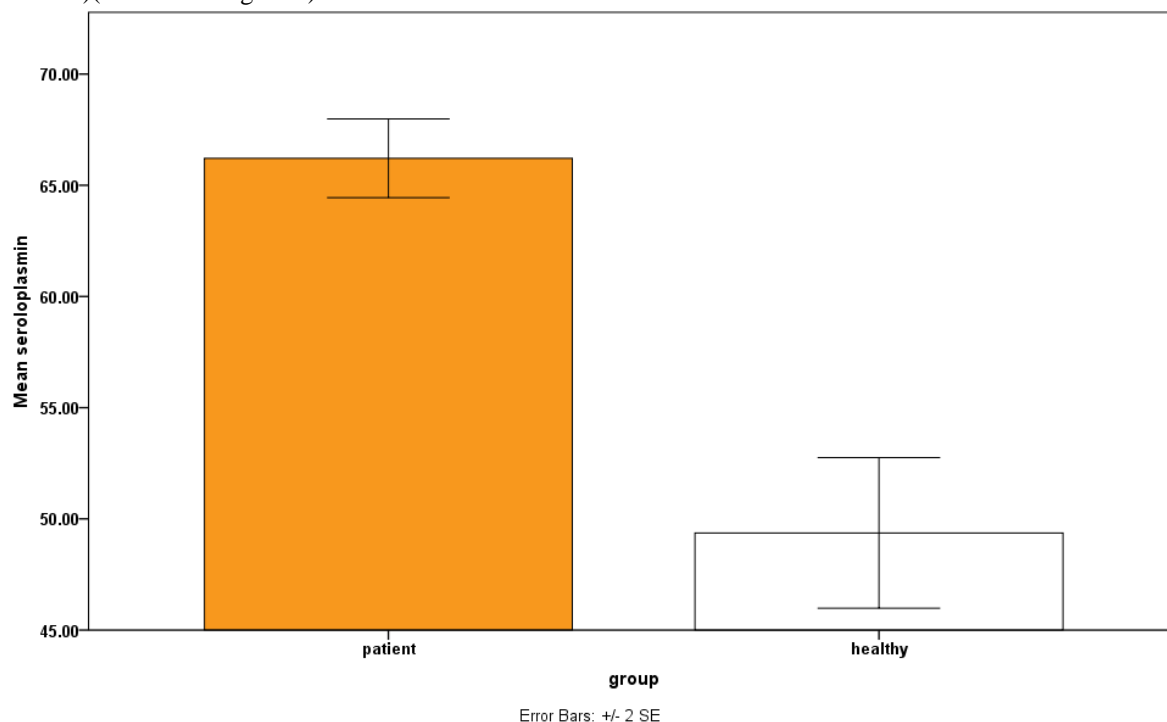


Figure 3: Mean serum ceruloplasmin levels in Strangles affected horses and healthy horses

Mean serum selenium levels in patient horses was $124.85 \pm 2.22 \text{ ng/l}$ and in the control group was $134.21 \pm 1.99 \text{ ng/l}$. Difference of means of serum selenium level was statistically significant ($P=0.003$) (table 1 and figure 4).

