Study Iron Homeostasis of Infected and Non-Infected Iraqi Camels with Trypanosomiasis

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ABSTRACT

Trypanosomiasis is one of the common parasitic diseases, which infects the dromedary camels and decreases the numbers of these animals in Iraq. To get the best knowledge of the changes of iron status in camels infected with trypanosomiasis, and an attempt to take advantage of these variables as markers for infection, this study was designed. In the current study, blood samples were collected from 155 dromedary camels (total samples 155), where 33 (21.29%) were infected, according to the status of infection with *Trypanosoma evansi* that depends on blood smear examination as a golden test. Results denote significant differences in infection ratio by sex and age, from total of 132 male tested, 29 (21.96%) infected, distributed into 12 (41.37%) of age ≤ 2 years and 17 (58.62%) of age ≥ 2 years. Of the 23 females tested, 4 (17.39%) animals were infected at age ≤ 2 years. Furthermore, the results of this study demonstrated a significant (P<0.05) decrease in total serum iron, transferrin saturation, ferritin, whereas there was an increase in total iron-binding capacity and unsaturated iron-binding capacity in the infected male and female camels of different ages. Analyzed data of iron status parameters denoted that the cutoff point test between sensitivity (97) and specificity (100) for serum iron is ≤ 67.26, for transferrin saturation is ≤17.23 between the sensitivity and specificity (100) and (≥378.66), for total iron-binding capacity between the sensitivity and specificity (93.9 and 96.7) respectively. Also, the cutoff point test between the sensitivity (100) and specificity (96.7) for unsaturated iron-binding capacity is (≥301.27) and ferritin concentration has a cutoff point is (≤ 249.88) for the sensitivity (100) and specificity (99.2). It could be concluded from what was stated in the results of the current study, that the measurement of the concentration of serum ferritin could be considered as a good (reliable) marker for the *Trypanosoma. evansi* infection.

Keywords: Dromedarycamels, Iron Homeostasis, *Trypanosoma evansi*

Introduction

Trypanosomiasis is the name of two types of protozoal parasitic disease (American and African trypanosomiasis) in humans and many animals infected by this parasite which goes back to the genus *Trypanosoma* (1). It is represented as one of four main protozoal causes (*Trypanosoma, Babesia, Theileria, and Anaplasma)* that decrease the numbers of camels in Iraq (2). One of the trypanosome species is the *Trypanosoma evansi* (*T. evansi*) that causes Surra disease in animals, especially in camels. *T. evansi* has been diagnosed in Iraq since 1938 in camels, dogs (3), buffalo, and cattle (4). Camel's anemia in trypanosomiasis is a common sign associated in each form of disease, acute and chronic forms. Anemia in acute infection occurs as a result of parasite contribution factors in red blood cells destruction. Increase in red blood cells phagocytosis in trypanosomiasis (5). The RBC$_s$ suffer from mechanical destruction, oxidative stress of cell wall lipid peroxidation and the parasite vesicles to extracellular that can fuse to erythrocytes causing more phagocytosis, thus anemia may occur (6). The development of anemia in trypanosomiasis results from various factors among them, the most important, is the iron homeostasis dysregulation, although the molecular mechanism is not yet explained (7). Anemia signs in many different chronic diseases, especially in trypanosomiasis, are attributed to an imbalance between erythrophagocytosis and erythropoiesis that is linked to confused iron homeostasis and changed iron recycling by macrophages and iron

sequestration. There is a strong suggestion implies that the foreword of iron is hampered in the chronic form of trypanosomiasis and iron processing pathways lead to iron sequestration (8). In Iraq during the non-tropical seasons, Surra disease became a silent illness because of delay detection, which causes more effects, accordingly finding another diagnostic marker for the infection will be useful in controlling the disease. The current study aimed to study and knowledge the changes of iron status and confirms the main type of anemia that appears during the infection of dromedary camels with *T. evansi*.

