Research Article

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Diagnostic study to Bovine Theileriosis by using PCR technique

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Abstract

The present study performed in 51 cows from different age, sex and breed. The blood examination of all these animals showed high level of parasitemia varied between 17-52% and observed all parasite stages. The genomic diagnosis showed a high specificity and sensitivity to whole blood PCR test for diagnosis *Theileria annulata* moreover recorded a high percentage of infection by *Theileria annulata* 88.23% and lower percentage of infection 11.76% by other *Theileria* spp.

Keywords: Bovine, Theileriosis, *Theileria annulata, Theileria* spp. PCR technique

Introduction

Bovine theileriosis is a tick – borne protozoa disease caused by eight species of *Theileria* that lead to severe and mild infection in the host.

These species are *Theileria parva* , *T. annutata* ,*T. mutans* ,. *T. lowrenci, T. velifera, T. tarigtrgi, T. sergenli* and *T. orientalis* two of them *T. parva* and *T. annulata* cause lymphopraliphorative disease with high morbidity and mortality rate in cattle commonly known East coast fever and Tropical theileriosis. While the other species like *T. sergenil, T. buffili* and *T. orientalis* caused mild or asymptomatic disease in the cattle also known benign theileriosis (1 ,2). The main method which used to diagnosis *Theileria* parasite include Giemsa – stained blood and lymph node smears, ELISA, Indirect immunofluorecent antibody test (IFAT) and PCR/ DNA- probe test (3).

Blood smear and lymph smear examination smear are examined for schizonts and piroplasms same animal may have low parasitameia and therefore infection may not be detected on blood smear examination thus a negative result should not be regarded as confirmation that infection is absent but rather that parasitaemia if present was too low to be detected microscopically different. *Theileria* spp. piroplasms do differ morphologically but their morphology also varies during the course of infection rendering this method diagnostically fallible (4). Morover the immunological test (ELISA) and(IFAT) represintave the modern test with high sensitivity and specifity. However cross-reactiivity with antibodies directed against other *Theileria* spp limits the specifity of the ELISA and IFAT test.

Polymerase chain reaction is a biochemistry and molecular biology technique (5). It is used to amplify a region of DNA that lies between two regions of a known sequence. This reaction requires two short single-stranded DNA primers that anneal to opposite strands of the template DNA and flank the region of amplification (ROA). Additionally, two other types of biological molecules are required for this reaction: DNA polymerase and the four (dNTPs) deoxynucleoside triphosphates (6). PCR is commonly used in medical and biological research labs for a variety of tasks, such as the detection of hereditary diseases, the identification of genetic fingerprints, the diagnosis of infectious diseases, and the detection or identification of the causative agents (7). PCR is one of very important diagnostic techniques widely used in veterinary parasitology infection disease and because high specificity and sensevity of PCR technique this test is the favorite test to diagnosis the blood parasite infection particularly the infection by *Theileria* spp. (8,9).

There are many applications to PCR test in diagnosis, classification and detection *Theileria* spp. Many studies used PCR to diagnose the infection by *Theileria.* spp., *Babesia* spp. and *Anaplasma* spp. are moreover able to diagnose animal complain of the mixed infection by all hemi protozoa (10-13). While the other research used PCR test to diagnose and classify the infection by different species of *Theileria* parasite because the PCR characterized by high specificity to diagnose the genus and species of parasites (14,15). However all the modern epidemiological study to bovine theileriosis used the PCR test because the high sensitivity to PCR and

used to avoid the cross reaction that occurs when using the serological test (3, 16, 17). Moreover, PCR characterized by high sensitivity is able to diagnose bovine theileriosis when the level of parastemia is upper than 0.0001% this lead to use PCR to detection *Theileria.* spp. in blood of carrier animal (18, 19). One of very important advantage of PCR test is being able to diagnose *Theileria.* spp. by using different source of specimen some study diagnosis *Theileria* spp. from the whole blood samples or by using the blood extract from the digestive system of ticks wall the other research isolated and identified the parasites from the tissue culture (3, 12,13,18,19). Moreover, (15) used the PCR technique to isolate and identify the sequence of the gene responsible for the resistance against the infection by bovine theileriosis from resistance and susceptible cattle breeds.

