

Estimation of some biochemical parameters and trace elements in sheep infested with *Taenia hydatigena* cysts in Sulaymaniyah province/Iraq

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Abstract

This study was carried out in Sulaymaniyah province, Iraq to estimate the concentration of some biochemical parameters and trace elements in the serum of sheep infected with the metacestodes of *Taenia hydatigena*. The present study included the measurement of some biochemical parameters as total protein, albumin and globulin and evaluation of trace elements as zinc, copper and iron using automatic analyzer and spectrophotometer respectively. For this purpose, up to 40 serum samples from sheep infected with *T. hydatigena* cysts and 10 serum samples from non-infected group had been used. The findings of the current study showed significant elevated levels in both total protein and globulin compared to non-infected group, where the total protein and globulin levels were 8.04 ± 0.275 g/dl and 5.90 ± 0.321 g/dl respectively, compared to the non-infected group 6.686 ± 0.409 g/dl and 4.124 ± 0.479 g/dl respectively. While, the mean serum albumin in infected sheep was significantly decreased 2.14 ± 0.224 g/dl compared to non-infected one 2.562 ± 0.152 g/dl. About the results of trace elements, the serum Cu was significantly increased in infected group 1.42 ± 0.466 mg/L compared to non-infected one 0.90 ± 0.171 mg/L, while the mean serum Zn concentration was significantly decreased in infected group 0.37 ± 0.230 mg/L compared to non-infected group 0.70 ± 0.108 mg/L. Although, the mean serum Fe of infected sheep 1.42 ± 0.388 mg/L was slightly lower than non-infected one 1.26 ± 0.490 mg/L, statistically there was no significant difference between them. It was concluded that *T. hydatigena* cysts had significant effects on serum total protein, globulin, Zn and Cu.

Keywords: *Cysticercus tenuicollis*, Serum, Spectrophotometer

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تقدير بعض المعايير الكيموحيوية والعناصر النادرة في الأغنام المصابة باكياس الديدان الشريطية المُعَدَّة في محافظة السليمانية / العراق

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الخلاصة

أجريت هذه الدراسة في محافظة السليمانية العراق لتقدير تركيز بعض القياسات الكيموحيوية والعناصر الضئيلة في مصل الدم للأغنام المصابة باكياس داء المثانة الكيسية. الدراسة الحالية شملت بعض القياسات الكيموحيوية مثل البروتين الكلي، الزلال والكلوبيولين وتقييم العناصر الضئيلة مثل الخارصين والنحاس والحديد باستخدام المحلل التلقائي والجهاز الطيفي الضوئي. لهذا الغرض تم استخدام 40 عينة مصل الدم من الأغنام المصابة بالطفيلي و 10 عينة مصل الدم من اغنام غير مصابة. اظهرت نتائج هذه الدراسة زيادة معنوية في مستوى البروتين الكلي والكلوبيولين كان (8.04 ± 0.275) و (5.90 ± 0.321) g/dl على التوالي مقارنة بالمجموعة غير المصابة (6.686 ± 0.409)

و(4.124 ± 0.479) g/dl على التوالي. بينما معدل زلال المصل انخفض معنويًا (2.14 ± 0.224) g/dl مقارنة مع تلك غير المصابة (2.562 ± 0.152) g/dl. بالنسبة لنتائج العناصر الضيئلة تبين زيادة معنوية لتركيز النحاس في المجموعة المصابة (1.42 ± 0.466) mg/L مقارنة مع غير المصابة (0.90 ± 0.171) mg/L بينما معدل مصّل تركيز الزنك انخفض معنويًا في المجموعة المصابة (0.37 ± 0.230) mg/L مقارنة مع المجموعة غير المصابة (0.70 ± 0.108) mg/L. أن معدل تركيز الحديد في الاغنام المصابة لم يتغير معنويًا من الاغنام الغير المصابة. وفي الاستنتاج وجد ان الطفيلي له تأثير معنوي على تراكيز البروتين الكلي والكلوبيولين والخاصين والنحاس.