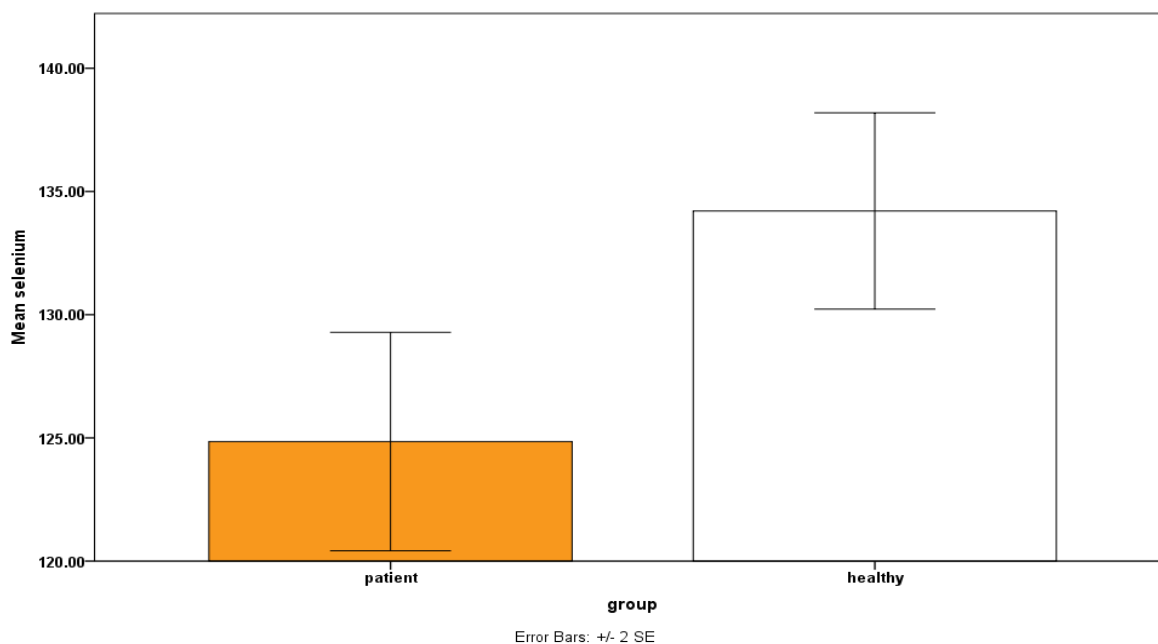


Figure 4: Mean serum selenium levels between group of patients and control group

Table 1: Comparison of mean serum zinc, copper, ceruloplasmin and selenium in horses with Strangles and control group

Serum parameter	group	number	mean	Standard deviation	P value
zinc($\mu\text{g/dl}$)	Patient	21	84.98	1.15	0.001
	healthy	18	93.00	1.27	
copper($\mu\text{g/dl}$)	Patient	21	74.45	1.76	0.547
	healthy	18	73.13	1.26	
ceruloplasmin (mg/dl)	Patient	21	66.21	2.34	0.002
	healthy	18	49.37	2.93	
(ng/l)selenium	Patient	21	124.85	2.22	0.003
	healthy	18	134.21	1.99	

In examining the correlation between serum copper and ceruloplasmin in horses with Strangles, this fact was determined that a significant relationship exists. The increase in serum copper coincides with ceruloplasmin increase ($P=0.002$ and $r=0.938$). In the control group, with an increase in serum copper levels, ceruloplasmin increased, but this increase and correlation was not statistically significant ($P=0.166$ and $r=0.367$).

Table 2: correlation between serum copper and ceruloplasmin in patient and control groups

group	Mean serum copper	Mean serum ceruloplasmin	Correlation coefficient	P value
Patient	74.45 \pm 1.76	66.21 \pm 2.34	0.938	0.002
control	73.13 \pm 1.26	49.37 \pm 2.93	0.367	0.166

Horses with Strangles were at three age groups, 1-3 years ($n=8$), 4-6years ($n=9$) and more than 9 years ($n=4$). Mean serum zinc levels in mentioned groups were $84.41\pm 2.03 \mu\text{g/dl}$, $86.33\pm 1.47 \mu\text{g/dl}$, $83.12\pm 3.39 \mu\text{g/dl}$, respectively. The difference of means between these three groups was NOT significant ($P=0.581$) (table 3 and figure 5).