Materials and methods

This study was designed to include the examination 51 cows complied of acute form of bovine theileriosis by amplification of *Theileria* spp. DNA from blood sample obtained from infected animal and then test by using PCR technique depending on two types of primers. The first was derived from the gene encoding **the SSU r RNA** gene used to diagnose *Theileria* spp. The second primer was derived from the gene encoding the **30-kDa** major *Theileria annulata* merozoite surface antigen (18).

DNA extraction and purification.

DNA was extracted by using (Promega DNA Purification Kit USA).

Method of PCR reaction (SSUrRNA gene detection)

The detection of chromosome encoded to diagnosis *Theileria* spp. was done using primers by thermal cycler. The PCR amplification mixture for the detection of SSUrRNA contained 12.5 μl of green master mix (which contains bacterially derived Taq DNA polymerase, dNTPs, $MgCl_2$ and reaction buffer at optimal concentration for efficient amplification of DNA templates by PCR), 5 µl of DNA, 2µl of each forward and reverse primers, 3.5 µl of nuclease-free water and 10 µl of mineral oil.

The PCR tubes were transferred to preheatedthermocycler and start the program (Table 2).

Table (1). The sequences of the primers used in the study.

primer		Sequence	Position	characteristic
SSUr RNA gene	Forward 989	AGTTTCTGA CCTATCAG	278-294	*T. annulata* specific
	Reverse 990	TTGCCTTAA ACTTCCTTG	1376-1359	*T. annulata* specific
30-KDa gene	Forward N516	GTAACCTTT AAAAACGT	234-250	*Theileria* spp.
	Reverse N517	GTTACGAAC ATGGGTTT	954-938	*Theileria* spp.

Table (2): PCR program for SSUrRNA gene

Step	Temp C°	Time / min.	No. of cycles
Denaturation	95	3	1
Annealing	94	1	30
	60	1	
	72	1	
Extension	72	3	1

Method of PCR reaction (30-KDa gene detection)

The detection of chromosome encoded to *Theileria annulata* merozoite surface antigen was done using primers by thermal cycler. The PCR amplification mixture for the detection of 30-KDa gene contained 12.5 μl of green master mix (which contains bacterially derived Taq DNA polymerase, dNTPs, $MgCl_2$ and reaction buffer at optimal concentration

for efficient amplification of DNA templates by PCR), 5 µl of DNA, 1µl of each forward and reverse primers, 5.5 µl of nuclease-free water and 10 µl of mineral oil.

The results of the PCR were performed in post amplification area. Ten µl from amplification samples were directly loaded in a 1% agarose gel containing 0.25 μl /25ml, ethidium bromide with adding loading buffer in electrophoresis and the products were visualized by UV transillumination.

Agarose gel electrophoresis Two concentrations of agarose gel were prepared (1.5% and 1%) as we needed. The concentration of 1% agarose was used in electrophoresis after DNA purification process, while 1.5% agarose used for gene detection.

The PCR tubes were transferred to preheated thermocycler and start the program(Table3).

Table (3): PCR program for 30-kDa gene

Step	TempC°	Time / min.	No. of cycles
Denaturation	95	3	1
	94	1	
Annealing	55	1	30
	72	1	
Extension	72	3	1

Results

Genomic diagnosis of bovine theileriosis: Microscopically the examination of thin blood film to 51 animals showed parasitemia in infected cattle ranging from 17-52%. The piroplasms and schizont, detection inside the erythrocytes and all these forms were classified as *Theileria* spp. The 51 specimen (100%) were positive of *Theileria* spp. infection examined by using of PCR technique. The whole DNA extracted and purification successfully by used special DNA extraction kit from promiga company used to extract the DNA from Whole blood (Fig 1).

