

Study of Clinical ,Hematological and serological Diagnosis of Ovine Theileriosis in Basrah province

Tareq Rifaht Minnat and Israa Abdulwadood MA.
Collage of Veterinary Medicine /Basrah University
Basrah /Iraq

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ABSTRACT

The present study was carried out to determine prevalence of ovine theileriosis in the sheep in Basrah province, with studying clinical signs and hematological parameters supported by serological diagnosis of *Theileria lestoquardi* by ELISA technique.

The survey study extended from 15 July 2010 to 30 May 2011. A total of 1466 sheep of different age ,sex and breed examined randomly from different areas in northern, southern, eastern, western and central of Basrah provinc. A total number of sheep exanimated clinically are 1466 and 460 sheep of them showed a clinical signs such as the fever, enlargement of superficial lymph nodes , respiratory signs ,tachycardia, paleness of mucus membrane . The blood examination showed the high level of parasitemia varied between 12-80% and observed all parasite phase; erythrocyte stage 64.8%, lymphocytic stage 22.62% and erythrocytes & lymphocytic stage 12.58%.

The study showed the high prevalence of ovine theileriosis in northern area with 65.82% followed by southern area with 58.94% and lower prevalence of theileriosis in the central of province with 52.98%.

The hematological parameter showed the macrocytic hypochromic and normocytic normochromic anemia in sheep infected by *Theileria lestoquardi* with significant decreases in Hb 7.8 ± 0.4 (g/l) , PCV 26.81 ± 2.991 (%), MCHC 28.99 ± 1.8 (%), RBCC 5944545 ± 62.38 (10/mm), WBCC 5870.0 ± 709.76 (10 /mm), in the lymphocytes 3602.95 ± 450.5 (absolute) and neutrophils 1166.8 ± 235.72 (absolute),while recorded a significant increase in MCV 4545 ± 6.27 (%) while significant increase in monocytes and eosinophiles. The serological diagnosis by indirect ELISA test showed a high prevalence of Malignant theileriosis by *Theileria lestoquardi* 93.14% ,while the lower prevalence of benign theileriosis by *Theileria ovis* 6.85%.

INTRODUCTION

Ovine theileriosis is an important hemoprotozoal disease of sheep and goats in tropical and subtropical regions (Altay *et al*, 2007a) due to at least six species of *Theileria* including *Theileria ovis*, *Theileria separate*, *Theileria recondite*, which are considered as being low or non-pathogenic to the small ruminants (Alani and

Herbert, 1988). *Theileria lestoquardi* (*Theileria hirci*), and *Theileria spp.* (*China 1*) and *Theileria spp.* (*China 2*) that recently was reported from north of China (Ahmed *et al.*, 2006 ;Niu *et al.* ,2009).

T. lestoquardi and *Theileria china* unlike the other three species are highly pathogenic (Altay *et al.*,2005).

Hummandi,(1978) show that high incidence of the disease is recorded in the middle and south parts of Iraq as well as the transmitted vectors which causes the malignant ovine theileriosis in sheep are hard ticks as (*Haylamma anatolicum anatolicum*),which are widely in all of Iraq.

Al-Hasnawi,(2009) showed that Basrah province was endemic area by bovine theileriosis. The infection distributed in all parts of Basrah and concentrated in the Al-Hartha and Shat- Al-Arab. there are three clinical forms which can be observed when animal infected by ovine theileriosis especially malignant *Theileriosis* which assumes acute, sub acute and chronic forms . The acute disease is characterized by fever. other symptoms appear which include inappetence, cessation of rumination, rapid heartbeat, dyspnea, weakness, listlessness and swollen of the superficial lymph nodes. Marked anemia and icterus develop in a few days (Guo *et al.*, 2002) and very high mortality in 3-6 days which ends with death. In subacute and chronic cases, signs are generally less marked except for anemia and emaciation (Radostits *et al.*,2007).

this study conducted to study the clinical ,hematological and serological diagnosis of ovine theileriosis .

