Heavy Metal Concentrations in Parasitized Cows and Sheep with *Echinococcus* as Environmental Bioindicators

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Abstract—Heavy **metals (aluminum [Al], arsenic [As], cadmium [Cd], mercury [Hg], and lead [Pb]) levels in cow and sheep liver samples with or without *Echinococcus* infection were studied. These species of parasites are protected their hosts' tissues from the deleterious effects of heavy metals through absorbing them into their bodies and therefore act as environmental bioindicators. Liver samples of cow and sheep were collected from slaughterhouses in Erbil and Koya cities. Parasitized cow liver with *Echinococcus* parasite had lower levels of toxic metals (Cd and Pb); cadmium levels in infected cows (0.333 ppb) were 6 times lower than in the uninfected cows (2.17 ppb) ($P < 0.001$). This was the same case for lead level: 3.447 ppb was 1½ times as lower as in uninfected cows (4.406 ppb) ($P < 0.05$). The same lower levels of toxic metals (Cd and Pb) were detected in parasitized sheep liver; cadmium levels in infected sheep (0.333 ppb) were 8 times lower than in the uninfected sheep (2.65 ppb) ($P > 0.005$), whereas lead level (3.447 ppb) was 14 times as lower as in uninfected sheep (47.72 ppb) ($P < 0.001$). In addition, arsenic level (7.0 ppb) was more than 1 time lower than in the uninfected sheep (8.8 ppb). Conversely, cows and sheep with *Echinococcosis* yielded higher concentrations of other metals in comparison to their uninfected counterparts: Al (159.8 ppb uninfected and 247.7 ppb infected and 318.7 ppb uninfected and 409.7 ppb infected), As (4.8 ppb uninfected and 5.84 ppb infected), and Hg (25.5 ppb uninfected and 26.4 ppb infected and 25.8 ppb uninfected and 27.27 ppb infected) for cow and sheep liver, respectively. This could support the hypothesis that tapeworms are able to absorb toxic heavy metals from the host body into their tissues as well as to modify other element concentrations in the host body. Conclusively, cows and sheep, as well as the infectious parasite *Echinococcus,* can be exploited as potential accumulation bioindicators of terrestrial environmental pollution with heavy metal (Cd and Pb) and (Al, As, and Hg) levels, respectively.**

***Index Terms*–Bioindicators, Cow, *Echinococcus*, Germinal layer, Heavy metals, Liver, Sheep.**

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I. Introduction

Echinococcosis is one of the parasitic diseases, which causes infection by tiny tapeworms of the genus *Echinococcus*. Echinococcosis can be classified as either alveolar echinococcosis (AE) or cystic echinococcosis (CE). The hydatid disease is also another name of the CE infection. The infection of such type of disease can be occurred by the larval stage of *Echinococcus granulosus* parasite. The long tapeworm with an approximated 2–7 mm found in dogs (definitive host) and sheep, goats, cattle, and pigs (intermediate hosts). The humans mostly infected by the CE, which is very harmful by enlarging cysts in the liver, lungs, and other organs that often grow unnoticed and neglected for years [1].

The infection of the larval stage of *Echinococcus multilocularis* can cause the AE disease, which was found approximately 1–4 mm-long tapeworm in the coyotes, foxes, and dogs (definitive hosts). *E. multilocularis* has intermediate hosts, which is small rodents. The AE disease is very rare in the human but relatively common in the animals in endemic areas. The AE disease threatens human health more than the CE disease by forming parasitic tumors in the lungs, liver, brain, and other organs that could be fatal without proper treatment [2]. The most common brain parasitic infection is brain echinococcosis, which occurred in the rare location, and in all cases, can be represented from 1% to 2% with hydatid diseases.

The heavy metals are most toxic and harmful metals among the other elements, which are naturally occurring elements with the different concentrations in all ecosystems. There are a large number of heavy metals exist in nature. They can be found in the elemental form and different chemical compounds (ref).

