Journal of Animal and Poultry Production

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Available online at: www. jappmu.journals.ekb.eg

Cattle Theileriosis: Prevalence and Comparative by Using ELISA and Blood Smears Techniques in Sulaimani Province - Kurdistan Region / Iraq.

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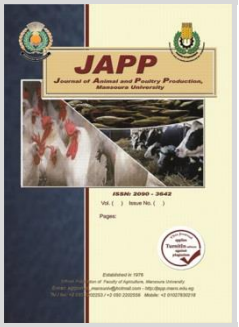

ABSTRACT

Isolated one hundred fifty adult female cattle (local breed) one hundred infested with a hard tick (Ixodidae) and show some clinical signs for a search of theileriosis such as; enlargement pre-scapular lymph node and pale of the eye mucus membrane, fifty of them use a control (Non infested cattle) during the period of April to November 2018 in Sulaimani province. Indicated the highest sensitivity (prevalence) of *Theileria annulata* when examined by ELISA technique 63% (63/100), the prevalence rate of macroscopically detection to *T. annulata* merozoite was 48% as the following, in mixed lymph node biopsy cells and blood smears technique was 22%, the blood and lymph node biopsy smear examinations were 15% and 11% respectively. Distinguished the total prevalence rate in control cattle was 6% and 4% in the ELISA technique and microscopically method respectively. Sensitivity values and positive predictive noted a highly in ELISA serolog-ical test 63% and 95.45% respectively, while in mixed lymph node biopsy and blood smears examined 22% and 100% respectively. During the study collected 1368 hard tick and identified to three genera in infested cattle of Ixodidae; Hyalomma species are the highest number percentage (44.8%), followed Boophilus species (28.6%) and the Rhipicephalus species is the lowest percentage (26.6%). This study conducted to find out the prevalence the local cattle with theileriosis (*T. annulata*) by the microscopic examination for both blood and lymph biopsy smear (merozoite) and compared with the (ELISA) technique.

Keywords: Non- infested Cattle, Infested cattle, Giemsa staining methods, (ELISA) technique.

INTRODUCTION

Protozoan, Theileria annulata is a major factor in the occurrence of tropical theileriosis, which is endemic in most parts of the world. (Brown, 1990), described by enlargement of superficial lymph nodes and potential fever (Demessie and Derso, 2015). Transmission of this disease occurs during infected with infested ticks in particular of the genus Hyalomma (Uilenberg, 1981). Different parts of Iran the cattle of are affected by T.annulata, the native cows are very sensitive to this infection than non-native cows. (Kirvar *et al.*, 2000). The local carrier cattle are the major causes of spreading the infection among the ticks and cattle (Ilhan *et al.*, 1998). Blood smears are the common method for identifying and characterizing types of peroplasm, but it is not a sensitive method when compared to ELISA and PCR (Shayan and Rahbari, 2005). ELISA test has been extensively methods used for diagnosis of theileriosis and evaluation of immunity in vaccinated (Hashemi-Fesharki *et al.*, 2006) the ELISA test became more sensitive but less precise, recently the PCR method is more accurate when comparison with the other serological tests such (ELISA) (Kachani *et al.*, 1994 and Kachani *et al.*, 1996). At present the Molecular techniques are considered as the most sensitive and specific diagnostic assays for the diagnosis the most piroplasma species which infection the animals (Azizi *et al.*, 2008).

MATERIALS AND METHODS

Animals sample.

A total of 150 local female cattle (local breed); one hundred of them infested naturally with hard tick and it showed some specific signs of theileriosis; fever, enlargement pre-scapular lymph node and pale of mucus membrane examined and using fifty cattle for control (Non-infested cattle) were isolated clinically from various flocks between April to November 2018 searched for existence of theileriosis in surrounding areas for sulaimani province - Kurdistan region / Iraq. For each cattle,

- Blood smears from the peripheral blood were quickly fixed by methanol (99%) for 5 min and stained in Giemsa stain (diluted at 5% with buffer solution for 30 min and flushed with tap water, then left to dry subsequently, the stained blood smears were examined with an oil immersion lens) it was described by (Adam *et al.*, 1971).
- Lymph node biopsy smear were prepared; prepared directly from pre-scapular lymph node.

Tick collection and identification

Ticks were calculated and identified according to (Walker *et al.*, 2003), using a dissecting microscope and magnifying-hand lens and the binocular microscope.

Enzyme linked immune - sorbent assay techniques- ELISA.

