



Detection of *Theileria spp.* in blood samples and estimation of haematological and biochemical changes in sheep in Al-Diwaniya province

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Abstract:

The present study was carried out to detection the prevalence of *Theileria spp.* in sheep in Al-Diwaniya province and assess the hematological and biochemical changes occurred in sheep naturally infected with *Theileria spp.* Blood samples were collected from (132) sheep from five regions in Al-Diwaniya province. Result revealed that the overall prevalence was (26.51%). Ratios of prevalence were significant differences among different regions, the highest prevalence was (42.85%) in Al-Diwaniya while, the lowest (9.09%) in Sumar.

The clinical signs were recorded in *Theileria* -infected sheep were enlargement of prescapular lymph nodes, loss of appetite, pale mucous membranes, and congestion of mucous membranes, coughing and diarrhea. In addition, present of ticks on the various parts of infected sheep. Statistically significant increase ($p < 0.05$) in the body temperature of diseased sheep compared with uninfected sheep. Haematological study revealed that statistically significant decreases ($p < 0.05$) in red blood cell counts, haemoglobin concentration and packed cell volume with non-significant increase ($p < 0.05$) in white blood cell counts and recorded significant increase ($p < 0.05$) in the MCV and MCH values, while the MCHC value was non significant differences in the infected sheep when compared with uninfected sheep.

The biochemical study revealed that statistically significant decrease ($p < 0.05$) were observed in serum total protein, albumin and non significant increase ($p < 0.05$) were recorded in globulin level, alanine aminotransferase (ALT) and creatinine, with significantly increase ($p < 0.05$) in the levels of aspartate aminotransferase (AST), cholesterol and urea in the infected sheep when compared with uninfected sheep.

Key words: *Theileria*; Sheep- protozoa; Haematological parameters; Biochemical parameters; Clinical signs; Prevalence; Al-Diwaniya province.

الكشف عن *Theileria spp.* في عينات الدم وتقييم التغيرات الدموية والكيموحيوية في الأغنام في محافظة الديوانية

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الخلاصة:

أجريت الدراسة الحالية للكشف عن انتشار طفيلي *Theileria spp.* في الأغنام في محافظة الديوانية ولتقييم التغيرات الدموية والكيموحيوية التي تحدث في الأغنام المصابة طبيعياً بـ *Theileria spp.* جمعت (132) عينة دم من الأغنام من خمسة مناطق في محافظة الديوانية. أظهرت النتائج أن نسبة انتشار الإصابة الكلية (26.51%) اختلفت نسب الإصابة معنوياً باختلاف المناطق كانت أعلى نسبة انتشار للإصابة في الديوانية (42.85%) وأقلها في سومر ونسبة (9.09%). العلامات السريرية المسجلة في الأغنام المصابة بطفيلي *Theileria* هي تضخم العقد اللمفية أمام الكتفية، فقدان الشهية، شحوب الأغشية المخاطية، و احتقان الأغشية المخاطية، السعال، و الإسهال إضافة الى وجود القراد على أجزاء مختلفة من جسم الأغنام المصابة. أظهر التحليل الاحصائي وجود زيادة معنوية ($p < 0.05$) في معدل درجات الحرارة في الأغنام المصابة مقارنة بالأغنام غير المصابة. كما أظهرت الدراسة الدموية الى انخفاض معنوي ($p < 0.05$) احصائي في العد الكلي لكريات الدم الحمراء، تركيز خضاب الدم وحجم الخلايا المضغوطة مع زيادة غير معنوية ($p < 0.05$) في العد الكلي لكريات الدم البيضاء وسجل زيادة معنوية في قيم معدل حجم الكرية، معدل خضاب الكرية و بينما لا يوجد فرق احصائي بمعدل تركيز خضاب الكرية في الأغنام المصابة مقارنة بالأغنام غير المصابة. إحصائياً أظهرت الدراسة الكيموحيوية الى انخفاض معنوي في البروتين الكلي و الالبومين وزيادة غير معنوية ($p < 0.05$) في مستوى الكلوبيولين، وخميرة الالنين ناقلة الأمين (ALT) و كرياتينين ومع زيادة معنوية ($p < 0.05$) في مستويات خميرة الاسبارتيت ناقلة الأمين (AST)، الكولسترول و اليوريا في الأغنام المصابة مقارنة بالأغنام غير المصابة.

الكلمات المفتاحية: الثايليريا؛ أوالي-الأغنام؛ المعايير الدموية؛ المعايير الكيموحيوية؛ العلامات السريرية؛ الأنتشار؛ محافظة الديوانية.

Introduction:

Theileria is a tick-transmitted protozoan parasite belongs to phylum Apicomplexa (1). Theileriosis associated with *Thieleria spp* in cattle, sheep and goats as well as in wild and captive ungulates (2). *Theileria ovis* was widely distribution in sheep and goats has with being present in Africa, Asia, India, and parts of Europe, caused the benign form of theileriosis (3).