The volatile substances become linked to very fine particles, which can be widely imparted on the larger scale. The different properties of each form and compound of the heavy metals strongly affect the human by entering human food chains. The biochemical cycles and balance of some heavy metals drastically changed by human activates. The production of lead, zinc, and copper increased ten-fold from 1850 to 1990 [3].

The pioneer heavy metals of worry to the Cooperative Programmer for controls and detection of the Long-range

Transmission of Air Pollutants in Europe (EMEP) in the field of heavy metal pollution assessment are Hg, Pb, and Cd, because they are the most poisonous and have known serious effects on human health. The ecological exposure to high concentrations of heavy metals has been attached to various cancers and kidney damage. In Europe, there are more considerable measurement data available on Hg, Pb, and Cd heavy metals. There are 35 metals that concern us because of their professional and residential exposure, and 23 of them are heavy elements such as cerium, antimony, bismuth, arsenic, cadmium, chromium, copper, cobalt, gallium, iron, gold, lead, silver, tellurium, thallium, manganese, mercury, nickel, platinum, vanadium, uranium, and tin [4].

The study aimed at assessing the possible protective role of these parasites to their hosts' tissues from the deleterious effects of heavy metals through absorbing these toxic metals, as well as the role of these parasites as environmental bioindicators.

II. MATERIALS AND METHODS

A. Sample Collection

The materials used for the study included field and laboratory materials. The experimental work has been performed at the laboratories of the Medical Microbiology Department/Koya University.

About 100 samples of specific-infected liver, lungs, and intestine tissues of domestic animals (cow and sheep) with the helminthic parasites of the genus *Echinococcus* were collected for the purpose of detecting the concentration of heavy metals in the infected animal tissues. The meat of these animals is an important part of the traditional cuisine of the local population. In parallel, around 20 meat samples also have been collected from each type of uninfected animals from the same farms, as a control.

B. Sample Preparation

The samples were dried and digested with concentrated HNO_3 and concentrated H_2O_2. A blank digestion was prepared out by the same way for the control samples. After digestion, the samples allowed to cool down, filtered, and then top upped with distilled water to the mark of the volumetric flask. The concentration of heavy metals of parasitized digested domestic animal tissues was determined using inductively coupled plasma–optical emission spectroscopy (ICP- OES) [5].

C. ICP-OES

ICP-OES is also referred as ICP atomic emission spectrometry. In recent years, ICP-OES and ICP-mass spectrometry (ICP-MS) have become very popular and common tools for the routine analysis of samples because they have some distinct advantages over other existing techniques, such as great sensitivity, selectivity, wide dynamic range, and simultaneous multi-elemental capability with very low limits of detection. ICP-OES is usually used for multielement determination of trace, minor, and major elements, while ICP-MS is preferred for multielemental analysis at ultratrace levels [6-8]. Another advantage of the use of ICP-OES is that the effects of matrix usually are smaller than that in ICP-MS because ICP-OES involves measurement of emitted light while which is a completely passive process. However, in ICP-MS, ions must be extracted physically from the plasma [9]. Nowadays, ICP-OES is a recognized technique for the multielement determination in a wide sample range, such as environmental, geological, and biological [10-15].

The principle of the method is excitation of atoms and ions into high energy level using high temperature. This excited state is not stable because of the atoms or ions natural tendency to return to its ground state. When excited atoms or ions return to the ground level, they typically release energy. This energy is referred to as emission radiation, and its intensity depends on the temperature, number of atoms or ions in excited states, and the atom or ion type. A single element has a number of emission lines that can be used for quantitative analysis. However, the most frequently used lines depend on their sensitivity and on the presence of possible interferences [16].