It is one of the important techniques which are used to diagnosis bovine theileriosis (*Theileria annulata*). This technique dependent on the surface protein of Theileria spp. (TaSP) is used ELISA test to detect circulating antibodies against tropical theileriosis this test described by (Kachani *et al.*, 1996).

Statistically analysis

In this study the sensitivity and specificity are important measures of the diagnostic accuracy of a test but both positive predictive value and negative predictive value vary according to disease prevalence. The sensitivity values suggestion positive result of prevalence of disease while the specificity values means the negative results, the positive predictive value helping

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DOI: 10.21608/jappmu.2020.118219

to knowledge the actually have the disease and the negative predictive value described the do not have disease in isolated cattle (infection and control cattle), all these values were used by the researcher Akobeng, (2007) according the Table-1.

Table 1. Testing table foe detectable the sensitivity, specificity and predictive values.

Test (+) or (-)	Infected cattle clinically	Healthy cattle (control)	Total number of (+) or (-) test
Test positive (+)	A	b	Positive
Test negative (-)	C	d	Negative
Overall	E	f	Examined

-Sensitivity values {a/ (a + c)}. -Specificity {d/ (b + d)}.
-The positive predictive value {a/ (a+b)} -Negative predictive value {d/(c + d)}.

RESULTS AND DISCUSSION

Results

In this result 1368 hard tick were collected directly from 150 local cattle breed infested naturally with hard ticks during the period April 2018 to November 2018 in Sulaimani province, in table (2) it shows three genera of Ixodidae; Hyalomm *spp*., the highest number percentage (44.8%), followed Boophilus *spp*., (28.6%) and the Rhipicephalus *spp*., (26.6%) is the lowest.

Table 2. Identification and distribution rate of hard tick in cattle (local breed) in Suliamani Province.

Hard tick genera (Ixodidae)	No. of hard tick collected	%
Hyalomma species	613	(44.8%)
Boophilus species	391	(28.6%)
Rhipicephalus species	364	(26.6%)
Total	1368	100

In table {3} shown positive number for detection T. annulata in infested cattle 63% and non-infested cattle with hard ticks (control) 6%, When used sensitivity (positive result), specificity (negative result) and predictive value to estimate probability of T. annulata according to prevalence (Akobeng, 2007), the sensitivity and specificity values were 63% and 94% respectively, positive predictive value (actually have the disease) and negative predictive value (result who do not have disease) were 95.45% and 55.95% respectively in different localized area surrounding which Sulaimani province from 150 sera samples.

Table 3. Prevalence rate (Sensitivity), specificity and predictive values of theileriosis in cattle (Local breed) by using ELISA test in Sulaimani province.

ELISA test	No. of cattle examine%	Test is positive%	Test is negative%
Cattle infested with hard ticks	100	63 (63%) a	37 (37%) c
Non-infested cattle with hard ticks (control)	50	3 (6%) b	47 (94%) d
Total	150	69 (46%)	81 (54%)

-Sensitivity values {a/ (a + c)} = 63%,
-Specificity {d/ (b + d)} = 94%
-Positive predictive value {a/ (a + b)} =95.45%
-Negative predictive value {d/ (c + d)} = 55.95%.

In table (4) investigated *Theileria annulata* (merozoite) microscopically by Giemsa staining method in cattle (local breed), showed a higher number and percentage in infected cattle with *T. annulata* in mixed methods (Lymph node biopsy cells and Blood smears) 22 %, followed the blood smears samples is 15 % and the a minor appearance in lymph node biopsy smears was 11 %, with a total prevalence of all three results examine with Giemsa staining method 48%. In healthy cattle investigate 4% of cattle infected with theileriosis when examined microscopically by blood smear.

Table 4. Prevalence rate (Sensitivity) specificity and predictive values of theileriosis in cattle (Local breed) in Sulaimani province.

Methods of Examined	Unhealthy cattle infested (100 suspected cattle with theileriosis)		Non-infested cattle (50 control cattle)	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Mixed lymph node biopsy and blood smears	22 (22%) a	78 c	0 b	50 d
Blood smears	15 (15%) a	85 c	2 (4 %) b	48 d
Lymph node biopsy smears	11 (11%) a	89 c	0 b	50 d
All examined	48 (48%) a	52 c	2 (4 %) b	48 d

In this table (5) shown the highly sensitivity (prevalence) in Mixed lymph node biopsy and blood smears 22% followed by blood smears and Lymph node biopsy smears 15% and 11% respectively. Specificity values and positive predictive noted a highly in both mixed lymph node biopsy and blood smears, and Lymph node biopsy smears 100% in infested and non-infested cattle.