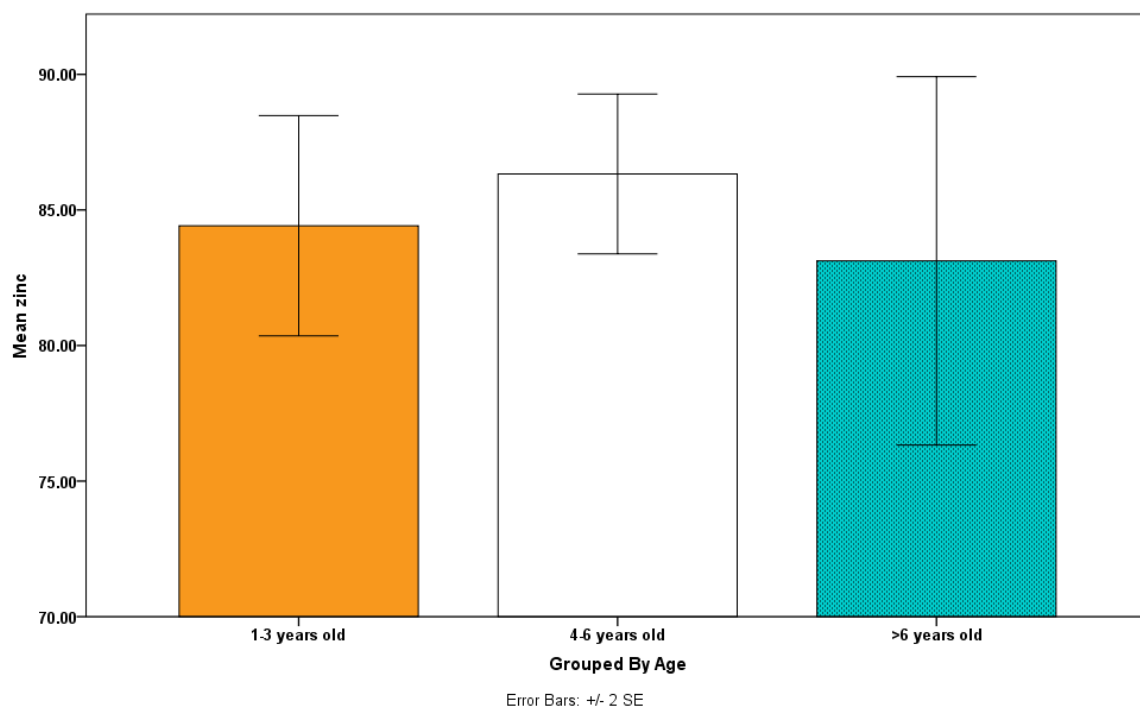


Figure 5: Mean serum zinc levels in horses with Strangles in different age groups

Mean serum copper levels among different age groups in the Strangles affected group showed no significant changes ($P=0.228$). These means in 1-3 years group was 70.83 ± 1.56 $\mu\text{g/dl}$, in 4-6 years group was 76.57 ± 3.62 $\mu\text{g/dl}$ and in more than 6 years group was 76.89 ± 2.83 $\mu\text{g/dl}$ (table 3 and figure 6).