III. RESULTS AND DISCUSSION

The study aimed to detect and evaluate the concentration of five heavy metals (aluminum [Al], arsenic [As], cadmium [Cd], mercury [Hg], and lead [Pb]) in the flat worm parasite *Echinococcus* that inhabits the liver of domestic animals, cow and sheep, and compare their levels with both infected and healthy liver tissues of such animals. The parasites were present in the infected tissues in two forms (Germinal layer [GL]).

A. Heavy Metals Variation among Cow Liver Tissue and Echinococcus Parasite

Level of heavy metals (Al, As, Cd, Hg, and Pb) in infected cow liver tissues with *Echinococcus* parasite in the form of GL was measured. The data revealed significant differences between means of studied groups as related with GL, infected liver tissue, and healthy control, which were 0.33, 0.33, and 2.171 ppb, ($P < 0.001$) and were 3.48, 3.48, and 4.4 ppb ($P < 0.05$), for the Cd and Pb, respectively (Table I). Hence, interestingly, parasitized cow liver with *Echinococcus* had lower levels of toxic metals (Cd, Pb); cadmium levels in infected tissue (0.333 ppb) were 6 times lower than in the uninfected tissue (2.17 ppb) ($P < 0.001$). This was the same case for lead level: 3.447 ppb was 1½ times as lower as in uninfected cows (4.406 ppb) ($P < 0.05$).

The results showed no significant differences between means of studied groups as related with Al, As, and Hg. The results were compatible with a study of heavy metal levels in red fox small intestine samples with or without *E. multilocularis* infection [17]. Brožová, *et al.* [17] revealed that infected red foxes with *E. multilocularis* showed lower levels of toxic metals (Cd and Pb); twice as low as cadmium levels were recorded in infected foxes in comparing to uninfected foxes. This was the same case for lead level, between infected and uninfected red foxes.

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Oppositely, higher concentrations of other metals were yielded in parasitized cow liver samples in comparison to their uninfected counterparts: Al (159.8 ppb uninfected and 247.7 ppb infected), As (4.8 ppb uninfected and 5.84 ppb infected), and Hg (25.5 ppb uninfected and 26.4 ppb infected) (Table I).

As we are in the era of intensive development of industry, the question that often arises: What is the level of environmental pollution? And to what extent is such case being important that need our sincere attention? These questions need to be answered clearly and the problem should be highlighted, and urgent solution should be followed up. Heavy metals are considered as one of the most hazardous constituents of xenobiotics according to recent reviews of the issue [18].

The toxic effects of heavy metals have direct influence on the constituents of the immune system in addition to modification of the immune response of the host. Definitive hosts such as cows and sheep and some of their parasites such as *Echinococcus* can be exploited as potential accumulation bioindicators of terrestrial environment pollution of heavy metal [18].

B. Heavy Metals Variation among Sheep Liver Tissue and Echinococcus Parasite

The data showed similar results to the liver of cows in investigated sheep liver samples as related to the type of concentrated heavy metals. Our results showed that parasites and sheep liver tissue bioaccumulate heavy metals at different rates, which is varying according to the infection. Lead (Pb) has recorded significant differences between means of studied groups, which was 3.48, 3.48, and 47.7 ppb ($P < 0.001$) in the GL, the infected sheep liver tissue, and healthy control, respectively, (Table II).

As related with cadmium, lower level was detected in parasitized sheep liver (0.333 ppb) which is 8 times lower than in the uninfected sheep liver (2.65 ppb) ($P = 0.07$). Arsenic was also detected in infected sheep liver at 7.0 ppb which is more than 1 time lower than in the uninfected sheep liver (8.8 ppb). No significant differences have been showed between means of studied groups as related with the other four metals (Al, As, Cd, and Hg).

As shown in cow's liver, the remaining two metals exhibited higher concentrations in parasitized sheep liver samples in comparison to their uninfected counterparts: Al (318.7 ppb uninfected and 409.7 ppb infected) and Hg (25.8 ppb uninfected and 27.27 ppb infected). These were supported by the findings of [19], when they found that trematode parasites accumulated higher levels of As, Cu, Se, and Zn in comparison to their alligator hosts, whereas Fe, Cd, and Pb concentrations were higher in alligators comparing to parasites.