Materials and Methods

Study Design and Sample Collection

A total of one hundred and fifty five blood samples (about 20 ml) were collected through jugular vein puncture of dromedary camels in different ages (1-8 years) of both genders (males and females) from AL-Najaf and Al-Kut provinces, Iraq. The blood samples were collected during the period from November 2018 to July 2019. Each blood sample was divided into two parts, the first part, about 3 mL, was put into EDTA tube for blood smears which were stained by Giemsa stain to detect *Trypanosoma* parasite. The second part, about 17 mL, was put into a plastic tube for serum adequate after centrifugation of blood samples at 3500 rpm. for 15 minutes and then serum was stored at -18 °C to determine iron status parameters (9).

Iron Statue Measurements

Total Serum Iron Concentration (μg/dl)

Total serum iron concentration (TSI) was measured by a spectrophotometric method using an enzymatic assay kit (human, Germany), and calculated by the following equation (10).

$$\text{TSI (μg/dl)} = \frac{\Delta \text{ A sample}}{\Delta\text{A standard}} \times 100 \text{ (equation 1)}$$

Total Iron Binding Capacity (μg/dl)

Total iron-binding capacity (TIBC) is amounting of iron that serum transferrin binds when there is excess of Fe^{+3}. A special iron kit (human, Germany) was used for determination of TIBC by colorimetric method at 600 nm according to the company recommendation, and calculated by the equation described by (10).

TIBC (μg/dl)=Iron binding to transferrin concentration × 3 (equation 2)

Unsaturated Iron Binding Capacity (μg/dl)

Unsaturated iron binding capacity (UIBC) was calculated from the following equation (11).

UIBC (μg/dl) =Total serum iron concentration (μg/dl) -TIBC (μg/dl) (equation 3)

Transferrin Saturation (%)

Transferrin saturation (TS) was calculated by equation 4 as described by (10).

$$\text{TS (\%)} = \frac{\text{Total serum iron}}{\text{Total iron binding capacity}} \times 100 \text{ (equation 4)}$$

Serum Ferritin Concentration (μg/L)

Total serum ferritin was measured routinely by using a commercial ferritin kit, provided by Spectrum Company, Egyptian, and calculated according to the standard curve equideral equation (11).

Statistical Analysis

Data were analyzed using SAS program (12). The nested model was used to study the effect of status, sex within the status, and age within the status on some parameters. Means were compared as pairwise comparisons. Comparisons among proportions were performed using the Chi-Square test. $P \leq 0.05$ was considered significant.

Results and Discussion

Prevalence of Trypanosomiasis in Different Age and Sex

Trypanosomiasis was confirmed by the presence of the trypomastigote stage of the parasite in Giemsa stained blood smear, as spindle flagellated form (Figure 1). Accordingly, trypanosomiasis in examined samples was (33 +ve.) for the parasite and (122 ‾ve). Results revealed that from 132 males examined, 29 males were trypanosomiasis infected (21.96%) and from 23 females 4 were infected (17.39%) as shown in Figure 2.