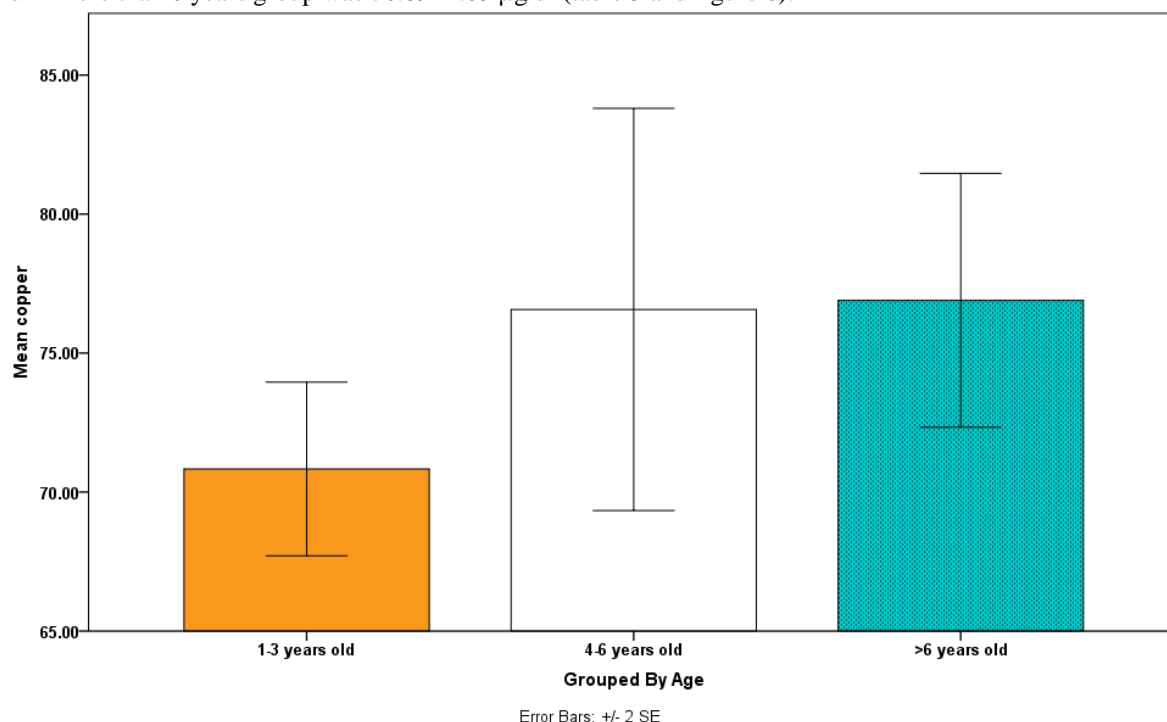


Figure6: mean serum copper levels in horses with Strangles in different age groups

In horses with Strangles mean serum ceruloplasmin levels among different age groups were 66.62 ± 1.27 mg/dl , 62.04 ± 1.82 mg/dl and 67.51 ± 2.58 mg/dl , respectively. These differences in means among different age groups was NOT significant ($P=0.122$)(table 3 and figure 7).

