Some haematological and biochemical parameters assessments in sheep infection by Haemonchus contortus

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Abstract
The study was conducted on (100) sheep naturally infested with Haemonchus contortus, (1-3 years old), in Tikrit city, from 1st January 2014 to 31st May 2014. Affected sheep exhibited clinical signs included, emaciation, weakness, pale mucous membranes, rough, easily detached and lusterless coat. Ten ml of blood samples were collected from the jugular vein of all the sheep’s using disposable syringes, then take 2.5 ml from blood sample was used to determine CBC the Red blood cells (RBC), white blood cells (WBC), Haemoglobin estimation (Hb), Packed cells Volume (PCV). The study showed reduction in RBC, hemoglobin, hematocrit and neutrophils (4.7±0.68), (6±0.74), (18±0.19), and (20±0.73) respectively. And revealed leukocytosis; Lymphocyte, eosinophil’s and monocyte (11.6± 0.41), (65± 0.51), (10± 0.64) and (5± 0.29) respectively in infected sheep, and the remind samples were centrifuged at 3000 rpm for 10 minute and serum was separated and stored at -20 °C until used for measure the biochemical change by use spectrophotometer. The results showed a significant decrease (P < 0.05) in the rates of total protein concentration, albumin, iron, and zinc (5.02± 0.59 g/dl), (1.7 ± 0.45g/dl), (89.3 ± 12.41 µg/dl), (42.37± 16.22 µg/dl) respectively in the serum of infected sheep according the compared with normal sheep.

Key word: Haemonchus contortus, some hematology, biochemical parameters assessments, sheep

Introduction
The most common parasite of sheep includes: H. contortus, Oesophagostomum, Ostertagia, Chabertia, Nematodirus, Trichuris, Moniezia and Fasciola. The most important of these is H. contortus [1]. H. contortus, found in the abomasum of sheep and goats, causes blood loss resulting in decrease in erthrocytes, hemoglobin, packed cell volume, body weight and wool growth [2,3]. H. contortus penetrates the surface of the abomasal mucosa to feed on the blood of the host [4]. The average blood loss due to H. contortus infection is 0.03 ml/parasite/ day [5]. Even relatively light infections in adults cause various degrees of anaemia [6]. H. contortus causes hematological and biochemical alterations [7,8]. Eosinophils, another type of polymorphonuclear leukocytes, also perform an important parasiticidal function, notably through production of enzymes that poison and kill the helminths [9]. Decrease in protein [7,8].[10] observed a significant decrease in total serum protein in an experimental infection with H. contortus in Barbari goats. The albumin decrease level in sheep infection with H. contortus [11].[12] reported hypo-albuminaemia and associated it to protein losing gastroenteropathy of Haemonchus infection in small ruminants.

H. contortus causes iron reduction in serum [13].[14] noted decrease in the serum iron levels of lambs receiving a single dose of 10000 L3 H. Zinc concentrations were significantly affected by parasite infection, H. contortus causes decrease in zinc level [15].

Materials and methods
Animals: A total at 100 stool and blood sample were collected from infected sheep with H. contortus and for control 10 samples from non-infected. From different local area in Tikrit city between January to may of 2014.

Stool collection: Stool samples were collected rectally using disposable gloves wherever possible, with minimal contamination from bedding, soil or plant material [16]. They were labelled and transferred to laboratory for examination. Faecal samples of infected sheep were qualitatively examined daily by wet preparation direct smear and concentration use is sugar floatation technique and the fecal sample cultured at 27°C for 8 days [17]. The larvae were extracted from faeces by Baermann funnel method [18].

Haematological examination:
Ten ml of blood samples were collected from the jugular vein of all the sheep’s using disposable syringes, then take 2.5 ml from blood sample was transferred in to container contain EDTA was used to asses CBC the Red blood cells (RBC), white blood cells (WBC), Haemoglobin estimation (Hb), Packed cells Volume (PCV) by the methods as described by [19, 20]. and the remind of the samples were centrifuged at 3000 rpm for 10 minute and serum was separated and stored at -20 °C until used.

Biochemical examination:
Total serum proteins: measured in the serum spectrophotometrically using Biolabo SA kits made in French according to the methods described by [21]. Serum albumin: measured in the serum spectrophotometrically using Biolabo SA kits made in French according to the methods described by [21]. Serum iron: measured in the serum spectrophotometrically using Biolabo SA kits made in French according to the methods described by [21].
Serum zinc: measured in the serum spectrophotometrically using Spectrum kits made in Egypt according to the methods described by [22].

Statistical Analyses:
Results were analyzed statistically using the program (SPSS) for estimate, standard error and analyzed the data using a test (Independent sample t-test). Note that the significant difference for all the tests at the level of probability (p<0.05) [23].

Result

The range of hematological measures was significantly different between infected and uninfected controls. The present study revealed a marked reduction in RBC, hemoglobin, hematocrit and neutrophils (4.7± 0.68), (6± 0.74), (18± 0.19), and (20± 0.73) respectively (Fig.1). The result of the hematology parameter appear increase in WBC, Lymphocyte, eosinophil's and monocyte (11.6± 0.41), (65± 0.51), (10± 0.64) and (5± 0.29) respectively (Fig.2).