MATERIALS AND METHODS

The study conducted in Basrah province ,the area of the study is divided into: northern, southern, eastern, western and central region .

A total number of (1466) sheep for different age, sex and breed were clinically examined randomly belonging to different locations in Basrah province during the period from July 2010 to May 2011. The clinical signs and hematological and serological studies represented infected group and 150 sheep as a control group.

Also Blood sample was collected from jugular vein of each animal showed clinical signs, and used for making blood film. 10 ml of blood sample was collected by puncture the jugular vein, 5 ml of blood placed in tube with EDTA for heamatological examination and 5 ml without EDTA was allowed to coagulant and spirited the serum by using centrifuge (3000 rpm) for used in ELISA test .The serum were stored at -20°C until use.(Aktas *et al.*,2005).

1-Hematological Examination.

1- Red Blood Cell Count (RBCC) done according to (Schalm *et al.*, 1975) .

2-Total White Blood Cell Count (WBCC)done according to(Dasie and Lewis, 1984).

3-Differential White Blood Cells Count.

4-Hemoglobin Concentration (Hb) : according to (Coles,1986).

5- Packed Cell Volume (PCV): according to (Dasic and Lewis, 1984).

2-Blood smear:

The blood smear is made from blood collected from Jugular vein in EDTA after sterilizing animal jugular vein site with alcohol 70%. thin blood film was prepared (Chaudhri&Gupta, 2003) .

3-Lymph Samples

Lymph samples were collected from the enlarged superficial lymph node specially prescapular lymph node by puncture and diatheses the lymph to making lymph film according the procedure of (Al- Robayi, 1999).

4- Serological diagnosis (ELISA test):

The total number of serum samples 1466 were collected from clinically infected sheep in different ages. And 875 of 1466 serum samples were positive to microscopical examination used by ELISA test, in eight geographical areas in Basrah province , The Serum samples which previously labeled and stored at $-20\text{ }^{\circ}\text{C}$.

RESULTS

The total number of sheep examinant clinically are 1466 in different ages, sex and breeds. 460 of 1466 sheep in acute form showed clinical signs varied from rise of body temperature($40.4-41.8\text{C}^0$), increasing respiratory rate (50-90/mint.),dyspnea, interrupted coughing, increasing heart rate (90-140/mint.) as in table (2). The size of lymph nodes specially the superficial prescapular lymph node was varied from slightly to large enlargement .The appetite of infected sheep ranged from loss appetite to anorexia, lameness, salivation, nasal discharge and lacremation; pale-yellowish and congested mucus membranes yellowish urine; some time associated with corneal opacity, semi-solid to pasty feces and yellowish diarrhea or constipation, ticks infestation . In chronic form the sheep showed emaciation and dehydration and anemia table (1). Some time the disease occurred as subclinical form (carrier) which reached 415 of 875 (47.42%).

The study showed high level of parastemia in infected sheep varied from 12-80 % and the RBC can be infected by(1-4) parasites in maximum limit; all forms of *Theileria hirci* piroplasms including rod, round, ring and anaplasmod form with abnormalities in erythrocyte structure were observed. While in the WBCs specially lymphocytes the macroschizont and microschizont stage (koch blue body) observed in blood smear and schizont in lymph smear. Moreover the examination showed 22.62% of blood cells infected by schizont

lymphocytic stage , 64.8% infected by erythrocytes stage and 12.57% showed the erythrocytes and lymphocytic form table(3).

The result of the study showed a high prevalence rate in the North area of the province 65.82% ,South area 58.95% followed by West area 56.12% followed by East area 55.47% and then Center area of the province 52.98% with significant difference in $P < 0.05$ table (4).