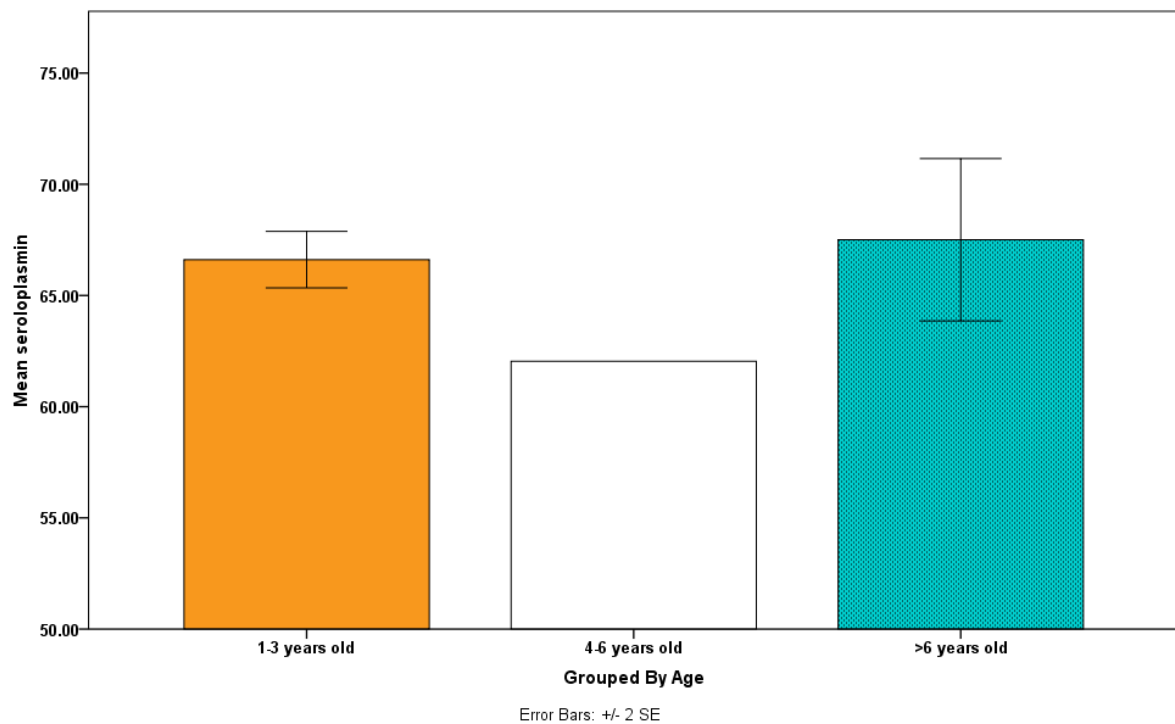


Figure7: mean serum ceruloplasmin levels in horses with Strangles at different age groups

In Strangles affected horses mean serum selenium levels in three age groups were 125.82 ± 3.46 ng/l, 121.99 ± 3.37 ng/dl and 129.36 ± 5.83 ng/dl, respectively. Difference of means among groups was Not significant ($P=0.478$)(table 3 and figure 8).

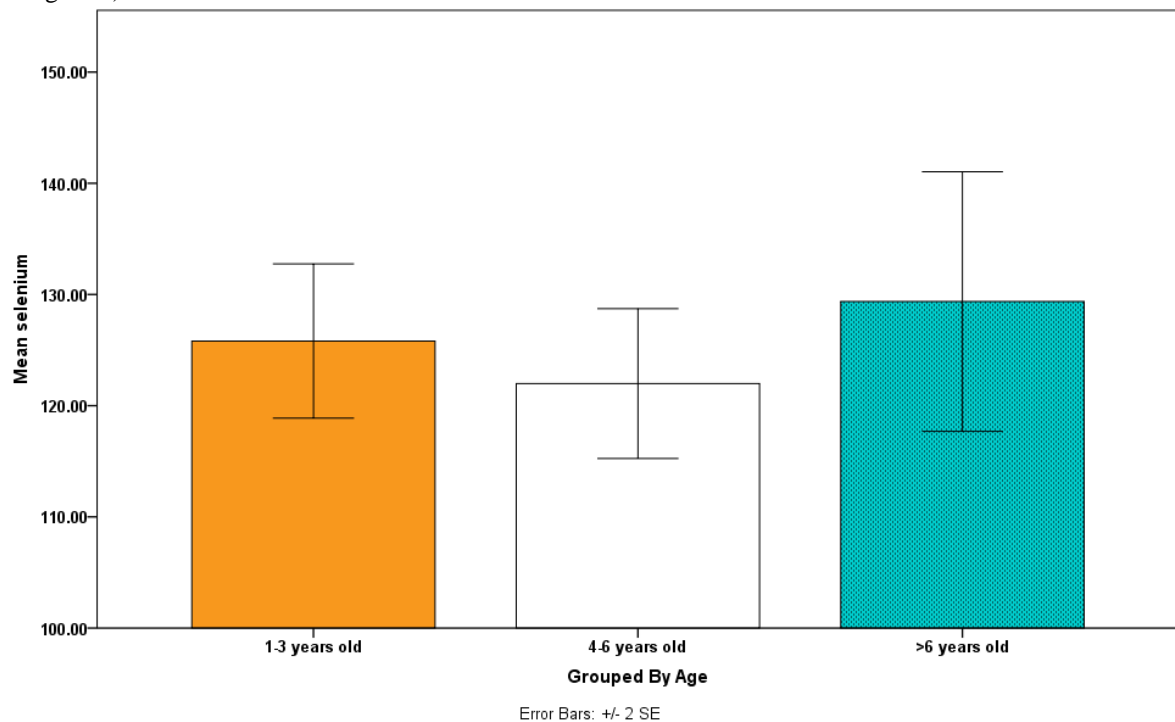


Figure8: mean serum selenium levels in horses with Strangles in different age groups