Results in Figure 3 show the prevalence of trypanosomiasis in different ages of males and females. Results revealed that males at age more than two years were more susceptible to infection (28.33%) than younger (16.66%). There were no significant differences between males and females under two years of age (16.66%, 17.39%, respectively).
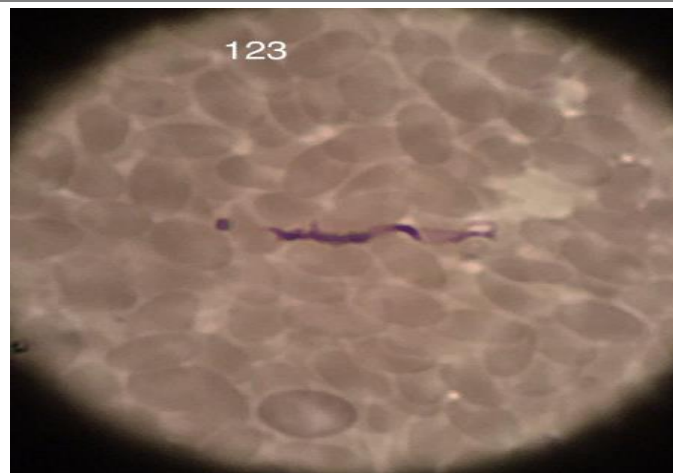
Figure 1. Infected blood film stained with Giemsa that examined by microscopy under oil immersion (100X)
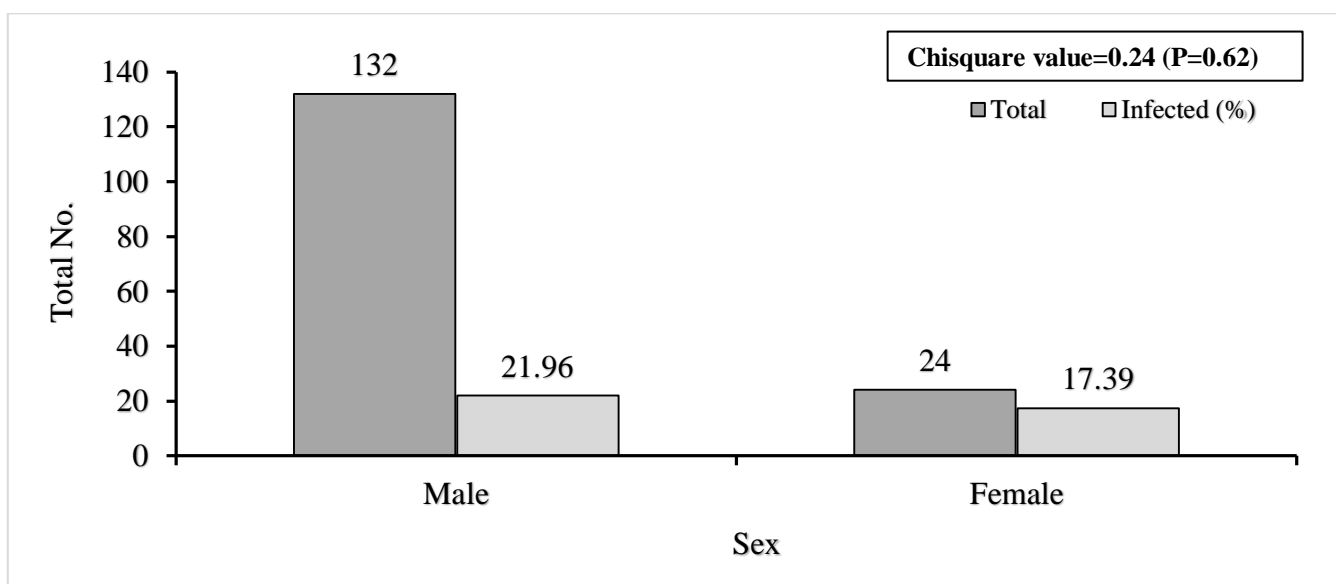
Figure 2. Infected rate of *Trypanosoma evansi* in camels according to sex, n= 132 for males, n=23 for females

The application of microscopy assay for the specific detection of trypanosomes in camel blood samples was established in the current study.

The results revealed that the male camels were significantly more infected (21.96%) than females (17.39%). The results agree with a local study done on Iraqi camels (2), and with other studies at the regional level of Iraq (13, 14). However, our results disagree with (15, 16). The reasons cannot be explained exactly, but might be attributed to the large number of samples collected from males especially in the Najaf region because males are more likely to be slaughtered than females in slaughterhouses; also, males are exposed to different types of stress such as transportation more than females. However, the last study indicated to the sex-based difference could be the result of behavioral differences, with one sex coming into contact with sources of infection more than the other. However, it has also been ascribed to the association between testosterone and the immune system. Although the exact mechanism of action is unclear, evidence suggests an immunosuppressive role for testosterone, which would hamper the elimination of parasites (17).