Introduction

Taenia hydatigena is a universal and boundless parasite of canids that can taint an extensive variety of warm-blooded animals with its larval stage which is generally alluded to as *C. tenuicollis* (1). Cysticerci are typically found on the omentum, mesentery, peritoneum and less regularly, on the pleura and pericardium. The migrating larvae can be discovered for the most part in the liver parenchyma inside 7-10 days and may cause hepatitis cysticercosa in youthful animals (2). Most diseases are perpetual and asymptomatic and are not generally distinguished until slaughter (3). In overwhelming contaminations, the migrating larvae could create serious annihilation of the liver parenchyma with eosinophilic invasion and extreme irritation that could be lethal (4). Notwithstanding constituting an animal wellness issue, the parasitosis is a wellspring of financial misfortune for the meat business (1,5). There are several of studies demonstrating the relationship of trace elements with parasitic diseases (6,7). Some infectious diseases or chronic inflammation leads alteration in the level of trace elements but the reason is unknown yet and it needs more study (8,9). There are two sorts of trace element abnormalities and it's generally known to be linked to dietary deficiency (10) or the imbalance due to different diseases (11). In all cases, serum elemental analysis is an experimental choice to evaluate the trace element status (12). In recent years, it is becoming increasingly clear that greater priority should be given to *C. tenuicollis* because of its economic impact due to condemnation of offal's containing these larvae, particularly in resource poor countries (13,14). Loss in quantity or quality of meat or offal will have financial implications, with reduced payment for carcass contamination or diseases or infected tissues. Diagnosis of ovine cysticercosis in animals depends on the finding the gross cysts during meat examination or necropsy technique, while there are many valuable tests can be beneficial to diagnose the disease in livestock such as Enzyme Linked Immuno-Sorbent Assay (ELISA), hematological and biochemical tests (15). To the best of our knowledge, no previous study was conducted on *T. hydatigena* cysts in our region especially Sulaymaniya province, so it is decided to carry on the study to evaluate some biochemical and trace elements parameters in sheep infected with *T. hydatigena* cysts.

Materials and methods

Animal selection, ante and post mortem inspection

The study was carried out at Modern Sulaymaniyah Slaughterhouse in Sulaymaniyah province from 1st of April to 30th June 2017. Before the slaughtering animals were recorded for their breed, sex and age. The study was done on native breed of sheep. All the animals were originated from same farm, and only adult males were used. The study was done on two groups of sheep, infected animal and non-infected animals (control). Forty sheep carcasses infested only with the cyst of *C. tenuicollis* were selected randomly and the following organs were checked carefully to other parasitic diseases including lungs, gastrointestinal tract, liver, heart. The control group composed of ten of healthy animals that are free from any diseases (16).

Blood sample collection and smear preparation

Blood samples were collected from animals before slaughtering and biochemical and trace elements parameters were done only for those animals that had cysts after post mortem examination. About 10 ml of blood sample was taken from the jugular vein of each sheep using sterile syringe with needle volume 22 gauges. Two ml of blood sample was directly put in anticoagulant tube, for blood smear technique and the rest of blood was put in plain tube without anticoagulant, and then the serum was separated from blood by centrifugation at 3000 rpm for 20 minutes and stored at - 20 °C till it was used for trace elements and protein profile evaluation. The Giemsa stained blood smears were prepared and examined according to the procedure described by Zajac and Conboy (17) for detection of any blood parasites.

Fecal sample collection and examination

The fecal specimens were collected directly from rectum of sheep, then floatation and sedimentation techniques were done for each specimen according to the methods described by Zajac and Conboy (17) for detection of any gastro intestinal parasites.

Serum total protein, albumin and globulin estimation

An automatic analyzer was used to measure the serum total protein and albumin of both infected and non-infected groups of sheep (Cobas c311; Hitachi, Japan) at the laboratory of Baxshin Private Hospital/Sulaymaniyah city.

The concentration of serum globulin was determined mathematically by subtracting the value of albumin from total protein concentration (18).