IV. Conclusion

We can conclude that *Echinococcus* tapeworm parasites have a noticeable role in protecting their host tissues from the adverse effects of some heavy metals. The study has highlighted the positive protective role of these parasites rather than just as pathogenic destructive agents. The results of the present study indicate that *Echinococcus* cestode parasites are useful markers of environmental pollution

TABLE I
Level of Heavy Metals (Al, As, Cd, Hg, and Pb) in *Echinococcus* Parasite in the Form of GL and in Infected Cow Liver Tissues with the Parasite

Heavy metal	N	Mean±SD	*P* value
Al			
Echinococcus parasite	10	247.7427±110.76845	
Infected cow liver tissue	11	156.8954±125.50611	0.098
Control	8	159.8115±12.36485	
Total	29	189.0265±107.18400	
As			
Echinococcus parasite	10	5.8418±3.79105	
Infected cow liver tissue	11	7.7865±3.00998	0.106
Control	8	4.8045±1.44784	
Total	29	6.2933±3.15616	
Cd			
Echinococcus parasite	10	0.3330±0.00000	
Infected cow liver tissue	11	0.3330±0.00000	0.000
Control	8	2.1718±0.81024	
Total	29	0.8402±0.92932	
Hg			
Echinococcus parasite	10	25.4390±2.19807	
Infected cow liver tissue	11	26.4815±2.35630	0.461
Control	8	25.5530±1.26829	
Total	29	25.8659±2.04450	
Pb			
Echinococcus parasite	10	3.4470±0.00000	
Infected cow liver tissue	11	3.4470±0.00000	0.003
Control	8	4.4063±1.14882	
Total	29	3.7116±0.72134	

**The level of heavy metal was measured in part per billion (ppb), GL: Germinal layer*

TABLE II
Level of Heavy Metals (Al, As, Cd, Hg, and Pb) in *Echinococcus* Parasite in the Form of GL and in Infected Sheep Liver Tissues with the Parasite

???	N	Mean±SD	*P* value
Al			
Echinococcus parasite	11	409.7406±368.52453	
Infected sheep liver tissue	18	537.3582±848.07847	0.622
Control	12	318.7394±216.90447	
Total	41	439.1334±601.24377	
As			
Echinococcus parasite	11	7.0115±4.22029	
Infected sheep liver tissue	18	6.3472±3.94196	0.283
Control	12	8.8023±4.27065	
Total	41	7.2440±4.14458	
Cd			
Echinococcus parasite	11	0.7095±1.24886	
Infected sheep liver tissue	18	0.3330±0.00000	0.079
Control	12	2.6508±4.97017	
Total	41	1.1124±2.86550	
Hg			
Echinococcus parasite	11	27.2768±1.91989	
Infected sheep liver tissue	18	26.7228±1.80924	
Control	12	25.8129±2.32480	0.217
Total	41	26.6051±2.02917	
Pb			
Echinococcus parasite	11	3.4470±0.00000	
Infected sheep liver tissue	18	3.4470±0.00000	0.000
Control	12	47.7203±41.06584	
Total	41	16.4050±29.65958	

**The level of heavy metal was measured in part per billion (ppb). GL: Germinal layer*

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with heavy metals such as Al, As, and Hg. Furthermore, the present results suggest usage of cestode worms in the assessment of the environmental deterioration by such metals, whereas cows and sheep are likely good biological indicators of Cd and Pb levels within the terrestrial environment.

Due to the importance of the work and the highly impact of the deleterious heavy metals on the domestic animals and consequently their effects on individuals in particular and the environment that we all share in general, therefore it is highly recommended to extend the study to involve many other organs and animals of both terrestrial and aquatic habitats.

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