In addition, the results in the present study showed that the highest rate of infection was recorded in camels at ≥2 years of age (28.33%), while the lowest rate of infection was in young camels at <2 years of age (16.67%). This result agrees with the results of previously published studies conducted on Iraqi camels (18), Iraqi bovine (4), and with trypanosomiasis-associated camel studies done in Egypt (19) and Saudi Arabia (20).
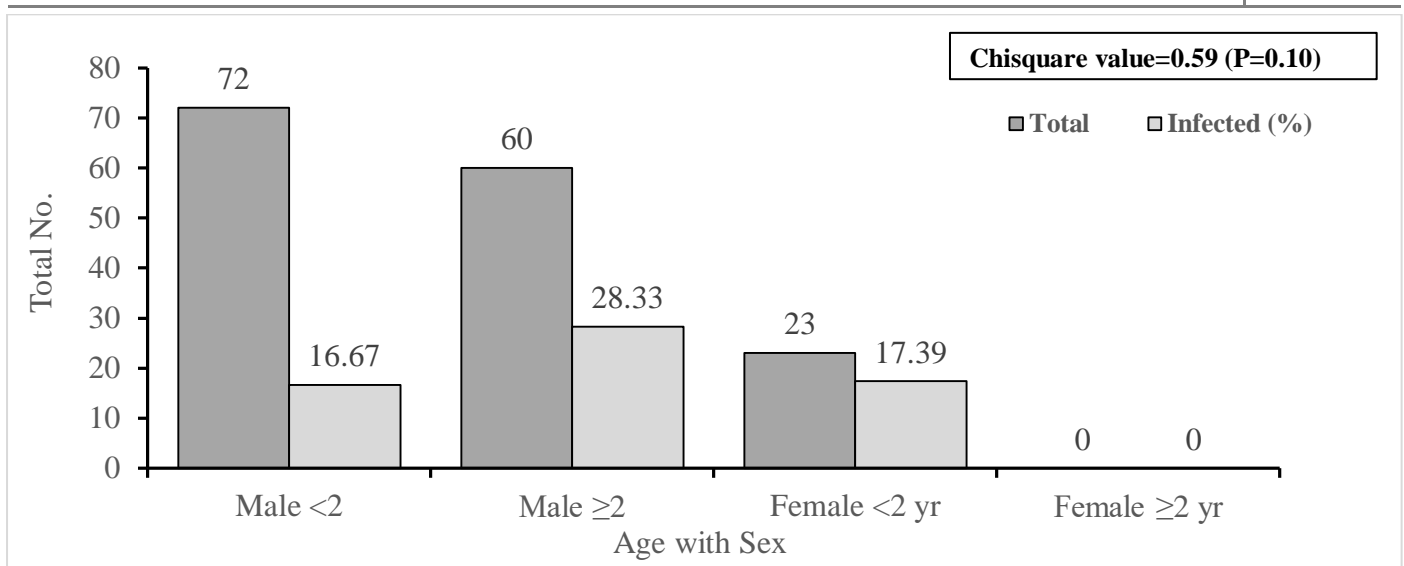
Figure 3. Comparison between males and females of Iraqi camels in the infection rate with *Trypanosoma evansi* according to age. n= 132 for males, n=23 for females

The authors reported that the high infection rates in adult camels might be due to the heavy stress expos through transportation of goods, poor management, and immune status of animals, which is depressed with progressive in age and disease infection that may predispose to *T. evansi* (19). Also, it is thought that the presence of maternal immunity during the first 6-12 months of life plays a vital role in protection against different infections and this form of passive immunity is greatly diminished after two years of age (21).

Iron Status in Camels

Results in Figure 4 showed that there was a significant ($P<0.05$) decrease in total serum iron, transferrin saturation, and ferritin concentration, correlated significantly ($P<0.05$) with the increase in total iron-binding capacity and unsaturated iron bounding capacity of transferrin in camels infected with *T. evansi*.