Table3: Comparison of mean serum zinc, copper, ceruloplasmin, and selenium in horses with Strangles dissociated by different age groups

serum parameters	Age group	Number	Mean	Standard deviation	P value
zinc($\mu\text{g/dl}$)	1-3years	8	84.41	2.03	0.581
	4-6years	9	86.33	1.47	
	>6years	4	83.12	3.39	
copper($\mu\text{g/dl}$)	1-3years	8	70.83	1.56	0.228
	4-6years	9	76.57	3.62	
	>6years	4	76.89	2.83	
ceruloplasmin (mg/dl)	1-3years	8	66.62	1.27	0.122
	4-6years	9	62.04	1.82	
	>6years	4	67.51	2.58	
selenium(ng/l)	1-3years	8	125.82	3.46	0.478
	4-6years	9	121.99	3.37	
	>6years	4	129.36	5.83	

DISCUSSION

Based on the results of the experiments it was shown that serum zinc and selenium levels decrease and serum ceruloplasmin levels increase in Strangles. In this study it is determined that mean serum zinc levels in horses with Strangles is significantly lesser than healthy horses ($P=0.001$). Mean serum copper levels showed no significant changes in this experiment. In this study it was found that ceruloplasmin as an acute phase inflammatory protein increases in horses with Strangles ($P=0.002$). In our study, serum levels of zinc were reduced but no significant changes was seen in copper levels. In a study of horses infected with equine herpes virus type I mean levels of serum zinc and copper decreased in compare with normal horses but there was an increase in serum iron levels. And there were no significant changes in serum cobalt between two groups [19]. Serum copper levels in patients with liver fibrosis caused by hepatitis B virus have been reported more than in normal subjects [20,21]. And also has been reported in patients with AIDS [15,22]. In calves with pneumonia a decrease in serum zinc and copper levels has been suggested [18]. The results of our study are consistent with the mentioned studies to some extent.

In one study diets of pregnant mare's supplemented with supplements containing copper, zinc, iron, manganese, cobalt, iodine and selenium which caused an increased blood levels of these elements and subsequently increased concentration of these elements in milk. In this study it was found that foals born also have high serum levels of iron, zinc, copper and cobalt [23]. In a survey of infectious disease in the Brazilian water buffalo serum copper, phosphorus, cobalt and zinc were measured and a significant increase in serum copper and zinc has been reported[24]. In the group of infected horses an increase in serum ceruloplasmin levels was coincide with an increase in serum copper levels and this was a positive and significant correlation($P=0.002$ and $r=0.938$). But in the group of healthy horses this correlation was not significant ($P=0.166$ and $r=0.367$). In a study of the newly born foals, serum ceruloplasmin levels was 17 ± 0.8 mg/dl and in seventeen months old foals it was 43.7 ± 5.8 mg/ml. Mean serum zinc levels in the mentioned times was 73.2 ± 13.1 $\mu\text{g/dl}$ and 38.3 ± 5.9 $\mu\text{g/dl}$, respectively[25]. In this study it was found that selenium levels in horses with Strangles is significantly lower than in healthy horses ($P=0.003$). Antioxidant actions of selenium are apparently obvious and it seems that in Strangles disease because of oxidative agent's use of this element will be more than usual. So, serum levels of this element are reduced and animal may requires selenium as an antioxidant in supportive therapy. In the groups of patient horses based on age, changes in the mean serum levels were not significant for any of the parameters. Age of infected animals in a change in serum zinc, copper, selenium, and ceruloplasmin was not considerably important.

In conclusion that serum levels of zinc and selenium decrease in horses with Strangles and mean of serum ceruloplasmin increases as an inflammatory agent in treatment of these animals injection of supplemental components with zinc and selenium is recommended.

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