Serum zinc, copper and iron concentration estimation

To measure the level of Zn, Fe, and Cu elements in serum, Inductive Coupled Plasma Optical Emission (ICP-OES) Spectrophotometer (Perkin Elmer DV2100, USA) was used at the quality control laboratory of Kurdistan Institution for Strategic Studies and Scientific Research / Sulaymaniyah city. In the present study, 213.857 nm, 324.752 nm, and 259.939 nm wavelengths were utilized to asses Zn, Cu and Fe elements, respectively (Table 1) and the emission graphs of measured elements are illustrated in (Fig. 1).The Blank and standards solutions were calibrated. The serum samples were diluted with deionized water after blood centrifugation in a clean pretreated test tube by automatic pipette before analysis (19).

Table 1: Standards used for zinc, copper and iron (ppm = µg/ml)

Standard name	ppm
Std 1	1
Std 2	3
Std 3	5

Optimization of the serum samples

In order to optimization of serum samples, a preliminary study was done on five infected and three non-infected groups of sheep sera to find the best dilution to perform the study. In this study deionized water was used to dilute sheep sera at ratios of 1:50, 1:100 and 1:200, for determining of Zn, Cu and Fe, respectively. The 1:50 dilution was chosen because there was no statistically difference between 1:50 and 1:100 dilutions.

Statistical analysis

Both SPSS version 24 and MS Excel (2010) software were used for statistical analysis. The results of trace elements and protein profiles are stated as mean ± standard deviation (SD). Student’s t-test was used to find statistical differences between study groups and P <0.05 was regarded as significant value.

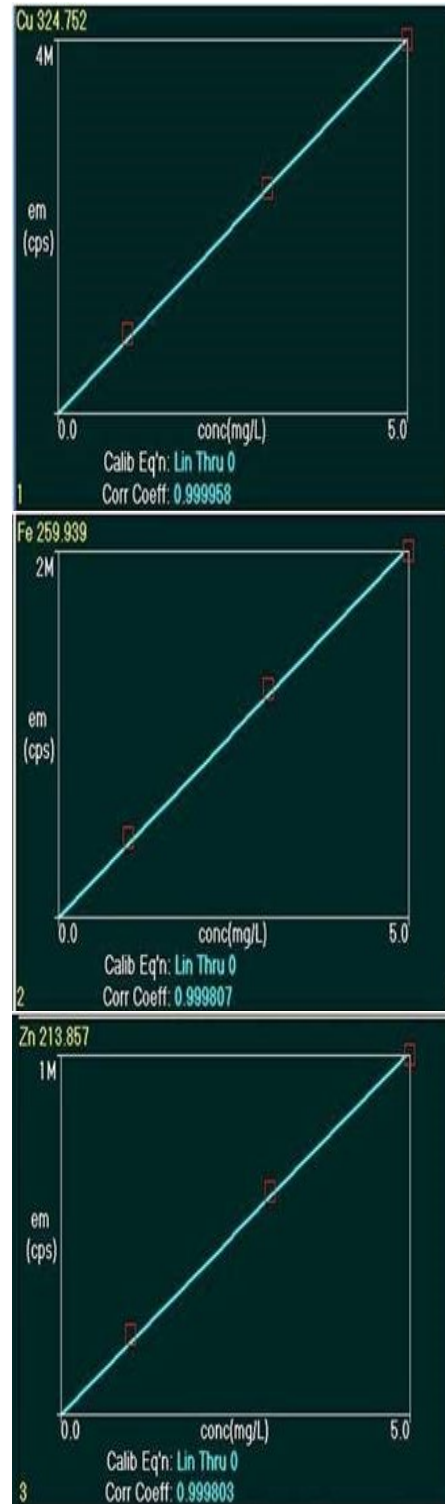


Figure 1: The emission graphs of Cu (1), Fe (2), and Zn (3) for all standards as a function of concentration.

Results

Biochemical and trace elements parameters

All changes in biochemical and trace element parameters recorded in this study represent the effect of *C. tenuicollis* only and not from other parasitic infection. For this purpose, blood smear and fecal examination were used to detect infected animals and separate them from non-infected group. In blood smears examination, no blood parasites were seen in both infected and non-infected groups with *C. tenuicollis* and also in fecal examination, no gastrointestinal parasites were observed in both groups.

