Using of Polymerase chain reaction (PCR) technique and microscopic examination of stained blood smear for Detection of Tropical bovine theileriosis among carrier cattle in Al- *Nasiriyah* city.

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I. Abstract

Tropical bovine *theileriosis* was recognized as one of the most important cattle diseases in Iraq. The target purpose from present study is to detect Tropical bovine *theileriosis* among carrier cattle in Al- *Nasiriyah* city by using each of PCR technique and blood smear method . Out of 60 Blood samples, Blood smear examination revealed 40 (66.67 %) samples to be positive for intra erythrocytic Theilera when staining with Geimsa stain .The specificity of the *T. annulata* primer for *cytob1 gene* was examined with DNA from*T. annulata*, the expected 312-bp was generated from *T. annulata* DNA. The *cytob1 gene* was detected in 48 samples (80%), while in negative results in 12 samples (20%). The sensitivity of PCR techniqueis greater more than light microscopy examination.

Key words: theileriosis**,** cattle parasites**,** blood smear,protozoa**.**

II. Introduction

Theileria annulata parasites ,the causative agents of Tropical bovine theileriosis transmitted by ticks of the genus Hyalomma(Robinson, 982). This hemoprotozoal disease is widely distributed in the tropical and subtropical regions of the world (Mehlhorn and Schein, 1984), especially in middle east , middle asia ,North Africa and Southern Europe (Jongejan and Uilenberg, 1994). Infection of cattle with this disease lead to significant economic losses due to both mortality and reduced production(Urquhart *et al.* , 1996; Kivaria, 2006). Recovery from the disease results persistent carrier state which have an effective role in the maintenance of the life cycle by persistent *transmission* of the parasites to *Hyalomma* ticks(Ilhan *et al.* , 1998) . resistant cattle showed subclinical form of the disease (Hoghooghi-Rad *et al.* , 2011). Infected animals may show a general clinical features include fever , changes in behaviour such as depression and dullness ,anorexia, swelling of the

lymph nodes ,anemia and difficult breathing (dyspnea) with nasal and ocular discharges as well as drop in milk production (Soulsby, 1989; Rady *et al.* , 2010).

Classical diagnosis of the disease based on clinical sings and examination of stained blood and lymph node biopsy smears with light microscope(Aktas *et al.* , 2006). smear method allow detection of parasites in acute cases and has low sensitivity to detect the parasites in carriers and subclinical forms due to low level of parasitemia or low number of infected erythrocytes (Shayan and Rahbari 2005; Nayel *et al.* , 2012). Polymerase chain reaction (PCR) have been successfully used to diagnose theileriosis especially to detect the parasites in carrier animals (D'Oliveira*et al.* , 1995).

The purpose of this study was to investigate the presence of *Theileria annulata* in clinically healthy cattle in Al- Nasiriyah city by using each of PCR and blood smear method.

Materials and Methods

Samples:-

A total of 60 bloods samples were collected from jugular vein of randomly selected cattle which were found in abattoir of Nasiriyah city southern of Iraq. Also a thin layer smears of blood were prepared from the marginal ear vein of each cattle .Collected blood samples were brought in ice-pack containers to laboratory.

Microscopic examination:-

A thin layer smears of blood were fixed with methanol on slides for five min. and then stained for 45 min in Giemsa stain diluted with buffer (5%). Slides were examined for intra-erythrocytic forms of *Theileria* spp. piroplasms at oil_ objective magnification.

DNA Extraction:-

A volume of 200 µl fresh blood was extracted by DNA extraction kit (Bioneer) as recommended by manufacturer.

PCR assay:-

A PCR assay targeting *cytob1*, gene an element of *Theileria annulata*(Bilgic *et al.* , 2010) was used for the detection of *T. annulata*in clinical samples (blood). The primers that used in (Forward: 5-ACT TTG GCC GTA ATG TTA AAC–3/Reverse:5-CTC TGG ACC AAC TGT TTG G–3) was used to amplify a 312 bp variable region .