Theileria separata is present only in Africa, *Thieleria hirci* (synonym *T. lestoquardi*) is the more virulent form of malignant ovine theileriosis is endemic in Middle East and is probably present in regions of north Africa and parts of china (4).

In addition, a different species of *Theileria* (*Theileria* sp1. called *T. luwenshuni* and *Theileria* sp2. called *T. uilenbergi*) reported are a part of pathogenic parasites for small ruminants in China (5). In acute infections of malignant ovine theileriosis is characterized by fever, very high mortality in 3-6 days, anemia, jaundice, and enlargement of lymph nodes (2). Recovery form disease is often slow and subacute signs are recurrent fever and anemia (4).

Al-Abood *et al.* (6) recorded the infected rate of blood parasite (*Theileria*

and *Anaplasma*) in sheep in Basrah was (17.5%). *Thieleria spp.* was found in (18.29%) of sheep in the East and Southeast Anatolia in Turkey (7). The aim of the present study was to detection of *Theileria spp.* prevalence in sheep in the Al-Diwaniya province and assess the haematological and biochemical changes occurred in sheep naturally infected with *Theileria spp.* in Al -Diwaniya province.

Materials and Methods:

This study was carried out in Al-Diwanyia province during the period from January 2011 to October 2011. A total (132) of blood samples were collected from randomly selected sheep herds located in Al-Diwanyia province includes Al-Diwaniya, Afak, Al-Hamza, Al-Digara and sumar. The sheep (male and female the age range from 2-4 years old) were clinical examined for presence of superficial lymph nodes enlargement, change of appetite and examined of conjunctival and eyes mucus membranes, diarrhea, coughing and ticks present on different parts of body and rectal temperature was measuring by using a digital thermometer.

Blood samples obtained from jugular vein of sheep, about (10) ml of blood was taken from each animal, 3 ml was placed in a K2-EDTA tube used to evaluate some of blood parameters include Red blood cell count (RBC), White blood cell count (WBC), Packed cell volume (PCV), Haemoglobin concentration (Hb), indices (mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were estimated as described by (8,9) and for the preparation of blood films were fixed in absolute methyl alcohol (5min) and stained with 10% Giemsa (30min), microscopically examined under oil immersion lens (8).

Other 7ml was put into coagulant free tube to take the serum; sample was centrifuged at 3000 rpm for 10 min (9). The serum to estimate the biochemical parameters, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, were determined by Reflotron[®] Plus (Roche, Germany). While other biochemical parameters include total protein, cholesterol, albumin and urea were determined by using spectrophotometer and using commercially diagnostic test kits supplied by (Spinreact, Spain, Cromatest, Spain, Fortess diagnostics, UK) following their user's manuals. Globulin was calculated by subtracting the serum albumin values from total protein values (10).

Statistical analysis: Using (SPSS) version 14.0 data were analyzed using T-test analysis. Using Chi-square (X^2) test, $p < 0.05$ was accepted to be statistically significant.

Results:

In the present study showed the overall prevalence of *Theileria spp.* was (26.51%) in sheep from Al-Diwaniya province.

Ratios of prevalence were different with different regions, there were significant differences at level ($p < 0.05$) among different regions in sheep infected with *Theileria spp.* the highest prevalence was (42.85%) in Al-Diwaniya while the lowest (9.09%) in Sumar (Table 1).

The clinical signs of theileriosis in sheep were loss of appetite, gross enlargement of the prescapular lymph nodes, pale mucous membranes, and congestion of mucous membranes, diarrhea, coughing and ticks were found on various parts of the body (Table 2). Statistically significant increase ($p < 0.05$) in body temperature of *Theileria*-infected sheep compared with control group (Table 3). Hematological examination revealed that statistically non-significant increase ($p < 0.05$) in WBC counts between infected sheep compared with control group. There were significant decreases in RBC counts, Hb concentration and PCV percentage in the diseased sheep compared with control group. While the MCV and MCH values were revealed significantly increased ($P < 0.05$) in the infected sheep compared with the healthy sheep. The MCHC value was non-significant difference between diseased sheep compared with the control group (Table 3).

The statistical analysis revealed that the levels of total protein and albumin were significantly decreased ($p < 0.05$) in the infected sheep compared with the control group. While non-significant increase ($p < 0.05$) in the level of globulin, alanine aminotransferase (ALT) and creatinine in the infected sheep compared with control group. There were significant increases ($p < 0.05$) in the levels of aspartate aminotransferase (AST), cholesterol and urea between infected sheep and control group (Table 4).

Table (1): prevalence of *Theileria spp.* infection in sheep according to five regions in Al-Diwaniya province.