The present study cleared in Table 1, that there were no effects of gender and age on iron status parameters of healthy camels with exception of ferritin, where females showed a significant decrease in ferritin concentration. Infected camels with *T. evansi*, suffered from a significant decrease ($P<0.05$) of TSI, TS, and ferritin concentration and significante ($P<0.05$) increased of TIBC and UIBC.
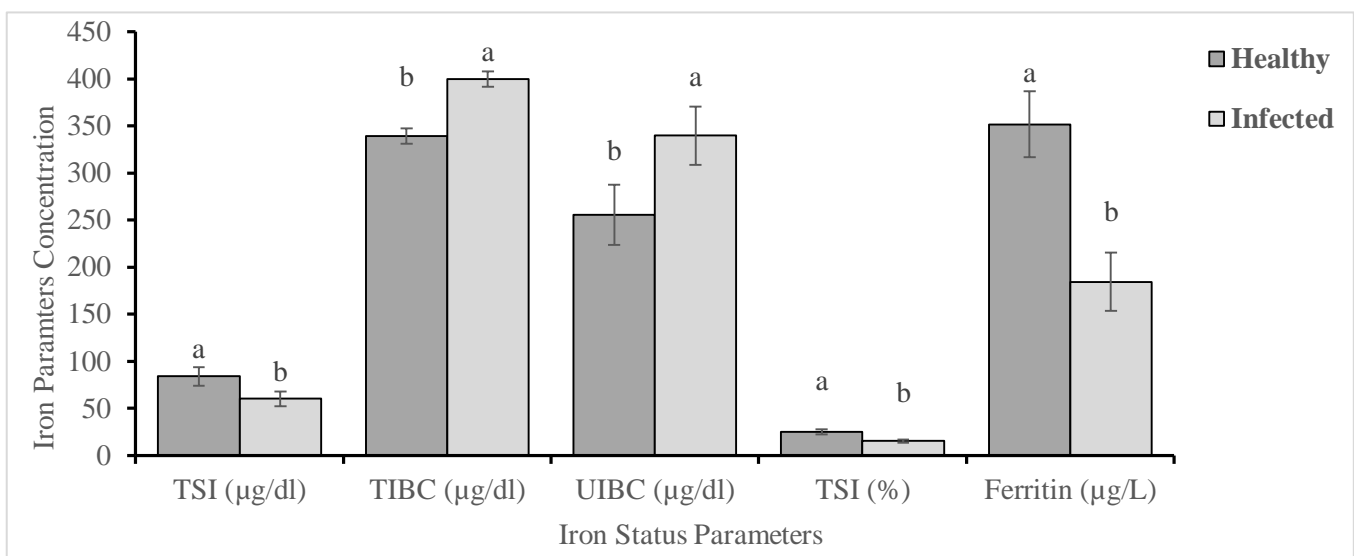
Figure 4. Effects of infection with Trypanosoma evansi in camels on TSI, total serum iron; TIBC, total iron-binding capacity; UIBC, unsaturated iron-binding capacity; TS, transferrin saturation. Mean±SE. a-b Bars for each parameter lacking a common letter differ significantly at $P \leq 0.05$

Table 1. Total serum iron (TSI), total iron-binding capacity (TIBC), unsaturated iron-binding capacity (UIBC), transferrin saturation (TS), and ferritin in *Trypanosoma evansi* infected male and female of different ages compared to healthy Iraqi camels [1]

Status	Sex	Age (years)	No	TSI (µg/dl)	TIBC (µg/dl)	UIBC (µg/dl)	TS (%)	Ferritin (µg/L)
Healthy	Male	<2	60	84.2±1.11 [a]	338.1±3.39 [b]	253.9±3.82 [bc]	25.1±0.46 [a]	363.1±7.41 [ab]
		≥2	43	84.0±1.74 [a]	341.3±3.77 [cb]	257.2±4.87 [b]	24.8±0.68 [a]	371.5±5.34 [a]
	Female	<2	7	86.6±2.76 [a]	335.7±7.43 [bc]	249.0±9.20 [b]	25.9±1.20 [a]	343.7±11.0 [b]
		≥2	12	79.9±2.83 [a]	339.1±8.62 [b]	259.2±11.0 [c]	23.9±1.46 [a]	328.1±12.5 [b]
Infected	Male	<2	12	59.7±1.65 [b]	396.8±4.22 [a]	337.1±5.47 [a]	15.0±0.52 [bc]	187.0±11.2 [c]
		≥2	17	59.9±0.98 [b]	404.8±6.02 [a]	344.8±6.44 [a]	14.8±0.37 [bc]	176.8±7.07 [c]
	Female	<2	4	61.7±1.06 [b]	386.6±6.64 [a]	324.9±6.93 [a]	15.9±0.42 [b]	187.0±11.2 [c]
		≥2	-	-	-	-	-	-
LSD			155	7.6917	21.317	25.429	3.1802	17. 764

