Influences of Some Ectoparasites on Vitamin B_{12} and Iron Levels of the Local Goats (*Capra hircus*).

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Abstract

Vitamins and trace elements have an essential role in animal feeding and vitality, and despite their scarcity in animals and humans, their ratio varies according to disease and physiological circumstances. In the current experiment, 50 infested goats with external parasites (ticks, lice, and mixed infestation) and 50 control goats were examined. The huge load sites of the louse were chest and shoulders, while ticks were ound around the ear of infected goats. Females of both ectoparasites; tick and louse were widely seen on the infested goat males male. The vitamin B12 level was reduced in all infested goats (mixed, tick and lice) by 183.08±1.03pg/dl, 184.06±3.63pg/dl, and 187.91±2.12pg/dl, respectively, and showed significant differences at $p \leq 0.05$ level when compared to the control goats, however, there were no significant differences among them (mixed, tick and lice). With regard to iron levels, mixed infestation, tick, and lice-infested goats show a decrease in their levels; 45.34±5.23µg/dl, 61.27±8.22µg/dl and 67.31±7.38µg/dl respectively, with the presence of significant differences at $P<0.05$ level versus control goat, also mixed infestation has significant differences with lice infestation at $P<0.05$ level. The present result reveals that vitamin B12 and iron levels are reduced either as primary or secondary effects of these ectoparasites.

Keywords: Ectoparasites, Vitamin B_{12}, Iron, Local Goat.

Introduction

One of the most vital domestic animals are goats. They are distributed throughout the world and in the Kurdistan region, as Khan *et al.* (2016) mentioned in their study. Their importance stems from their ability to adapt to various environmental conditions and utilize poor feed materials. The goat is usually called the "poor man's cow" due to its products (meat, milk, and hair) and its contribution to the rural economy (Radhika *et al.*, 2011). Goat as a whole small ruminant, is affected by a number of ectoparasites which can adversely affect their health (blood and their other related organs) and productive ability (milk, hair, meat production), which can be seen as a direct and indirect impact of those ectoparasites as mentioned in the study by Adang *et al.* (2015). As stated, that in the previous study, which was done by

Abdalrahman and Mustafa (2018), it reported that some types of ticks and lice were the most ectoparasites that impinged goats with adverse effects. In particular, tick infestation has an influence on most hemato-biochemical parameters in the mammalian blood. One of the most essential trace elements is iron, which is vital in various metabolic processes either in animals or in the human body (Ems and Huecker, 2018), these elements is highly required in animal and human activity and health, also Deen *et al.* (2004) illustrated that deficiency in any element is crucial and led to the discovery of an undesirable pathological situation. Parasite diseases lead to the reduction of iron and some trace elements (Seyrek *et al.,* 2009), and Mohammed *et al.* (2016) stated that during any infection, serum trace elements alter their concentration. Louse as another ectoparasites have adverse effects on the animals, and its infestation led to a decline in iron and some trace elements (Ahmed *et al.,* 2009). Vitamin like trace elements are essential for any living cell, and during any infection their concentration alters, in the study revealed that vitamin A was decreased in the infested animals with ectoparasites compared to healthy animals (Beigh *et al.,* 2013). Vitamin B12, which is referred to as cyanocobalamin, plays a vital role in normal brain and nervous functioning (Miller *et al.,* 2005; Yamada, 2013). In spite of the correlation between vitamin B12 and parasites is rarely determined, but in the study by Kleppa and Stuen (2003), it was found that infected animals with parasites had a high-level deficiency of some type of vitamin B-complex. In particular, Vitamin B12 level is affected by endoparasites, while as a secondary infection, it is affected by ectoparasites, as a result of decrease of cobalt level in serum, which is essential for Vitamin B12 synthesis. A study in infested animals with lice and endoparasites showed a decline in some vitamins compared to healthy animals (Tanritanir *et al.,* 2009, cited in Beigh *et al.,* 2013). The present study aims to determine the effect of ectoparasite infestation on Vitamin B12 level as a new record and emphasize on the relationship between iron level in the goat's blood with ectoparasitic infestation.

