Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 12, Issue 4, September 2017
ISSN 1992 – 0849

Determination the Level of Serum Iron in the Ewes Infected with *Fasciola hepatica*.

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Abstract:

[The current study was performed from the period of August 2016 to January 2017 in Kirkuk city to clear up the taking place of iron deficiency with different clinical appearance of ovine fascioliasis. Eighty ewes were distributed to two groups; patient group including 40 ewes that were had clinical signs of fasioliasis and 40 healthy ewes were typified as control group, based on clinical symptoms the patient ewes were portioned out to subacute and chronic groups that comprised of 20 and 20 continuously. Fecal samples of ewes were tested in order to differentiation the *Fasciola hepatica* infected away from non-infected, venous blood samples were collected from the ewes for particularization and comparison of iron levels in serum samples of the groups. Results of present study showed occurrence of significant decrease ($P \leq 0.05$) in iron level in the blood serum of infected ewes compared with perfect control group, whereas the existence of significant divergence was not observed ($P \geq 0.05$) in iron level in subacute and chronic infected ewes group. It was drawn a conclusion that ovine fascioliasis conduces to iron deficiency irrespective of its clinical forms. It is necessary to provide the infected sheep with iron preparations to facilitate their cure from fasscioliasis].

Key words: [Iron Deficiency - *Fasciola hepatica* – Ewes].

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تقدير مستوى حديد المصل في النعاج المصابة بالوريقة الكبدية.

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الخلاصة:

[اجريت الدراسة الحالية في الفترة من اب ٢٠١٦ و لغاية كانون الثاني ٢٠١٧ في مدينة كركوك لتوضيح حدوث نقص الحديد مع مختلف المظاهر السريرية لداء وريقات الاغنام. ثمانون نعجة وزعت الى مجموعتين; مجموعة المرضى المتضمنة ٤٠ نعجة اللاتي كانت تمتلك علامات سريرية لداء الوريقة و ٤٠ نعجة معافات من المرض اللاتي مثلت مجموعة سيطرة، قسمت النعاج المريضة بناء على الاعراض السريرية الى مجموعتين تحت الحادة و المزمنة اللتان اشتملتا على ٢٠ و ٢٠ على التوالي. تم فحص نماذج براز النعاج لاجل تمييز المصابين بالوريقة الكبدية من عدم المصابين، جمعت الدم الوريدي من النعاج لتحديد و مقارنة مستويات الحديد في نماذج مصل الدم للمجاميع. اظهرت نتائج الدراسة الحالية حصول انخفاض معنوي ($P \leq 0.05$) في مستوى الحديد في امصال دم النعاج المصابة مقارنة مع مجموعة السيطرة السليمة، في حين لم يلاحظ وجود اي اختلاف معنوي ($P \geq 0.05$) في مستوى الحديد في مجموعتي النعاج المصابة تحت الحادة والمزمنة . تم الاستنتاج ان داء وريقة الاغنام يودي الى نقص الحديد بغض النظر عن اشكالها السريرية. ان من الضروري تزويد الاغنام المصابة بمكملات الحديد لتسريع شفائها من داء الوريقة].

الكلمات المفتاحية: [نقص الحديد - الوريقة الكبدية - النعاج].

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Introduction:

The world food economy is being increasingly driven by the shift of diets towards animal-based products such as meat and milk. Globally livestock production is the largest user of agricultural land, thus leaves a significant imprint on the environment **[1]**.

Trematode parasites live in the liver, fore stomachs or blood vessels of a wide range of animals and humans. Most of them have a special economic and veterinary significance **[2]**. Liver fluke disease or Fascioliasis is caused by the parasitic trematoda, *Fasciola hepatica*, which is important liver fluke and has cosmopolitan distribution throughout the world, more commonly in temperate climates **[3]**. The parasite digests hepatic tissue and cause extensive parenchymal destruction with intensive haemorrhagic lesions, leading to sudden death due to acute form. When on the other hand the subacute form distinguished by inappetence, decreased weight gain or weight loss. The chronic form include decreased feed intake and weight gain, reduced milk yield, anemia, emaciation, submandibular edema and ascites **[4]**.The disease is an important limiting factor for ruminant production and causes several economic losses due to morbidity and mortality and also due to liver condemnation **[5]**.

Minerals perform four broad types of function in animals: structural, physiological, catalytic, and regulatory **[6]**. Iron is an essential mineral for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid synthesis, and electron transport **[7]**. It is a key player in hemoglobin synthesis and erythrocyte production **[8]**.

The intention of this work is to declare the association of iron deficiency with fascioliasis in sheep aside from the disease clinical status.

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Materials and Methods:

Study design:

The existing research was accomplished from the period of August to December 2016 in Kirkuk district. Eighty ewes were tested for iron concentration; patient group consist of 40 ewe infected with *F. hepatica* that diagnosed through faecal analysis, on the basis of clinical inspection **[9]**, 20 of abovementioned ewes have subacute signs of disease while chronic clinical symptoms were noticed in the remaining 20 ewes. In addition to that, 40 ewes selected as control group which presented negative result for faecal examination.