N	Regions	Total number of samples	Positive sample	%
1	Al-Diwaniya	14	6	42.85
2	Afak	29	4	13.79
3	Al-Hamza	29	12	41.37
4	Al-Digara	38	11	28.94
5	Sumar	22	2	9.09
Total		132	35	26.51

Calculated Chi square =11.16, Tabulated $X^2(0.05) =9.48773$

Degree of freedom=4, P-value: 0.02482348

Significantly different ($p < 0.05$) between this regions.

Table (2): Percentage of clinical signs in *Theileria spp.* infected sheep were recorded from total diseased number (n=35)

N	The clinical signs	Number of infected animals	%
1	Enlarged of the prescapular lymph nodes	13	37.14
2	Loss of appetite (inappetence)	13	37.14
3	Pale of mucous membranes	12	34.28
4	Congestion of mucous membranes	6	17.14
5	Ticks detected on different parts of body	18	51.42
6	Diarrhea	3	8.57
7	Coughing	2	5.71

Table (3): The Effect of infection with *Theileria. spp.* on temperature and haematological parameters in sheep.

N	Groups	Infected group n=35	Control group n=97
	Parameters		
1	Temperature °C	39.422±0.084 B	38.804±0.047 A
2	WBC × 10 ³ /μL	9.830±0.511	8.909±0.226
3	RBC × 10 ⁶ /μL	7.181±0.227 B	8.986±0.136 A
4	HB g/dL	8.851±0.247B	10.688±0.175 A
5	PCV%	31.825±1.789 B	35.945±0.583 A
6	MCV fL	45.477±3.270 B	40.177±0.554 A
7	MCH pg	12.405±0.233 B	11.923±0.096 A
8	MCHC g/dL	29.402±0.967	29.873±0.305

Values represent mean ±SE

Means within row with different letter differ significantly ($p < 0.05$) between groups.

Table (4): The Effect of infection with *Theileria. Spp* . on biochemical parameters in sheep.

N	Groups Parameters	Infected group n=35	Control group n=97
1	Total protein g/dL	5.301±0.299 B	5.902±0.132 A
2	Albumin g/dL	3.178±0.161 B	3.561±0.068 A
3	Globulin g/dL	2.668±0.211	2.412±0.117
4	AST U/L	143.360±8.195 B	125.970±3.117 A
5	ALT U/L	25.375±1.914	22.333±0.668
6	Cholesterol mg/dL	111.429±5.976 B	99.456±2.398 A
7	Creatinine mg/dL	0.722±0.041	0.656±0.029
8	Urea mg/dL	42.281±2.894 B	28.676±0.437 A

Values represent mean ±SE

Means within row with different letter differ significantly ($p < 0.05$) between groups.

Discussion:

The prevalence of *Theileria spp.* in sheep from Al-Diwaniya province was (26.51%). While, the infected rate of blood parasite in sheep in Basrah was (17.5%) (6). *Theileria spp.* was found in (11.9%) of sheep in south khorasan province in Iran and (18.40%) of sheep in around Kayseri (11, 12) Irshad *et al.* (13) reported (7.36%) prevalence of theileriosis in sheep in Pakistan.

In the five investigated areas in Al-Diwaniya province, the were significant differences among different regions in sheep infected with *Theileria spp.* the highest prevalence was (42.85%) in Al-Diwaniya while the lowest (9.09%) in Sumar. These results are in agreement with Al-Saeed *et al.* (14) was reported significant difference between the governorates of Kurdistan region in the *Theileria*-infected cattle. *Theileria spp.* can be transmitted by vector ticks (2). *Hyalomma a. anatolicum* is the most important vector for malignant ovine theileriosis (15). Dumanli *et al.* (16) showed the higher prevalence of tropical theileriosis in towns was found vector ticks indicate the positive correlation between the prevalence of disease and distribution of vector ticks. AL-Mahnaa (17) has shown the highest prevalence of ticks infected identified in ruminant in Al-Diwaniya city compared with other regions involved with study in Al-Diwaniya

province. Al-Saeed *et al.* (14) was related the influenced the intensity of tropical theileriosis and infestation levels in ticks by seasonal variation, breeding and management system in any region.

The clinical signs of theileriosis in sheep were enlargement of prescapular lymph nodes, loss of appetite, pale mucous membranes, coughing, diarrhea and ticks were found on different parts of infected sheep. These clinical signs were in agreement with those obtained by (18, 19). Statistically significant increase in body temperature of *Theileria*-infected sheep compared with control group the result was in agreement with (18).

Hematological examination revealed non-significant increase ($p < 0.05$) in WBCs between infected sheep compared with control group. Sandhu *et al.* (20) showed an initial non-significant leukocytosis in calves experimentally infected with *T.annulata* due to proliferation of lymphocytes in the lymphoid organs as defensive response to invading parasite. However, Al-Amiry(21) and Al-Obaidi and AlSaad (18) were showed significant increase in WBCs between *Theileria*-infected sheep compared with control group.