The PCR test was done by used two specific genes used to diagnose *Theileria* infection. The first one SSU r RNA 989/990 primer sit used to diagnose *Theileria* spp. the result of study showed all 51 animals were positive in microscopically examination was also positive to these tests (Fig.2,)

The Same group was positive to the first gene examination exanimate by use of the second gene 30-KDa N516/ N517 primer sit used to diagnosis *Theileria annulata.* The result of this test showed 45 animals 88.23% were positive to Theileria *annulata* infection(Fig.3). whereas 6 animals 11.76%were negative to infection by *Theileria annulata* but were infected by other species of *Theileria* (Table 4).

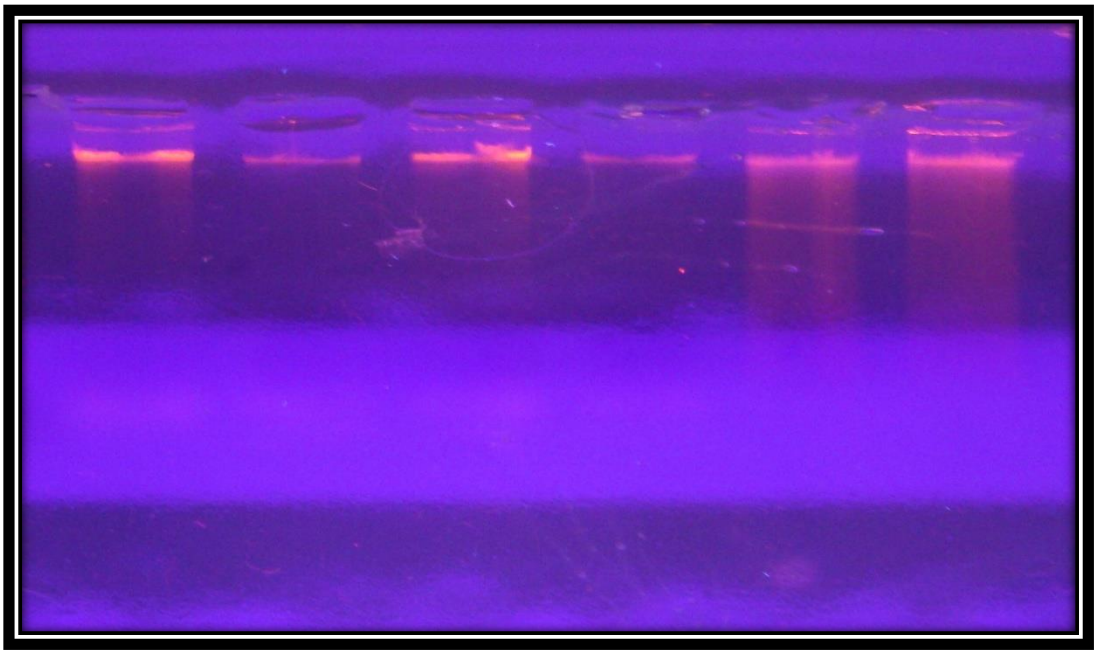

Fig. (1): Agar gel electrophoresis of amplified whole DNA

Table (4) result of genomic diagnosis.

Type of the genes	No. of infection animals	No. of positive animals	Percentage (%)
SSU r RNA gene *Theileria* spp.	51	51	100
30-KD gene *Theileria annulata*	51	45	88.23