Table (1) Clinical signs related to infected sheep by Ovine theilerosis

| Clinical signs | No. of sheep | Percentage of infection |
|---------------------------------------|--------------|-------------------------|
| Fever | 219 | 47.60 |
| Increase Respiratory rate | 310 | 67.39 |
| Increase heart rate | 325 | 70.56 |
| Nasal discharge | 400 | 86.95 |
| Salivation | 72 | 15.65 |
| Coughing | 122 | 26.52 |
| Dyspnea | 75 | 16.3 |
| Enlargement of prescapular lymph node | 455 | 96.73 |
| Lameness | 97 | 21.08 |
| Anorexia | 198 | 34.04 |
| Loss of appetite | 325 | 70.6 |
| Pale-Yellowish mucosal membrane | 380 | 82.60 |
| Eye congested | 108 | 23.47 |
| Lacrimation | 203 | 44.13 |
| Corneal opacity | | |

| | | |
|-------------------------|-----|-------|
| Dehydration | 13 | 2.82 |
| Emaciation | 223 | 48.47 |
| Anemia | 183 | 39.87 |
| Yellowish urine | 212 | 46.08 |
| Constipation | 91 | 19.78 |
| Yellowish-Soft diarrhea | 10 | 2.17 |
| Semi-Solid feces | 85 | 18.47 |
| Ticks infestation | 107 | 23.26 |
| | 430 | 93.47 |

Table (2) Range of body temperature ,pulse and respiratory rate of infected sheep compared with control sheep.

| Signs | Infected sheep Range | Control sheep Range |
|----------------------------------|----------------------|---------------------|
| Body temperature /C ⁰ | 40.4 - 41.8 | 38.8-40.0 |
| Heart rate / Mint. | 90 – 140 | 80-90 |
| Respiratory rate/ Mint. | 50 – 90 | 30 -40 |

Table (3) percentage of infected blood cells with different stages of parasite.

| Phase of parasites | No. of sheep | Percentage of infected blood cell % |
|--|--------------|-------------------------------------|
| 1- Lymphocytic stage (Koch s blue body) | 198 | 22.62 |
| 2- Erythrocytes stage | 567 | 64.8 |
| 3- Erythrocytes and Lymphocytic stage | 110 | 12.58 |

Table (4) Prevalence rate of ovine theileriosis in Basrah province .

| Location | No. of sheep exanimate | Infected sheep | |
|---------------------------------|------------------------|----------------|--------------|
| | | Positive | (%) |
| Northern area of Basrah | 553 | 364 | (65.82) |
| Eastern area of Basrah | 393 | 218 | (55.47) |
| Central area of Basrah | 134 | 71 | (52.98) |
| Western area of Basrah | 196 | 110 | (56.12) |
| Southern area of Basrah | 190 | 112 | (58.95) |
| | 1466 | 875 | 59.68 |
| $X^2 = 15.135$. $P < 0.05$ SD. | | | |

X^2 =Chi-Square SD.= significant difference & P= Probability value

The hematological analysis showed statistically a significant decreases ($P < 0.05$) ; in the mean of red blood cells count (RBCC), white blood cells count (WBCC), hemoglobin concentration (Hb) ,packed cell volume there is a significant increases found at Mean corpuscular volume (MCV) ($P < 0.05$) ; and abnormalities in the differential white blood cells showed decrease in lymphocyte and neutrophils count with significant difference ($P < 0.05$).While the monocyte and esonophilis count increased as statically a significant ($P < 0.05$).On the other hand the basophiles showed statistically an important change ($P > 0.05$) .

Table. (5) changes in some blood parameter in infected sheep with theileriosis.