Protein profiles estimation

C. tenuicollis had a clear effect on some serum protein in infected sheep. The serum total protein value in *C. tenuicollis* infected group was significantly ($P < 0.05$) higher than non-infected group, as shown in (Table 2). There was significant difference ($P < 0.05$) in serum albumin value between infected and non-infected groups.

The results indicated that *C. tenuicollis* had significant effect ($P < 0.05$) on serum globulin value.

Trace elements estimation

The results of preliminary study on serum Cu, Fe and Zn concentration measurement were shown in (Table 3). It seems from the results of this study that the *T. hydatigena* cysts had significant effect ($P < 0.05$) on the Cu and Zn at 1:50 and 1:100 dilutions respectively, but no significant effect ($P > 0.05$) on Fe concentration. On the other hand, there was no significant difference ($P > 0.05$) in the average value of these trace elements between infected groups (Table 4). It appears that the serum Cu value was significantly increased ($P < 0.05$) in infected group in comparison with non-infected group. While, serum Zn value was significantly decreased ($P < 0.05$). It was shown that serum Fe value in infected group was greater than non-infected control group but statistically no significant difference ($P > 0.05$) between them, as illustrated in (Table 5).

Table 2: Serum protein profiles (Mean \pm SD) in sheep infected with *C. tenuicollis* compared to non-infected sheep

Protein profiles	Infected group (n = 40)	Non-infected group (n = 10)	P-value of T-test
S. Total protein g/dl	8.04 \pm 0.275	6.686 \pm 0.409	*0.000473
S. Albumin g/dl	2.14 \pm 0.224	2.562 \pm 0.152	*0.000372
S. Globulin g/dl	5.90 \pm 0.321	4.124 \pm 0.479	*0.000281

Table 3: Preliminary study on serum trace elements level (Mean \pm SD) in sheep infected with *C. tenuicollis* compared to non-infected sheep

Dilution	Serum trace elements	Infected group (n = 5)	Non-infected group (n = 3)	P-value of T-test
(1:50)	Copper (Cu) mg/L	1.570 \pm 0.447	0.970 \pm 0.026	*0.019775
	Iron (Fe) mg/L	1.340 \pm 0.270	1.230 \pm 0.233	0.284971
	Zinc (Zn) mg/L	0.211 \pm 0.050	0.630 \pm 0.085	*0.002620
(1:100)	Copper (Cu) mg/L	1.542 \pm 0.408	0.967 \pm 0.029	*0.016970
	Iron (Fe) mg/L	1.372 \pm 0.275	1.220 \pm 0.226	0.217202
	Zinc (Zn) mg/L	0.203 \pm 0.043	0.617 \pm 0.085	*0.003375

Table 4: Preliminary study on serum trace elements level (Mean \pm SD) in sheep infected with *C. tenuicollis*

Serum trace elements	Infected group (n = 5) 1:50 dilution	Infected group (n = 5) 1:100 dilution	P-value of T-test
Copper (Cu) mg/L	1.570 \pm 0.447	1.542 \pm 0.408	0.460036
Iron (Fe) mg/L	1.340 \pm 0.270	1.372 \pm 0.275	0.428662
Zinc (Zn) mg/L	0.211 \pm 0.050	0.203 \pm 0.043	0.397116

Table 5: Serum trace elements level (Mean \pm SD) in sheep infected with *C. tenuicollis* compared to non-infected sheep

Trace elements	Infected group (n = 40)	Non-infected group (n = 10)	P-value of T-test
Copper (Cu) mg/L	1.42 \pm 0.466	0.90 \pm 0.171	*0.00044213
Iron (Fe) mg/L	1.42 \pm 0.388	1.26 \pm 0.490	0.26597930
Zinc (Zn) mg/L	0.37 \pm 0.230	0.70 \pm 0.108	*0.00014191

Discussion

Protein profiles estimation

C. tenuicollis infection in animals results in economical losses and decreasing the meat quality (13). In addition, it might cause alteration in the level of different blood parameters (15). The effect of *C. tenuicollis* on biochemical and hematological parameters is not well studied in animals, so this study tries to trace the changes in some blood parameters due to the parasitic infection.