[1] Mean±SE. [a-c] Means within a column lacking a common superscript differ significantly at P≤0.05

Results showed that there were no significant roles for age and sex as in the case of healthy camels on the iron status parameters.

The results of the present study denoted that the cutoff point test reflects the sensitivity and specificity for iron status parameters TSI, TIBC, UIBC, TS, and ferritin concentration precisely and detailed (Table 2, Figure 5); with the importance of ferritin concentration, that has a cutoff point of ≤ 249.88 for 100 and 99.2 sensitivity and specificity, respectively. The serum ferritin criterion was the most accurate value among others for sensitivity and specificity and it can be considered as a good marker for trypanosomiasis diagnosis. The cutoff point is the point of intersection between the reading value of the ferritin and the golden test ([+]ve appearance of parasite in blood smear).

Table 2. Estimation of cutoff point test between sensitivity and specificity for iron status parameters that use the main test for diagnosis trypanosomiasis in Iraqi camels

Test	Cutoff point	Sensitivity	Specificity	AUC	SE[*]	95% Cl[**]
TSI[†]	≤ 67.26	97	100	0.999	0.00169	0.974 to 1.000
TIBC	≥ 378.66	93.9	96.7	0.965	0.00790	0.951 to 0.998
UIBC	≥ 301.27	100	96.7	0.967	0.00239	0.971 to 1.000
TS	≤ 17.23	100	100	1.000	0.000	0.976 to 1.000
Ferritin	≤ 249.88	100	99.2	1.000	0.00217	0.973 to 1.000

[†]TSI, total serum iron; TIBC, total iron-binding capacity; UIBC, unsaturated iron-binding capacity; TS, transferrin saturation. AUC = Area under curve, [*]Hanley and McNeil, 1982, Binomial exact, [**]Cl = Confidence interval

The results of the current study indicated differences between non-infected and infected camels depending on the values of iron status parameters, which confirms the occurrence of iron deficiency anemia as a result of trypanosomiasis in Iraqi camels. As noted, there was a decrease in serum iron, transferrin saturation, ferritin concentration, and an increase in TIBC and UIBC in camels infected with *T. evansi*. In addition, the changes occurred in both genders (male and female), at the same level when compared with reference values of non-infected camels, also there were no differences between young (<2 years) and adult (≥2 years) camels responses to the infection.