There were significant decreased in RBC counts, Hb concentration and PCV percentage in the diseased sheep compared with control group which confirmed

anemia in the infected sheep ,These result was in agreement with other reports (6, 22,23) Bell- Sakyi *et al.* (24) showed the significant lower of PCV of sheep carrying *Anplasma* and or/*Theileria* than of sheep without parasite. In addition, Al-Sultan *et al.* (25) and Karawan (26) reported that decreased in RBC counts, Hb concentration and PCV percentage in *Theileria*- infected cattle. The pathogenesis of the anemia is not clear but occurs reasons hemolytic factor reported in the serum of acute affected cattle and may macrophages can damage red blood cells (2). In addition Nazifi *et al.* (23) showed that sever progressive anemia in ovine malignant thielerosis indicate increase exposure of RBCs to oxidative damage. Grewal *et al.* (27) indicated that *Theileria* infection led to increased oxidative stress to the animals and even increased activities of antioxidant enzymes could not decreased this oxidative stress this may be cause of elevated erythrocyte fragility due to membrane lysis and lower haemoglobin concentration.

The MCV value was revealed significant increase in infected sheep compared with the healthy sheep. Indicating macrocytic anemia. Razavi *et al.* (22) and Omer *et al.*(28) reported a significant increase in MCV value in malignant ovine theileriosis and in *T. annulata* infected cattle.

The diagnostic clinical chemistry findings are the levels of total protein and albumin were significantly decreased in the infected sheep compared with the control group. This observation is in agreement with studies were reported that a significant decrease in the levels of total protein and albumin in cattle infected with *Thieleria annulata* (29, 30).on other hand, Baghshani *et al.* (31)who revealed a significant decrease in albumin and non-significant decrease in total protein in sheep infected with *Theileria*. In addition, AlSaad *et al.* (32) who recorded a significant decrease in total protein in goat infected with *Theileria*. Singh *et al.* (33)

attributed the decrease in total protein content of serum due to hypoalbuminaemia may be to reduced synthesis because of the effect on the liver. A similar report was showed decrease of serum total protein this may be due to decrease dietary intake, diarrhea and it decrease synthesis in the liver caused by direct or indirect effect of parasite on the liver (18). While statistically non-significant increased ($p<0.05$) in the level of globulin in the infected sheep compared with control group. Binta *et al.* (34) demonstrated remarkable hyperglobulinaemia was evident in cattle infected with *Thieleria taurotragi* and *Thieleria mutans* ,but contradicts to the result showed significantly lower serum globulin in cattle naturally infected with *Thieleria annulata*(35).

Increased synthesis of APPs(acute phase proteins)may contribute to hyperglobulinemia that occurs in association with inflammatory responses to tissue injury and /or foreign antigens(10).In studies that described significant increase in APPs (haptoglobin ,serum amyloid A, ceruloplasmin and fibrinogen)with an increase in the parasitaemia rate in malignant ovine theileriosis and in bovine tropical theileriosis and showed highest sensitivity and specificity of serum amyloid A was a suitable indicator of inflammatory reactions in the both diseases (22,36).

There were significant increases in the levels of aspartate aminotransferase and non-significant increase in the levels alanine aminotransferase. This observation is in agreement with that of Baghshani *et al.* (31) showed the levels of AST and ALT were increased in diseased sheep with theileriosis although the only significant elevated of AST activity. On the other hand, Al-Obaidi and AlSaad (18) showed significant increase ALT and AST in sheep infected with *Thieleria hirci*. Shahnawaz *et al.* (37) who detected elevated in AST and ALT levels in cattle

infected with *Theileria annulata* indicate hepatic dysfunction .Lotfollahzadeh *et al.* (38) who reported the increased activities of serum enzyme indicated the hepatic injuries associated with *Theileria annulata* and *Babesia bigemina*.

In the present study, infected caused significant increase cholesterol This observation is in agreement with study was reported increased in cholesterol levels in cattle infected with *Theileria annulata* (37). The elevated of cholesterol attributed to liver damage result in a concurrent elevated of fats level with reduction of sugar and protein (39) .However, Baghshani *et al.* (31) recoded non-significant increase of cholesterol levels in sheep naturally infected with theileriosis.

The non-significant increase in creatinine level was observed in the present study. However, Col and Uslu (29) who reported an increased in the creatinine levels in cattle naturally infected with *Theileria annulata* , but contradicts to the result showed non-significant decrease in creatinine concentration in the infected sheep with theileriosis(31).

The significant increase in urea level between infected sheep and control group. These agree with previous studies that were showed increase in urea levels in sheep infected with *Theileria* (18, 31). An increased in urea concentrations may be related to kidney damage (29).

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