The PCR mixture (50 µl):-

DNA templates	10 µl
Mastermix	10 µl
Primer forward	2µl
Primer reverse	2 µl
D.W.	26 µl

The PCR conditions for *T. annulata* gene included 30 cycle of an initial denaturation of DNA at 94°C for 3followed by 30 cycles of denaturation (95 _C for 50 s), primer annealing (50 _C for 50 s) and extension(65 _C for 1 min). A final extension at 65 _C for 10 min was performed .For each reaction, 10 µl of PCR product was electrophoresed on a 2% agarose gel containing 10 µl /ml ethidium bromideat 100 V and visualised under UV light.

III. Results and Discussion

Blood smear examination is a common diagnostic method for bovine theileriosis in Iraq. Many problems associated with blood smear method such as false result and low sensitivity especially in carrier animals where there are a few number of infected erythrocytes (Shayan and Rahbari 2005),as well as the different species of *Theileria* look alike in most stages (piroplasm and schizont) so it is difficult to differentiate it by its morphological features((D'Oliveira*et al.* , 1995).

In this study blood smear examination revealed 40 (66.67 %) samples to be positive for intra erythrocytic Theilera when staining with Geimsa stain .The specificity of the *T. annulata* primer for *cytob1 gene* was examined with DNA from*T. annulata* the expected 312-bp (figure,1) was generated from *T. annulata* DNA. The *cytob1 gene* was detected in 48 samples (80%), while in negative results in 12 samples (20%) (Table ,1).

The high prevalence of bovine theileriosis was detected in this study may be due to the availability of intermediate hosts (ticks) and appropriate conditions in the environment (Srinavasan , *et al.* , 2000) in addition to presence of carrier animals which play a major role in continuance of parasites life cycle (Ilhan *et al.* , 1998). In Iraq it was found that the bovine theileriosis one of the problems facing the cattle industry(Al-Saeed, 2009). in the Kurdistan Region of Iraq (Al-Saeed, *et al.* , 2010) reported an infection rate of 68.9% with bovine

theileriosis by using PCR. In Basrah province (Al-Emarah , *et al.* , 2012) recorded 69.29% of examined cows were infected with erythrocytes stages of *T. annulata* by using blood smear examination.

In this study ,When data was tested for significance,The results showed a significant differences($p< 0.05$)between PCR and Blood smear method by using chi-square analysis test (SPSS , Statistical Package for Social Sciences version 18) .

The sensitivity of techniques based on PCR is greater more than light microscopy examination and PCR yields positive results longer than microscopic examination (Kain *et al.* , 1994).

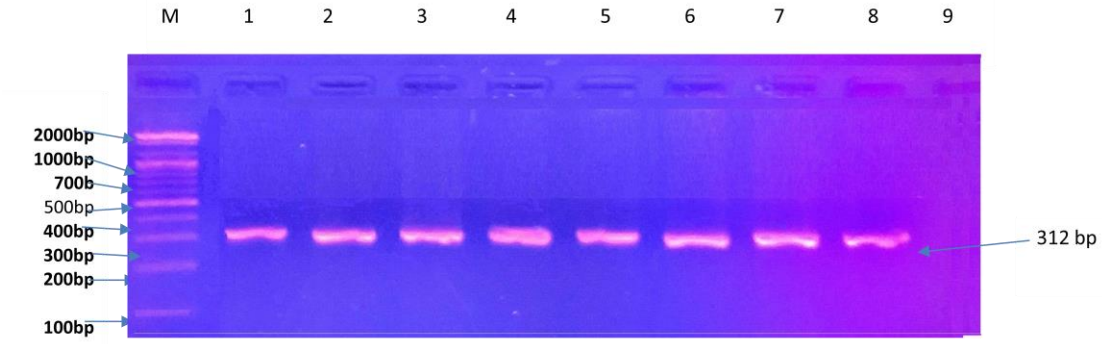

Figure (1): PCR products of *Theileria annulata* A *cytob1* gene on (1.5%) agarose gel for45 minute and 70 V. Lane M: DNA ladder100 bp. Lanes 1,2,3,4,5,6,7 and 8): positive results to *cytob1* gene. Lane (9): negative result .

Table(1) Microscopical examination of thin blood smears and polymerase chain reaction results.

	PCR		Microscopy	
Blood sample	No:	%	No:	%
positive	48	80%	40	66.67%
negative	12	20 %	20	33.33%
Totale				

	60	60

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