| Hematological parameter | Control sheep Mean ± Std. deviation | Infected sheep Mean ± Std. deviation | F value | Significant Differed |
|---|--|---|---------|----------------------|
| RBCC (10 ⁶ /mm) | 10814000 ± 108.27 | 5944545± 62.38 | 163.39 | P<0.05 S.D |
| WBCC (10 ⁶ /mm) | 8012.5 ± 397.577 | 5870.0± 709.76 | 73.67 | P<0.05 S.D |
| Hb (g/l) | 14.4 ± 0.52 | 7.8 ± 0.4 | 1051.36 | P<0.05 S.D |
| PCV (%) | 43.3 ± 1.8 | 26.81 ± 2.991 | 227.92 | P<0.05 S.D |
| MCV (fl) | 39 ± 4.33 | 45 ± 6.27 | 4.93 | P<0.05 S.D |
| MCH (pg) | 13.40 ± 1.57 | 13.12 ± 1.4 | 0.80 | P>0.05 S.D |
| MCHC (%) | 33.48 ± 1.41 | 28.99 ± 1.8 | 32.10 | P<0.05 S.D |
| Differential White Blood Cells Count (Absolute) | | | | |
| Lymphocytes | 4604.81±280.1 | 3602.95± 450.5 | 36.51 | P<0.05 S.D |
| Neutrophils | 2560.37± 358.1 | 1166.8 ± 235.72 | 113.04 | P<0.05 S.D |
| Monocyte | 319.92± 107.56 | 453.96± 93.95 | 9.294 | P<0.05 S.D |
| Eosinophils | 520.95± 175.81 | 627.47± 106.53 | 2.883 | P<0.05 S.D |
| Basophils | 23.135± 37.33 | 19.61± 28.36 | 0.60 | P>0.05 NS. |
| No. of sheep | 150 | 815 | | |
| S.D= Significant difference & NS.= Non Significant | | | | |

The total Number. of serum samples are using in ELISA test 875 that positive to blood smears examined. And 815 of 875 serum samples (93.14%) were positive by ELISA test identified to genus of *Theileria hirci* and 60 (6.85%) of serum samples were negative to ELISA ,the later suggested that infected by *T. ovis*, and recorded high prevalence rate of *T. ovis* in northern region (66.66%) followed by eastern region (18.33%) and lower prevalence rate (6.66%),(5%) and (3.33%) in western, central and southern region respectively.

The result of examining 875 serum samples obtained from different geographical area are shown in table (7) The highest prevalence rate of *T. lestoquardi* 98.21 % recorded in South area of province and lower prevalence 83.16% recoded in North area of province.

Table (6) prevalence of *Theileria hirci* in sheep by using ELISA test compared with microscopical examination.

| Location | No. of sheep examination | No. and percentage of microscopical examination | | No. and percentage of ELISA examination | |
|------------------------------------|--------------------------|---|---------|---|---------|
| | | Positive | % | Positive | % |
| Northern area of Basrah | 553 | 364 | (65.82) | 323 | (88.73) |
| Eastern area of Basrah | 393 | 218 | (55.47) | 207 | (94.95) |
| Central area of Basrah | 134 | 71 | (52.98) | 68 | (95.77) |
| Western area of Basrah | 196 | 110 | (56.12) | 107 | (97.27) |
| Southern area of Basrah | 190 | 112 | (58.94) | 110 | (98.21) |
| X ² = 19.348 P<0.05 S.D | | X ² = 20.404 P<0.05 S.D | | | |

X²=Chi-Square S.D= significant difference & P= Probability value

DISCUSSION

All clinical signs showed by infected sheep agree with results studies of (El-Azazy *et al*,2001; Yin *et al*.,2003; Haddadzadeh *et al*,2004 and Zia-ur-Rehman *et al*., 2010).

The corneal opacity was explained by (Hussine *et al*.,2004 and Osman &Al-Gaabary,2007) as a result of white blood cells infiltration .The prescapular lymph node enlargement in infected sheep could be explained by lymphoid hyperplasia in early stage of disease that occurs due to increases of proliferation of microschorizonts inside the lymphocyte caused inflammatory reaction in the infected lymph node (Al-Robayi,1994). Pale-yellowish of mucus membranes was explained by (Al-Robayi,1994) as a result of increase total bilirubin level in blood due to the infection, Singh *et al*.,(2001) show that paleness of mucous membranes exhibited the development of anemia and reduction of hemoglobin concentration and the total erythrocytes count was due to the distraction and the removed of the infected erythrocytes by reticulo-endothelial system.