The findings of the current study unveiled that biochemical parameters (serum total protein and globulin) were increased significantly in infected sheep compared with control group. Similar results reported in Duhok by Al-Bayati *et al* (20) who investigated that plasma total protein was increased in sheep infected with *C. tenuicollis*. The theories about alteration of protein level in blood plasma are controversial. In some studies, it's shown that during parasitic infection, larval stages of parasites absorb proteins from the host for their lives (21-24). On the other hand, it's found that the level of total blood protein increased due to inflammatory responses of the host (25). The outer layer of metacestodes, teguments, of some parasitic cestodes such as *Taenia crassiceps* (21) and *Echinococcus granulosus* (22) is composed of large amount of structural proteins and enzymes. It's revealed previously that the cyst absorbs structural protein from body fluid. This finding was also confirmed by Shepherd and McManus (23) using Immunoprecipitation and by Shapiro *et al* (24) using immunoblot analysis. Several host proteins were found in adult and cysts of the worm through these laboratory tests. The host inflammatory response was discovered to increase in acute and chronic parasitic infection which causes an increase in serum globulin (25). In other parasitic infection, a study conducted by Esmacilnejad *et al* (26) on *Babesia ovis* in sheep revealed comparable values with our results concerning total serum protein, albumin and globulin. The present findings are inconsistent with those found by Bamorovat *et al* (16) who found that no significant changes were seen in serum levels of total protein in sheep infected with *C. tenuicollis*. Also, its disagreement with that found by Radfar *et al* (27) who recorded that total serum protein was decreased significantly in infected goats compared with control group.

It seemed from the results of this study that serum albumin had significantly decreased in sheep infected with *C. tenuicollis* compared with non-infected ones. The migration of *C. tenuicollis* cause traumatic hepatitis (28). The same effect is also shown in liver fluke infection due to destruction of the liver cells during larvae migration. When the disease becomes chronic, it leads to reduce the amount of blood albumin due to decrease the ability of liver to produce albumin (29). The obtained results were accordant with those recorded by Esmacilnejad *et al* (26) signaled that

serum albumin had significantly decreased in sheep infected with *B. ovis* compared with healthy one.

Trace elements estimation

In this study, it was found that the mean serum levels of Cu were increased, while the mean serum Zn level was decreased in sheep infected with *C. tenuicollis* when compared with healthy ones. It appears from the findings of the study that the serum Fe value was slightly higher in infected cases than non-infected ones, but it was within normal range. As far as we know, no previous study had been published to show the correlation between trace elements and the effect of *C. tenuicollis* infection. Comparing our results with other parasitic infections, our finding is in agreement with that found by (30) they found that sheep infected with cystic echinococcosis had a decreased of Zn levels and an increased of Cu levels. The results could be explained that growing parasite lead to increase consumption of Zn and secretion of Cu into the circulation (7). Serum can store 90% of Cu in the bound form of ceruloplasmin, while it's unable to store Zn and the level of Zn could be decline easily. The raising level of Cu is in connection with the level of ceruloplasmin such as observed in acute phase reactant (31). Also, similar results reported by Dedes *et al* (6) who was showed that equine piroplasmiosis result in decreased plasma Zn level and increased Cu level. Furthermore, our recorded corroborate the opinion of (32) who reported that *Hepatozoon canis* caused an increased in serum Cu level and a decreased in Zn level. Contrary to our results, Seyrek *et al* (32) mentioned that serum Fe decreased in dog infected with *H. canis*. Many factors have been explained to reduce the level of Zn in the serum of animal including indirect effect of the host-parasite relationship or increased Zn demand by the parasite itself (33), and hormonal changes (34).

Conclusion

In conclusion, the serum total protein, globulin, and Cu values were increased in sheep infected with *T. hydatigena* cysts. While the serum albumin, and Zn values were decreased. In the light of this study, it is recommended to conduct further study to compare the effect of parasite on trace elements concentration in serum, cystic fluid and host tissues in other ruminants.

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