Table 5. Correlation between sensitivity and specificity, positive and negative predictive values among cattle in Sulaimani province.

Methods of Examined	Unhealthy cattle (100 cattle suspected with Theileriosis)		Non-infested cattle (50 control cattle)	
	Sensitivity values	specificity	Predictive (+)	Predictive (-)
Mixed lymph node biopsy and blood smears	22%	100%	100%	39.06%.
Blood smears	15%	96%	88.23%	36.09%.
Lymph node biopsy smears	11%	100%	100%	35.97%.
All examined	48%	96%	96%	48%

Discussion

The cattle that were isolated in this study for the purpose of detecting of theileriosis showed generally; fever, enlargement of superficial lymph node, pale-yellowish of mucous membrane and eye congestion and lacrimation. All these signs were agreed with results of most studies resembling (Hussein *et al*., 2007 and Alkhaledi, 2008). It is difficult to distinguish among Theileria species on the basis of the morphology of the piroplasm and schizont stages and in sometime confusion may emerge if mixed infections (Dumanli *et al*., 2005). In this results, identified three genera of Ixodidae in naturally infested cattle; Hyalomma, Boophilus and the Rhipicephalus, with a highly distribution number of Hyalomma spp., (44.8%), followed than Boophilus spp., (28.6%) and the Rhipicephalus spp., was (26.6%) is a lowest..This results were agreement with (Al-Saeed *et al*., 2010), which observed that Hyalomma species, it is dominant species in infesting cattle and give a more chance of bovine Theileriosis in Kurdistan Region, Iraq. The prevalence rate of Hyalomma spp.

49.82% (Mustafa, 2016) and 32% (Mustafa, 2017) in cattle (local breed) isolated randomly in Sulaimani province- Kurdistan Region The presence of hard ticks on the cattle demonstrate a major sign to diffusion for Theileriosis (Ghosh *et al.*, 2007). The distribution of Hyalomma ticks was greater than Rhipicephalus in northern Iraq (Omer *et al.*, 2012). The Genus Hyalomma are the common vectors of this protozoan parasite (Robinson, 1982). In this study, further investigation for detection of T. annulata by using ELISA technique and microscopic examination of Giemsa-stained blood and lymph node biopsy smear from 150 local cattle breed infested naturally with hard tick. In these results established has a much higher ability of T. annulata in ELISA test percentage 59.3%from 89/150 cattle local breed when comparison with microscopically detection to *T. annulata* was 32% (48/150). The positive percentage of mixed methods of blood and lymph smears method is a highest 14.7% (22/150), followed in blood smears was10% (15/150) and in lymph smears methods was 7.3% (11/150) is a lowest number prevalence in Sulaimani province. The percentage of T. annulata for mixed blood and lymph node biopsy smears is higher than two another Giemsa- staining because may be at the beginning when the infection (protozoan} enter the blood stream by vectors reach the lymph node; the protozoan growth and develop to schizont and left the lymph node cells to bloodstream become merozoite and invasion red blood cells, while in blood smears, showed the number of T. annulata was higher than lymph biopsy smears because the infection reached to chronic stage the low proportion of T. annulata, may be the beginning infection or the parasites exhaustion in lymphocytes (lymph node) and then let go into the bloodstream to invade erythrocyte. (Dehkordi *et al.*, 2012). The prevalence rate of infected cattle with T. annulata was 45.1%% when examined by blood smear, while the total sero-prevalence was 77.9% in Kurdistan region, while when examined with ELISA test showed the highly positive percentage is 75.8%, 88%, and 70% among cattle in Erbil, Duhok, and Sulaimani governorates, respectively (Omer *et al.*, 2012). The rate of infected cattle with *T. annulata* was (6.25%) when examined with blood smear in Iran (Hoghooghi-Rad *et al.*, 2011). The microscopic examination of blood smears is 27.2% (82) for Theileriosis in India (Kohli *et al.*, 2014). In blood smear examination the highest positive rate was 38.9%, the lowest rate was 5.1%, and the relative average positive rate was 13.7% Proved by (Li *et al.* 2016). Microscopic examination of Giemsa-stained thin blood smears 9.31% and PCR 11.44% (El-Dakhly *et al.*, 2018). The sero-prevalence of *T. annulata* in cattle was 10.6% (21 out of 198) in Central Anatolia, Turkey (Sayin *et al.*, 2003). Recorded that the *T. annulata* infection a higher percentage when examined with ELISA test (28%) than that in PCR technique (13%) in Egypt (Ibrahim *et al*., 2009). In china the bovine theileriosis showed the highest positive rate of was 98.3%, the lowest was 84.1%, and the average positive rate was 95.4% by iELISA (Indirect Enzyme-Linked Immunosorbent) with the specificity and sensitivity of iELISA test were 98.9% and 98.5% respectively. (Li *et al.* 2016). In Spain the prevalence rate for T. annulata from cattle were 22% tested by blood smears, 40% were positive by immunofluorescent antibody test, and 75% were positive by PCR (d'Oliveira *et al.*, 1995). The sensitivity and the specificity of schizont when tested by Indirect Fluorescent Antibody Technique (IFAT) were 88.9% and 97% respectively, with lower sensitivity of 63.9% when examined with blood smear (Darghouth *et al*., 2004). Serological screening is more adequate for the diagnosis of theileriosis during the chronic phase of the infection, where the animals serve as carriers, these animals have higher antibody titers, while the level of parasitemia is low and microscopically barely unnoticeable (Ilhan *et al.*, 1998). Recorded the specificity values and positive predictive a highly in ELISA serological test 63% and 95.45% respectively, while in mixed lymph node biopsy with blood smears method and lymph node biopsy smears 22% and100% respectively, may be the lymph node is a growth and proliferation habitat of Theileria species.