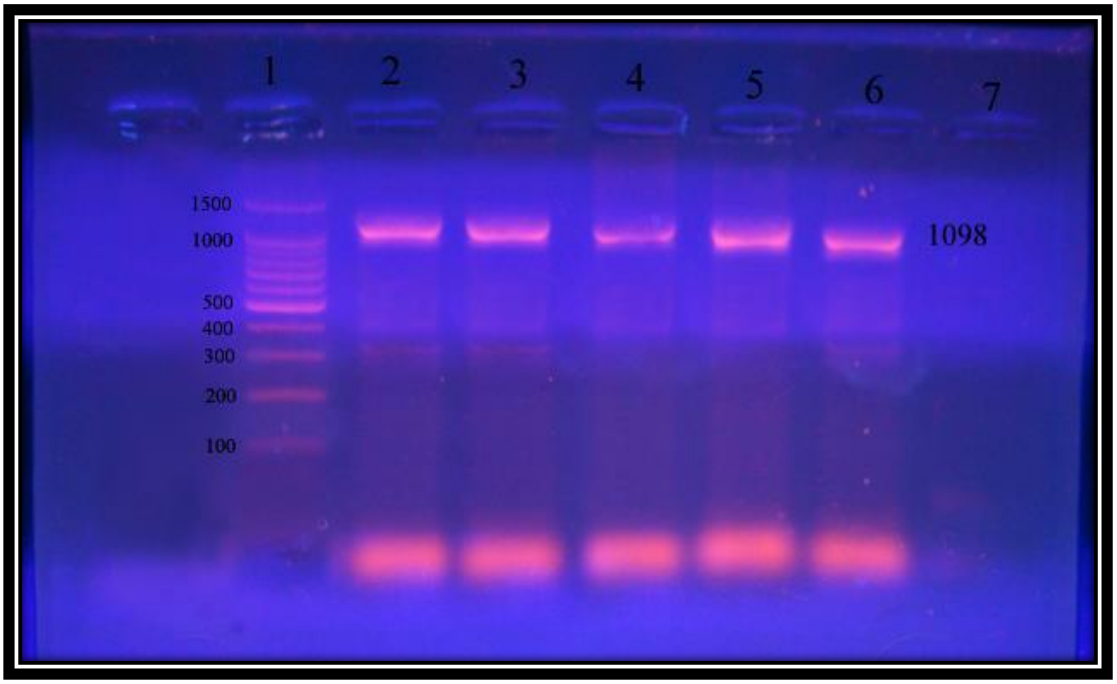

Fig. (2): Agar gel electrophoresis of amplified DNA from different *Theileria* species by using primer set SSU r RNA 989/990. Lane 1 DNA ladder. Lane 2,3,4,5,6 positives to (1098 bp).