Diarrhea seen in 18.47% of infected sheep was explained as a result to inflammatory reaction and ulceration to abomasal and gastro intestinal tract (Al-Robayi.,1994). On the other hand ,the study showed that 415 of 875 sheep infected by ovine theileriosis are characterized by subclinical signs. These result agreed with (Heidarpour Bami, *et al.*,2010) which revealed that subclinical infections are common in their study of infected sheep by ovine theileriosis.

Generally, the disease is widely distributed in all province include Northern, Southern Eastern, Western and Central and recorded higher prevalence rate 93.14% serologically by using (ELISA) test than microscopical observation 59.68%. However, result of the study of malignant ovine theileriosis in Basrah province agree with (Al-Hasnawi.,2009) how showed that Basrah province was endemic area by bovine theileriosis infection genus *T.annulata*. The infection distributed in all parts of Basrah.

The result of the study may indicate continuous transmission of *T.lestoquardi* in Basrah. This is supported by the fact that *Hyalomma anatolicum anatolicum* adult ticks were found to be active throughout most of year in the present study area as well as in the other parts of Iraqi country .

The present study showed that ovine theileriosis is highly affected in the blood parameters which represented by decrease TRBCC, TWBCC, PCV, Hb, MCH and MCHC, that causes anemia which differed in severity from mild to severe anemia in infected sheep. These agreement with result of

(Alsaad *et al.*,2009; Al-Hasnawi,2009 and Zia-ur-Rehman *et al.*, 2010). The study showed that *Theileria* infection complained from the type of anemia macrocytis hypochromic anemia, due to increasing of MCV, where, it gradually raised through mode of disease and concurrently with severity of anemia; this denoted that the bone marrow response to erythropoeisis and normocytic normochromic anemia were observed in infected sheep related to decrease of MCHC due to decrease in Hb concentration. These result were agree the with results of (Dhar and Gautam,1979) who showed that the infected animals by theileria appeared normochromic normocytic and hypochromic machrocytic anemia .

This study showed a significant decreases in hemoglobin concentration Hb and total RBCs count these results agree with most hematological studies as (Omer *et al.*,2002; Hussine *et al.*,2004; Nazifi *et al.*,2009 and Al-Hasnawi, 2009). Moreover there exist significant decreases in PCV and MHCH and significant increases in MCV with an significant statically change in MCH these result were agreement with (Al-Robayi, 1994; Alsaad *et al.*, 2006; Nazifi *et al.*,2009 and Al-Hasnawi, 2009). All these changes occur as a result of anemia, persistent loss of blood caused by permanent blood sucking ticks which play a role as well (Mbassa *et al.*,1994 and Durrani *et al.*,2008). The other important cause to make the anemia in ovine theileriosis was destruction of RBCs by reproductive of parasite inside RBCs which caused hemolytic anemia. As well as the modern research explains the mechanism of anemia that occurs due to the activity of antioxidant enzymes such as superoxide's dismutase

(SOD) was effective by parasites and results increased fragility of RBCs and thus ,acceleration of erythrocytes clearance by phagocytic cells (Grewal *et al.*,2005 and Asri & Datir 2006).

The present study showed that a significant decreases in total WBCC, this result agreed with the result of (Al-Hasnawi,2009 and Zia-ur-Rehman *et al.*, 2010). The decreased in white blood cells count might be explained by destruction of WBCs specially lymphocyte cells although occurs hyperlymphoplasia due to infection, in addition present of some lymphocyte destruction in blood smears which are related to reproductive of parasite inside lymphocyte. The differential WBCC show decrease in lymphocyte and neutrophils with a significant difference $P < 0.05$ this result agreement with (Zia-ur-Rehman *et al.*, 2010) . On the other hand there is significant increase in eosinophils and monocytes count; while basophiles don't show importing statically change these results were agree with (Singh *et al.*, 2001; Hussine *et al.*,2004 and Al-Hasnawi, 2009).