Material and Methods

Area and time of the study

The study was carried out in different locations: (Sulaimani province with its districts and sub-districts) in the Kurdistan region, which is located in the northeast of Iraq, and a survey was done in both April and May in the spring season and in September in the autumn season of 2018.

Animal sampling

In this study, more than 100 adult local black goats (Common black mountainous goats that present in Sulaimani-Kurdistan region of Iraq), were examined for presence or absence of ectoparasites, and after inspection of the site of the infestation (in the infested goats), they were selected and distinguished from control goats (free from ectoparasites) through the barely eye inspection. In the infested goat seeking for the type of the ectoparasites done, and ultimately for two major groups, were 100 goats selected for the study and divided into 50 control (non-infested) and 50 infested goats. Infested group divided to three subdivisions (tick infestation, louse infestation and mixed infestation) to determine their effects individually.

Entomological examination

Tick and lice or mixed (tick and lice) removed from the suspected parts of the goat as mentioned by Teglas *et al.* (2005) and Malekifard *et al.* (2015). The procedure for tick and louse collection was done as recommended by some references (Giri *et al.*, 2013). Ultimately,

lice and ticks are placed in glass vials which are 25 mm in diameter and 75 mm long, and filled with 70% alcohol concentration ethanol (TOSEL, Pharmaceutical Company, Ankara-Turkey) after labeling, they were stored in a cool place till examination. The Motic digital microscopy 111 2-4X magnification (Motic educator, China) was used for studying the minute parts of the ectoparasite's body, such as mouthparts (size, length, shape of base of capitulum, and shape of hypostome), ornamentation, legs, body shape, anal situation, and festoons of ticks as recommended by Barker and Walker (2014), also for louse, examination of size, body shape, head shape, and abdominal line were done according to Bates (2012), to determine ectoparasite's genus, species, and sexes..

Blood collection

Sterile syringes, which has an 18-gauge needle and a 10 ml capacity (Hoshin medical instrument Co., Ltd, P.R.C), were used for blood collection from the goat's jugular vein, and in the blood collection procedure, considering as many hygiene ways as possible to avoid spoilage and also protect animals from any secondary infections. About of 7ml of blood placed in gel and clot activator tube (ARTH AL.RAFIDAIN for medical equipment, Co.) were initially collected, and then samples were left to clot, and then centrifuged at 3000 rpm for 15 minutes through the laboratory centrifuge (Hettich, D-78532 Tuttlingen, Germany) Finally, serum samples were collected and frozen at −20°C until analyzed (Al-Bulushi *et al.*, 2017).

Verification test for endoparasites

Blood and fecal examined microscopically through digital microscopy, 40X, 60X and 100X, Motic educator, Japan., this procedure was carried out to ensure that control goats are free from endoparasites, especially piroplasmosis in the blood (through the Giemsa stain method), and to eradicate doubt in the blood of the infested goat by avoiding interaction effects between ectoparasites and endoparasites on their bloods, and emphasis on that, these effects occurs only through the infesting with ectoparasites.

Iron determination

Iron was determined by an automatic in-vitro apparatus (BioLis 24i premium analyzer. Tokyo BOEKI MEDISYS INC. ©Diamond diagnostic®), and determination in this apparatus was carried out by Beer-Lambert technique.

Vitamin B_{12} determination

Vitamin B12 was determined by an in-vitro diagnostic, medical device (Maglumi 1000 Chemiluminescence immunoassay system. Shenzhen New Industries Biomedical Engineering Co., Ltd. China), and utilizing a commercial kit (Maglumi Vitamin B_{12} CLIA., Lotus Global Co., Ltd. UK) for this test, which detects vitamin B12 binding protein level in the serum.

Data analysis

The data was analyzed and tested for mean, Standard Error and Confident interval 95%, through Chi-square test, for the freedom distribution of the ectoparasites on the goat's body, one-way ANOVA (Analysis of variance) with Duncan test was used for detecting significance effects between each group (control, tick, louse and mixed of them) at both levels ($P<0.01$) and ($P<0.05$) by the SPSS program, release 16.

Results and discussions

Through the verification test, were determined that all of 100 goats free from endoparasites, and they were available for the major following experiments, which they give the following results:

Entomological results

The results indicated that ear was the most infested site while teat was free from both ectoparasites, as described in Table 1.