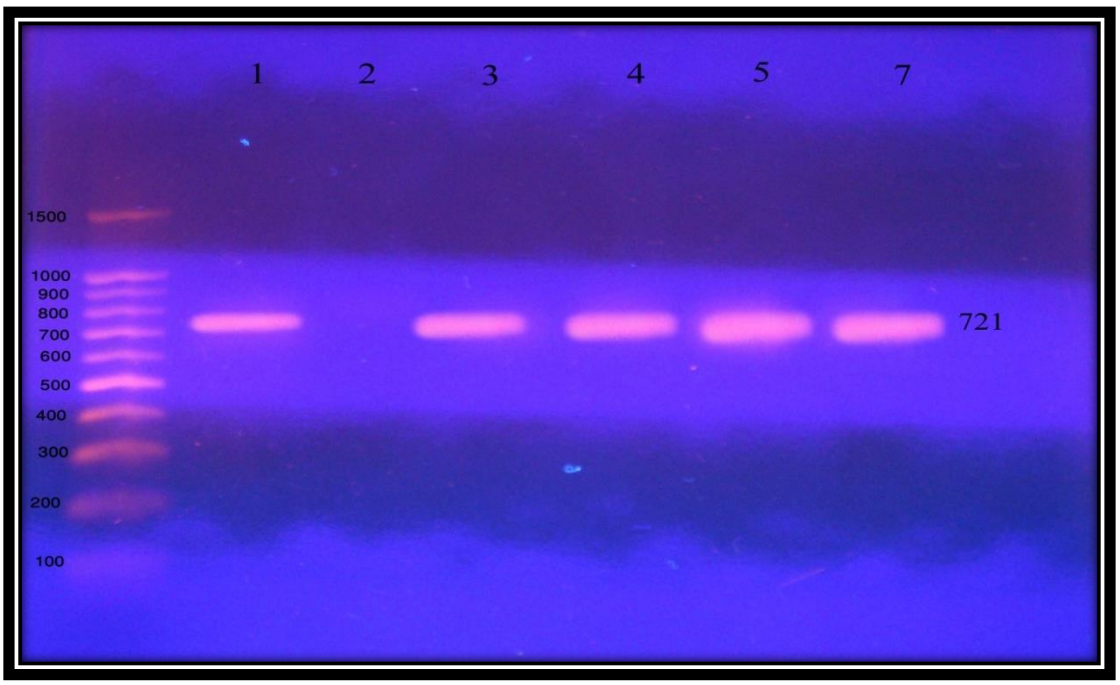

Fig. (3). Agar gel electrophoresis of amplified DNA from *Theileria annulata* by using primer set 30-KDa gen N516/N517. Lane 1 DNA ladder, lane 1,3,4,5,6 positive to 721 bp.

Discussion

The study showed high sensitivity and specificity to diagnose bovine theileriosis by using the whole blood PCR technique and recorded the high sensitivity to PCR technique bovine theileriosis compared with microscopic examination of Giemsa stained blood smears. These results were in agreement with previous study (14,16,18,20).

In the present study the whole blood PCR examination showed 88.23% of animal were positive to the infection by *Theileria annulata* and 11.76% were positive to the infection by other species of *Theileria*. However, the result indicates that the prevalence of *Theileria annulata* is very high in Basrah province cattle's and that species other than *Theileria annulata* are present in the same region. The high percentage of *Theileria annulata* infection determined in the present study is in agreement with results obtained by previous studies carried out in the Iraq (21-24). While the lower percentage of the other *Theileria* spp. disagreement with any study carrid out in Iraq. There are seven *Theileria* species that infected the cattle two of them *T. parva and T. annulata* are major importance (14, 25). The other five species are less pathogenic and some of them may confuse the epidemiology of *Theileria* (3). Moreover, it is difficult to differentiate *Theileria* solely on the basis to morphology of periplasm and schizont stages, and confusion may be raised in mixed infection that occurs and when used the IFA test cross reactions that have been observed among *T. annulata, T. parva, T. mutans* and *T. tourotrigi.*(17,25- 28). The geographical distribution to the benign *Theileria* spp .and available to vector specificity to the transmutation of the benign *Theileria* spp. explains the ability to infection Iraq cattle by benign *Theileria*. Moreover, many studies recorded the infection by benign in the south eastern Turkey and Iran (12, 29).

Conclusion

The genomic diagnosis showed a high specificity and sensitivity to whole blood PCR test for diagnosis *Theileria annulata*. Moreover recorded a high percentage of infection by *Theileria annulata* and lower percentage of infection by other *Theileria* spp.

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Conflict of Interest: The authors state that there is no conflict of interest..

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دراسة تشخيصيه للثليريوسز في الابقار باستخدام تقنية سلسلة تفاعل انزيم البوليميريز

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الخلاصة: تم إجراء الدراسة الحالية على 51 بقرة من مختلف الأعمار والاجناس والسلالات. أظهر فحص الدم لجميع هذه الحيوانات مستوى عالٍ من الطفيليات في الدم تراوحت بين 17-52٪ ولاحظت جميع مراحل الطفيلي، وأظهر التشخيص كما *Theileria annulata* للدم الكامل لتشخيص CRالجيني خصوصية وحساسية عالية لاختبار

سجل نسبة عالية من الإصابة بالطفيلي. 88.23 *Theileria annulata*٪ ونسبة إصابة أقل 11.76٪ ببقية أنواع الثليريا *Theileria* spp.