The results are in agreement with the study done by (22) on Iraqi camels and agree with what was mentioned in the review of (5). Where cleared anemia is a well-established infection-associated immunopathological feature of trypanosomiasis and the degree of the anemia is a reliable indicator of the severity of the infection. The immune system response during *Trypanosoma* infection consisting of a strong proinflammatory M1-type activation of the myeloid phagocyte system (MYPS) results in iron deprivation for these extracellular parasites. Yet, the persistence of M1-type MYPS activation causes the development of an anemia (Iron deficiency) as the most prominent pathological
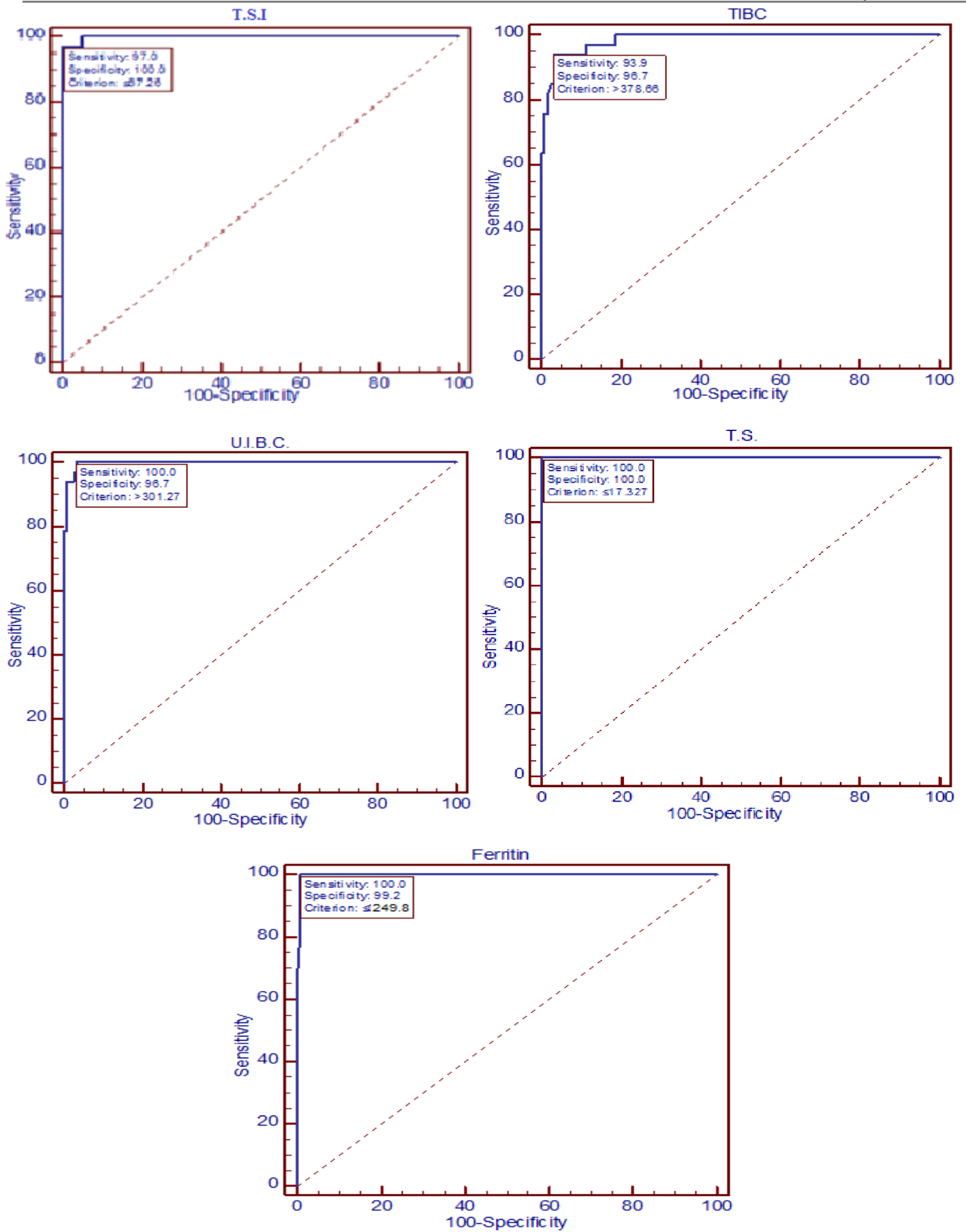
Figure 5. Cutoff point test with present Sensitivity and Specificity for iron status parameters

parameter in the mammalian host, due to enhanced erythrophagocytosis and retention of iron within the MYPS thereby depriving iron for erythropoiesis (5).

The TIBC and UIBC were increased in iron-deficient anemic animals due to the increase of the transferrin molecules which essential for iron transport from storage sites (23). High TIBC occurred in low serum iron and also UIBC increased in iron deficiency anemia due to the increase of the transferrin binding site unbound with iron (24-26).

TS decrease in iron deficiency anemia could indicate that the binding site of transferrin was unsaturated (27); plasma ferritin is a type of proteins directly and quantitatively correlated with the mobilizable storage iron. Ferritin has a remarkable capacity for uptake, storage, and release of iron. Estimation of serum ferritin is the most suited for estimating the body iron status. Decreased serum ferritin in the present study can be suggestive of iron deficiency anemia caused by trypanosomiasis but is also seen in conjunction with liver parenchyma damage (28).