Table.1: Distribution of ectoparasites on the goat's body in Sulaimani-Kurdistan region-Iraq

Infestation site		Type of Ectoparasite		
		Tick (*Rhipicephalus spp*)	**Lice (*Bovicola caprae*)**	**Total**
Ear %	Oi	151 (93.7%)	7 (3.3%)	158 (42.6%)
	Ei	68.6	89.4	
Chest & Shoulder %	Oi	0 (0.0%)	71 (33.8%)	71 (19.3%)
	Ei	30.9	40.18	
Back %	Oi	0 (0.0%)	58 (27.6%)	58 (15.6%)
	Ei	25.2	32.83	
Belly %	Oi	0 (0.0%)	63 (%29.8)	63 (%16.9)
	Ei	27.3	35.66	
Teat %	Oi	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Ei	5.2	6.8	
Tassel %	Oi	10 (6.3%)	11 (5.2%)	21 (5.7%)
	Ei	9.1	11.9	
Overall		161	210	371

$\chi^2(5)_{0.05}$ =9.49 and $\chi^2(5)_{0.01}$ =13.28, when χ^2(calculate)=163.28**, *Oi: observed data, Ei: expected data.*

In both ectoparasites as illustrated in table (1) presence of a highly significant difference ($P<0.01$) between the type of ectoparasites and various site**s** of infestation. Ear infestation in tick is the highest infestation site, and in both ectoparasites ear infestation reached to about of 42.6%, and it is agreeing with a result that mentioned by Mustafa (2011) in his study on the sheep, which shows that ear is the highest infestation site and close to 42%, due to it is suitable part of the body, also it have thin surface and near veins, especially marginal vein. As Muchenje *et al.* (2008) stated that tick chooses the moist, warm, undiscoverable and thin site of the body. Hair length and skin thickness have an exclusive association with the number of ticks that present on the infested site (Verissimo *et al.*, 2002), also this study agrees with the results of Soundararajan *et al.* (2014) they observed that ear infestation is about (69.79%), and also with Monfared *et al.* (2015) who stated that ear is the most infested site by ticks on goat with about (63%). in Ilam, Iran another study shows that most infestation sites are ear and eyes which take about 62.1% in goats, which partly agrees with the current result (Muhaidi *et al.*, 2010). In the lice infestation, the present study shows that, the chest and shoulder are the most infestation sites in goats, which is partly contrasted to the results of Tadie *et al.* (2018) who stated that

most distribution sites of lice on the animal are shoulder and skin of the neck, and reasonable facts behind this related to easiness movement in chest and shoulder hairs as its softness and not long which pointed by some references which illustrated in the scientific article by Ekanem *et al.* (2011). The current result shows that *Rhipicephalus* spp (*R. turanicus, R. sanguinus and R.Bursa*) in ticks and *Bovicola caprae* in lice were the most prevalent species on the surveyed goats, hence that current result agrees with Monfared *et al.* (2015) in Ilam province in Iran which found that *R. sanguinus* is most prevalet ticks on the goat, and in another study in Iran, which supports the present result, they showed that *Rhipicephalus spp* is most impinge with goat, and other genera (*Hyalomma spp* especially) are absent or less frequently on the goat when compared with sheep and cattle (Sofizadeh *et al.*, 2014, Fatemian *et al.*, 2018), while in contrast with another study they did not observe various genus of ticks (Mbaya *et al.*, 2008). In the current result there was no obvious observation of sucking lice, hence the current result agrees with the study in Nigeria by Adang *et al.* (2015) as they did not observe any sucking louse in their study.

Table.2 Female to Male ratio of ticks and lice of goats in Sulaimani-Kurdistan region of Iraq.