CONCLUSION

In conclusion, the diagnosis of *Theileria annulata* by ELISA techniques was more sensitive, and especially in diagnosis the chronic stage of disease, shows ability and effective of theileriosis infection in cattle by some values by using the analytical tests such as sensitivity, specificity and both positive predictive value and negative predictive value for detection for disease prevalence.

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داء الثايليريا في الأبقار: الأنتشار والمقارنة بأستخدام اختبار الاليزا وتقنيا ت مسحات الم واللمف في محافظة السليمانية – أقليم كردستان العراق

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تم عزل مائة وخمسين من أناث الأبقار (سلالة محلية) ، مائة منها مصابة بالقراد الصلب (Ixodidae) والتي أظهرت بعض العلامات السريرية للمرض مثل: توسيع العقدة الليمفاوية امام اللوحية، و شحوب الغشاء المخاطي للعين ، خمسين منها استخدم كسيطرة (أبقار غيرمصابة بالقراد) للبحث عن مرض الثاليريوسز خلال الفترة من نيسان لغاية تشرين الثاني 2018 في محافظة السليمانية. اظهرت أعلى حساسية (معدل انتشار) ل *T.annulata* عند فحصها عن طريق تقنية ELISA (63/100) 63٪ ، معدل انتشار merozoite الطفيلي *T. annulata* كانت 48٪ (100/48) عند فحصها عن طريق صبغة كيمزا على النحو التالي: نسبة اصابة الابقار ب *T. annulata* هي 22 ٪ (100/22) عندما كانت الطفيلي في خلايا العقدة الليمفاوية و المسحات الدمية معا" ، وثم يلي معدل الانتشارفي عينات المسح الدمية والخزع الليمفاوية 15 ٪ (100/15) و 11 ٪ (100/11) على التوالي. معدل الانتشار الكلي في الأبقار السيطرة لكل من الفحص عن طريق تقنية ELISA وطريقة الفحص المجهري 6 ٪ و 4 ٪على التوالي. واظهرت ان قيم الحساسية والتنبؤية الإيجابية عالية في كل من اختبار الاليزا وكانت 63 ٪ و 95.45 ٪ على التوالي ، وفي الاصابات المختلطة 22 ٪ و 100 ٪ على التوالي. خلال الدراسة تم جمع 1368 من القراد الصلب وتم تحديد ثلاثة أجناس من Ixodidae. النوع Hyalomma كانت أعلى نسبة (44.8٪) ، تليه Boophilus (28.6٪) و Rhipicephalus مي أقل نسبة (26.6٪) في الأبقار المصابة بالقراد الصلب. الهدف من هذه الدراسة هو معرفة مدى انتشار مرض الثيليريوسيس (*T. annulata*) في الأبقار المحلية عن طريق الفحص المجهري لخزعة الدم وخزعة العقدة الليمفاوية (ميروزويت) ومقارنتها بتقنية ELISA.