Discussion

H. contortus infection is known to cause significant changes of haematological parameters like Hb, PCV and RBC counts and which may cause an anaemia in infected animal [24, 25]. It is estimated that an adult H. contortus can suck 0.03 ml of blood/day [5], in addition to causing leakage of blood from the site of attachment. H. contortus, cause a decrease in RBC, Hb concentration and PCV [26, 3]. The decrease in RBC counts, Hb and PCV values in infected sheep may be because the bleeding of abomasum due to the injuries caused by the H. contortus similar to that described by [27]. [28] reported decreased in Hb, PCV, RBC, and neutrophils in cattle infection by parasite.

Infected sheep showed significant increase in WBC count may be due to the immune response of body against the parasites and resistance to infection as a means of self defense [29,30]. Or it may be due to increase sensitivity to the protein of the parasite, which is a foreign body from the animal's body [31]. Total Leukocyte Count was found significantly elevated in infected animals and the increase was mainly due to greater count of lymphocytes,
otherwise neutrophils count was noticeably reduced in infected compared to non-infected animals. The changes in Total Leukocyte Count in the infected animals clearly show immunopathological response of the host [32].

Increased lymphocyte percent (%) observed in the present investigation and that agreement with the findings by [33]. The results of our study are similar to [3] and with increased lymphocyte percent (%) may be because proliferation lymphocytes due to excretory secretory product of H. contortus significant increase (P<0.05) in the lymphocytes cells in infected young during first week of infection which was associated with hard depression in neutrophil cells, this was due to the infiltration of these cells in the abomasal infection spots, which play as a first defense line against the inflammatory process caused by larval activities [34].

Eosinophils are considered to be important elements in the response against H. contortus infections [35]. In this present study, there was an increased number of circulating blood eosinophils in the animals infected with H. contortus larvae. This was in agreement with [36]. The increase in the number of eosinophils is a common feature observed during infection with H. contortus [37, 38]. The significantly increased in monocyte percent (%) found in infected sheep may be due to appearance of monocytes as second line of defense after neutrophils [39]. This increase in monocyte cells may due to the stress in the infected animals [40].

In this appear hypoproteinaemia and hypoalbuminemia in sheep infection by H. contortus, because these parasites stimulate the proliferation of intestinal epithelial cells and replacement of abomasal acid-producing cells by immature cells, this consequently leads to the loss of large quantities of serum protein into the gut. In addition, haemodilution which occurs after abomasal haemorrhage can cause relative hypoproteinaemia and hypoalbuminemia [41, 42]. [12] reported that in parasitic gastroenteritis, excessive amounts of serum proteins leak into the parasitized stomach and intestines as a result of increased mucosal permeability, thereby resulting in severe hypoalbuminemia. Hypoproteinaemia with decreased levels of total serum protein and serum albumen is an important consequence of haemonchosis, which is responsible for protein loosening enteropathy [17].

The reduction of serum iron level in infected sheep could be attributed to the expanded erythropoesis to compensate for blood loss leading to depression of iron stores [13]. Zn concentrations fall in a variety of diseases associated with anorexia [43]. Zn deficiency is associated with increased adult worm burdens [44].

Reference

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تقدير بعض القيم الدموية و القيم الكيموحيوية في الأغنام المصابة بالهيمونكوس
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الملخص
أجريت الدراسة على (100) من الأغنام المصابة بطفيلي Haemonchus contortus، في عمر (1-3 سنة)، في مدينة تكريت، من كانون الثاني 2014 إلى أيار 2014. إذ عانت الأغنام المصابة من العلامات السريرية التي شملت الهزال والضعف، وشحوب الأغشية المخاطية، وخثونة الصوف، وسهولة نزه وفقدان لمعانه. وتم جمع 10 مل من عينات الدم من الزرود الدوائي من الأغنام المصابة باستخدام المحاقن، ثم تم أخذ 2.5 مل من عينة دم استخدامها في تحديد CBC، وكريات الدم البيضاء (WBC)، وكريات الدم الينية (RBC)، وتقدير الهيموغلوبين (خضاب الدم)، وحجم خلايا الدم المرصودة (PCV). حيث أظهرت الدراسة انخفاضاً معناياً (P < 0.05) في كل من عدد كريات الدم الحمر، الهيموغلوبين، وحجم الخلايا المرصودة والعدلات (4.7 ± 0.68 غرام/ديسي ليتر، 6 ± 0.74، 0، 18 ± 0.19، و 20 ± 0.73) على التوالي. كما أظهرت الدراسة زيادةً معنويةً في عدد كريات الدم البيضاء،خلايا الدم البيضاء، الحمضات والوحيدات (11.6 ± 0.41، 65 ± 0.51، 10 ± 0.64) على التوالي. ودرجة مئوية في حال استخدامه في تقدير القيم الكيموحيوية بواسطة المطياف الضوئي. أظهرت النتائج انخفاضاً معيناً (P < 0.05) في معدلات تركيز البروتين الكلي، الألبومين، وال الحديد، والزنك (5.02 ± 0.59 غرام/ديسي ليتر، 1.7 ± 0.45 غرام/ديسي ليتر، 89.3 ± 12.41 ميكروغرام/ديسي ليتر) على التوالي. وكثير من الأغنام المصابة كانت تظهر نقصاً في القيم الكيموحيوية من الأغنام سليمة.

الكلمات الدالة: الهيمونكوس، بعض القيم الدموية، قيم الكيموحيوية، الأغنام