Type of Ectoparasite	♀ Adult female	♂ Adult male	Female: Male
Tick (161) %	105 (65.21%)	56 (34.78%)	2:1
Lice (210) %	127 (60.47%)	83 (39.52%)	2:1
Overall (371) %	232 (62.53%)	139 (37.46%)	2:1

Table (2) shows the ratio of females to males of both ectoparasites (tick and louse) is approximately above 2:1. This result in ticks is not in agreement with the findings by Gorgani-Firouzjaee *et al.* (2013) as they detected a closed sex ratio (1.2:1) in *R. turanicus* in Urmia-Iran, while in agreement with the acquired results in the same place by Yakhchali and Hosseine (2006) as they observed an above 2:1 (female to male) ratio in goats infested by *R. turanicus R. bursa and R. sanguinus.* Khalil and Abdulwahed (2010) showed in their results that the tick female to male ratio is about 3:1, which is above the present results and partially concurs with them. This result might be due to the male needing a few blood meals than the female as it needs more meals to engorge and lay eggs. In louse infestation, in another study on *Bovicola caprae* on goats, through brushing method it was observed that the ratio of female to male was 2:1, due to short lifespan in males compared to females, and also in our opinion as like as many researchers think about it, might be related to the fact, that most of their nits being female, hence this result is in agreement with the current result (Kumar *et al.*, 1994), and the current result approximately concurs with another result as they observed a 2.5:1 ratio of female to male louse (Giri *et al.*, 2013).

Vitamin B12 and iron levels results

Table (3) shows a decline in Vitamin B_{12} levels in all infested goats (Mixed, Tick and Lice) 183.08±1.03pg/dl, 184.06±3.63 pg/dl and 187.91±2.12 pg/dl sequentially and shows significant differences at $P<0.05$ level, when compared to the control goat, whereas among them, there were no significant differences at $P<0.05$ level. In iron level mixed, tick and lice infested goats show descending in their levels; 45.34±5.23µg/dl, 61.27±8.22µg/dl and 67.31±7.38 µg/dl respectively, with the presence of significant differences at $P<0.05$ level

versus control goats, also mixed infestation has significant differences with lice infestation at P<0.05 level.

Table .3 Ectoparasites infestation (Mixed, Tick, and lice) effects on the vitamin B_{12} and iron in the local black goats:-

Dependent parameter	Control M±S.E (C.I %95)	Tick M±S.E (C.I %95)	Lice M±S.E (C.I %95)	Mixed M±S.E (C.I %95)
Vitamin B_{12} pg/dl	219.04 ± 1.76^{a} (208.20-222.03)	184.06 ± 3.63^{b} (173.06-188.68)	187.91 ± 2.12^{b} (179.08-189.14)	183.08 ± 1.03^{b} (175.76-186.32)
Iron µg/dl	84.98 ± 5.13^{a} (74.66- 95.31)	61.27 ± 8.22^{b} (38.49-84.05)	67.31 ± 7.38^{b} (52.13-82.49)	45.34 ± 5.23^{bc} (33.80-56.87)

(Means with a different letter within each row are different significantly at P≤0.05) M: Mean, S.E: Standard error, C.I: Confident interval, Pg: Picogram, µg: microgram and dl: Deci litre

The present study absolutely concurs with Hatem (2016), who observed a highly decrease in some trace elements statistically (p≤0.01) in the infected sheep. It also agrees with Rakhshandehroo *et al.* (2014) as they detected significant differences (P<0.05) in some trace elements, vitamin A and E in the infested goat. In all results in the current study, the impact of lice is less than both mixed infestation (lice and ticks) and tick-infested goat, this variation in the effects and minimal impact of louse might be related to the genus of lice that seen in this study, which is present merely biting louse and mainly have secondary effects on the blood parameters, because of this reason the decline of these elements (Iron and vitamin B12), seen as a secondary impact of louse. Our results corroborated by findings of Seyrek *et al.* (2009) as they observed a decrease in iron level with highly significant differences (P<0.01), also current result is in agreement with those who observed that a decline in Fe with high significance (P<0.01) in lice infestation buffalo compared to non-infested buffalo (Ahmed *et al.*, 2009). Hamed (2015) mentioned that rock type, erosion of soil surface and pesticide application to soil are other factors that influence the mineral level in the soil and consequently in plants and animals. As Seyrek *et al.* (2009) said that deficiency of trace elements under the imbalance category is related to disease and as Poppenga *et al.* (2012) mentioned, physiological state, parasites, and toxicity are among factors that affect trace element levels. Decreasing some trace elements (iron among them) and vitamins might be related to the parasitic infection itself as a strategic defense induced by Interleukin-1 and IL-2, which was pointed out in the study of Beigh *et al.* (2013) in the infested dog with some ectoparasites. There is no obvious and direct study showing a relationship between ectoparasites and vitamin levels in animals and humans, particularly in vitamin B_{12}, but as this vitamin is synthesized by cooperation of bacteria and cobalt elements, and as those components are affected by parasites, hence any alteration in their level have impacts on the vitamin B12 concentration or might be seen as a secondary effect of ectoparasites, and because they are vectors of some endoparasites which have direct effects on vitamin and other blood components. Zarebavani *et al.* (2012) observed that vitamin B_{12} declined slightly in the parasitic infection but had no significant differences (P>0.05) with a healthy one. Beigh *et al.* (2013) mentioned that infested animals show decreasing values of some vitamins and have a significant difference (P<0.05) when compared with a control group, which is most concurred with the present results. Despite low intake is a major reason for