The seroprevalence by using ELISA test was highest than microscopic observation in identification and diagnosis of ovine theileriosis genus of *T. lestoquardi* .These results agree with the result of (Guo *et al.*, 2007) which showed that 824 (66.77%) sheep infected by ovine theileriosis from 1234 sheep diagnosed by ELISA , (Yin *et al.*,2003) reported that 164 of the 173 (94.79%) samples examined were positive to *Theileria lestoquardi* by ELISA test

In present study two species of *Theileria*, *T. lestoquardi* and *T. ovis* observed, this result agree with Heidarpour Bami,et al,(2010). These results are explained by difficult difference between *T. lestoquardi* and *T. ovis* in microscopic examination due to *T. ovis* taken multi shape as *T. hirci* (Al-Robayi,1994 and Hashemi-Fesharki,1997). These findings agree with (Altay *et al.*, 2005) who reported that four *Theileria* species (*T. lestoquardi*¹, *T.ovis*², *T. separata*³ and *Theileria spp. China*⁴) can cause theileriosis in sheep. It is difficult to differentiate these species on the basis of the morphology of piroplasm and schizont stages, especially in mixed infections.

The present study showed that the distribution of benign theileriosis genus *T. ovis* is restricted in northern region rather than region of province, and recorded high prevalence rate in northern region (66.66%) followed by eastern region (18.33%) and lower prevalence rate (6.66%),(5%) and (3.33%) in western, central and southern region respectively. These findings agree with the result of (Zaemi *et al.*,2010) who reported that *T. ovis* is the dominant species in the north and north-west regions of Iran.

دراسة سريرية ودموية والتشخيص المصلي للإصابة بداء الثاليريا في أغنام محافظة البصرة

طارق رفعت منت ، اسراء عبد الودود

كلية الطب البيطري/ جامعة البصرة

الخلاصة

أجريت هذه الدراسة لتحديد انتشار *Theileria lestoquardi* بداء الثاليريا في محافظة البصرة ، اذ تم تقييم بواسطه تسجيل السويسرية والمعايير الدمية وكذلك اختبار الاليزا المصلي لطفيلي *Theileria lestoquardi* 2010 2011 1466 عينة من عشوائية من مناطق مختلفة لوحظت العلامات السريرية على 460 التي فحصت سريريا والتي تمثلت بالحمى ، تضخم العقد اللمفاوية السطحية ، علامات تنفسية وزيادة في ضربات القلب . وأظهرت الفحوصات الدموية ارتفاع في مستوى الطفيلنمية حيث سجلت نسبتها ما بين 12% - 80% ولوحظ جميع الطفيلي اذ كانت نسبة الطور الدموي 64.8% 22.62% 12.57% بينت نتائج الدراسة نسبة حدوث لداء الثاليريا في 65.8% تلتها جنوب البصرة 58.9% بينما اوطا نسبة حدوث كانت في مركز المحافظة 52.98% . أظهرت المعايير اللمية وجود فقر دم الكبير الحجم السوي الصباغ وفقر الدم سوي الحجم سوي الصباغ في الاغنام المصابة وانخفاض معنوي في قيم خضاب الدم (g/l) 7.8 ± 0.4 . وحجم الخلايا المرصوص $PCV 26.81 \pm 2.991$. تركيز خضاب الدم الكروي 28.99 ± 1.8 وعدد كريات الدم الحمر 5944545 ± 62.38 وعدد كريات الدم البيضاء 5870 ± 709.76 والخلايا اللمفاوية 3602.95 ± 450 1166.8±235.72 بينما لوحظ ارتفاع معنوي في الخلايا الحمضية وخلايا وحيدة النواة وكذلك زيادة في معدل بينما التشخيص المصلي للدراسة باستخدام اختبار الاليزا كانت لتشخيص النوع الخبيث *Theileria lestoquardi* 93.14% بينما اوطا نسبة كانت بالنوع الحميد للمرض *Theileria ovis* 6.85% .

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