Sample collection:

Under healthy circumstances, a single fecal samples were promptly picked up via rectum of each researched ewe by disposable plastic gloves and were deposited in air tight containers, labeled markedly with each ewe specifications as age, sex, breed and place with time of picking up, then were carried instantly inside cool package to Medical Laboratory Technical Department, Kirkuk Technical College where the samples were tested parasitologically without delay or preserved at 4 °C of refrigerator utmost of overnight prior to examination.

In respect of investigation of iron level, blood samples of the ewes were received from jugular vein into EDTA tubes two days after handling with fecal samples. Serum samples were segregated from blood at once by centrifugation, and then were stored at -20°C in plane tubes until dealing with them.

Sample examination:

The positive and negative results of *F. hepatica* ova presence were proven in fecal samples applying floatation technique in accordance with **[10]**. Direct enzymatic method was done on serum samples for estimation of iron level with spectrophotometer utilizing commercial kits (Biolabo, France).

Statistical analysis:

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The data obtained from results of iron level were resolved utilizing Microsoft excel 2010. The statistics were presented as average ± standard deviation (SD) and was defined by T test. The significant variation was drawn upon the value of $P \leq 0.05$ **[11]**.

Results and Discussion:

The comparison between patient and control groups with matter of serum iron level clarified in table 1. Generally, the levels of the mineral were below in patient group (143.26 ± 8.57µg/dl) than that in control group (187.13 ± 9.97µg/dl). The statistical divergence between the two groups was extremely significant ($P \leq 0.05$).

Table (1): levels (averages ± SD) of serum iron in patient and control groups.

Groups	Patient (N=40) Averages ± SD	Control (N=40) Averages ± SD
Iron level (µg/dl)	143.26 ± 8.57[a]	187.13 ± 9.97[b]

N = number

The different letters of each row reveals the existence of significant variations between groups at $P \leq 0.05$.

Table 2 manifests the level of iron in subacute and chronic groups; there is a not statistically significant variant result ($P \geq 0.05$) between two groups. The iron levels were (138.18 ± 5.41µg/dl) in the ewes that suffered from chronic fascioliasis, besides the (145.90 ± 8.55 µg/dl) levels that were recorded in the ewes had subacute findings of the disease.

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Table (2): levels (averages ± SD) of serum iron in subacute and chronic groups.

Groups	Subacute (N=20) Averages ± SD	Chronic (N=20) Averages ± SD
Iron level (µg/dl)	**145.90 ± 8.55^{c}	**138.18 ± 5.41^{c}

N = number
The similar letters of each row does not reveal existence of significant variations between groups at P ≥ 0.05.

Iron deficiency (ID) is a relatively common characteristic of severe bloodsucking parasite infestations in ruminants in temperate regions. It is caused by insufficient gastrointestinal iron absorption or by hemorrhage **[12]**. Iron deficiency anemia (IDA) is a more severe stage of ID as there is low hemoglobin in combination with ID **[13]**. The development of ID leads to depletion of the storage iron (ferritin and haemosiderin) in the liver hepatocytes and reticuloendothelial cells of the bone marrow before occurrence of IDA **[14]**. Anemia is one of most conspicuous signs of all ovine fascioliasis clinical forms **[15]**.

Data of current observation illustrate that *F. hepatica* infection of ewes leads to ID. Previous reports exhibited significant loss of hematological profile: red blood cells (RBCs) count, hemoglobin (Hb), packed cell volume (PCV) and mean corpuscular hemoglobin concentration (MCHC) levels in blood of sheep were positive for *F. hepatica* **[16,17]**. The reduction of serum iron is an important feature of normocytic and hypochromic anemia that occur in patients with a variety of chronic inflammatory and malignant diseases **[18]**.

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According to issues of present investigation and **[16,17]** papers, the *F. hepatica* infection in sheep may cause normocytic and hypochromic anemia due to ID, these results are consistent with findings of **[19]** when they demonstrated that cattle naturally infested with *F. hepatica* had a characteristic decline of serum iron concentration, blood levels of RBC, Hb, PCV and MCHC altogether, that contribute to come over of normocytic and hypochromic anemia in such cattle. An experimental trial testified the recognizing of normocytic and hypochromic anemia in rats inoculated with metacercariae of *F. hepatica* **[20]**.

Anemia of ovine fascioliasis may result from migration of immature flukes causes traumatic hepatitis and hemorrhage or when the adult flukes ingest the blood **[21].** In view of gastrointestinal blood loss or iron malabsorption result in ID and subsequently considered the underlying causes of IDA **[22]**, probably the migration of immature flukes may be the reason of IDA concerning the subacute cases of ovine fascioliasis, on the other hand about the sheep that undergo the chronic form of the disease, IDA may develop when the parasite consume the blood.

The general principles of treating animals with IDA include addressing the underlying disease, preventing further blood loss, correcting the anemia if severe and initiating iron supplementation **[23]**. Da Rosha *etal*. explained that injection of iron can be restored the health of anemic lambs rapidly through elevation of hematological profile values **[24]**.

Aforementioned results concluded that ID is a marked finding of various clinical symptoms of *F. hepatica* infestation in sheep. As well as anthelmintic therapy, medication of sheep with efficacious formulations of iron should be enforced to compensate the iron deprivation of the sick sheep.

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