vitamin B_{12} deficiency, but any disorder leading to ruined intestine and malabsorption is seen as another reason behind vitamin B_{12} lowering (Pawlak *et al.*, 2013). In another study, it was mentioned that during infesting with ticks, vitamin B_9 and vitamin B_{12} were increased in both control and infested animals, which goats not support our results, and they think it is due to leakage, dropped soluble and membrane-bound vitamins. (Kleppa and Stuen, 2003).

Conclusion

Through the current results, in both ectoparasites, the ear is the most infestation site, and in both of them present a 2:1 ratio of females to males on goats. Iron and vitamin B12 concentration decreased markedly in the infested goat with mixed and tick compared with the louse and control group. And ultimately more researches require for emphasizing the relationship between ectoparasites and Vitamins.

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تأثيرات بعض الطفيليات الخارجية على المستويات الفيتامين ب 12 و الحديد في دم الماعز المحلي (*Capra hircus*)

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الملخص

للفيتامينات والعناصر المعدنية النادرة دوراً مهماً في تغذية وحيوية الحيوانات ، على الرغم من ندرتهم في جسم الأنسان و الحيوان إلا أن نسبتهما تتغير وفقاً للحالة للمرضية و الفسيولوجية، و في التجربة الحالية اختيرت 50 رأساً من الماعز المصاب بالطفيليات الخارجية (القراد والقمل والإصابة المختلطة) و50 منها غير المصاب, أظهرت الدراسة بأن منطقة الصدر والكتف أكثر إنتشاراً للقمل بينما أكثر المناطق مصابة بالقراد كانت في الأذان. شوهدت أناث أكثر من ذكور فى كلا من القراد والقمل فى الماعز المصاب. أظهرت الدراسة إنخفاضاً في المستوى فيتامين ب 12 في جميع العنزات المصابة (مختلطة، القراد و القمل)183.08±1.03 ديسى لتر/بيكوجرام، 184.06±3.63 ديسى لتر/بيكوجرام و 187.91±2.12 ديسى لتر/بيكوجرام، على التوالي، مع وجود فروقات المعنوية عند المستوى $p\leq0.05$ مقارنة بالماعز من مجموعة السيطرة، في حين لاتوجد فروقات المعنوية فيما بينهم (الإصابة بالقمل، بالقراد او مختلط بينهم). مستوى الحديد في كل من الماعز المصاب بالإصابة مختلطة و كذالك الإصابة بالقراد أو القمل فقط انخفضت 45.34±5.23 ديسى لتر/ميكروجرام، 61.27±8.2 ديسى لتر/ميكروجرام و 67.31±7.38 ديسى لتر/ميكروجرام ،على التوالي مع وجود فروقات المعنوي عند المستوى $P<0.05$ مقارنةً مع مجموعة الشاهد (الغير مصابة)، وكذالك وجود فرق معنوي $P<0.05$ في الأصابة بالمختلط بالمقارنة مع مصاب بالقمل فقط. أظهرت هذة الدراسة بأن فيتامين ب 12 ومستوى الحديد انخفضا كآثار أولية أو ثانوية لهذه الطفيليات.

الكلمات المفتاحية: الطفيليات الخارجية، فيتامين ب 12، الحديد و الماعز المحلي.