Blood smear represented a golden test to diagnosis infected and non-infected state of Iraqi camels, but there is another statistical concept used in the present study as a new test to diagnosis trypanosomiasis in camels, named "cutoff point test" and each parameter of iron status have a specific value for this test. When this value close to the hundred this means the current test is the best to represent the infected and non-infected status of camels.

The results of the current study confirmed the changes of the body in camels infected with *T. evansi*, especially the changes of iron status, and iron deficiency anemia is the main type that appeared during the infection with *T. evansi* of dromedary camels. In addition, the cutoff point is a test that could be dependable to measure the sensitivity and specificity of iron status parameters and could represent one of the main tests for diagnosis of trypanosomiasis in Iraqi camels, which will play a very important role in controlling this parasitic disease.

Acknowledgments

Conflict of Interest

The authors declare that they have no conflict of interest.

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دراسة التوازن الحديدي في الإبل العراقية المصابة وغير المصابة بداء المثقبيات

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الخلاصة

يعتبر داء المثقبيات من الأمراض الشائعة التي تصيب الإبل العربي وتقلل من أعداد هذه الحيوانات في العراق. لمعرفة أفضل التغيرات التي تطرأ على حالة الحديد في الإبل المصابة بداء المثقبيات ،وفي محاولة للاستفادة من هذه المتغيرات كعلامات للعدوى صممت هذه الدراسة. الدراسة الحالية أجريت على عينات الدم التي جمعت من 155 جمل, بواقع 33 جمل مصاب (21.29٪) بهذا الداء. وفقًا لحالة الإصابة بمثقبيات أيفانساي التي تعتمد على فحص المسحة الدموية كاختبار ذهبي. تشير النتائج إلى اختلافات كبيرة في نسبة الإصابة حسب الجنس والعمر،من إجمالي 132 ذكر تم اختبارهم ، 29 (21,96 ٪) ذكر مصاب, موزعين الى 12 (41,37٪) ذكر يبلغ من العمر سنتان فما دون و 17(58.62٪) ذكر يبلغ من العمر سنتان فما فوق، ومن 23 أنثى تم اختبارها ،4 أناث (17.39٪) بمعدل عمر أصغر من سنتينفقط. أظهرت نتائج هذه الدراسة أن معاملات حالة الحديد في كلا الجنسين تأثرت نتيجة الأصابة بداء المثقبيات، فقد كان هناك انخفاض معنوي (p<0.05) لتركيز الحديد في المصل، و لتركيزالترانسفرين المشبع و تركيز الفرتين, بينما كانت هنالك زيادة معنوية (p<0.05) في السعة الكلية للحديد المرتبط والسعة الكلية للحديد الغير مرتبطبالنسبةللأبل المصابة من الذكور والإناث، ولمختلف الأعمار.تشيرالبيانات التي تم تحليلها وفقا لحالة الحديد إلى أن اختبار نقطة القطع بين الحساسية (97) والخصوصية (100) لحديد المصل هو (67.26≥) ونقطة القطع لتشبع الترانسفيرين (17.23≥) بين الحساسية والنوعية (100) و(378.66) هي نقطة القطع لسعة الحديد الكلية المرتبطة بين الحساسية والنوعية (93.9 و 96.7 على التوالي ،كما أن اختبار نقطة القطع بين الحساسية (100) والنوعية (96.7) لقدرة ربط الحديد غير المشبع هي (301.27 ≥), وتركيز الفيريتين له نقطة قطع (249.88 ≥) للحساسية (100) والنوعية (99.2). في الختام، استنتج من ما ورد في نتائج الدراسة الحالية فيما يتعلق بفحص تحليل Roc ومن قيمة نقطة القطع، أن قياس تركيز الفيريتين في الدم يمكن اعتباره علامة جديدة وجيدة لتشخيص الأصابة بداء المثقبيات.

الكلمات المفتاحية: الأبل العربية, التوازن الحديدي, مثقبيات أيفانساي