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MICROSCOPIC INVESTIGATION AND MOLECULAR PHYLOGENY OF TRYPANOSOMIASIS IN CATTLE OF WASIT PROVINCE, IRAQ

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Abstract

In Iraq, only limited data are available about bovine trypanosomiasis and it causes. To the best of our knowledge, this is the first-time trypanosomes have been molecular phylogeny identified in relation to cattle sources. Of 150 cattle subjected to present study, blood samples, clinical examination and case history data were collected during of October 2018 to March 2019 at different areas of Wasit province, Iraq. Using the microscopy and molecular polymerase chain reaction (PCR) assay, a totally 3.33% and 9.33% of study cattle were positives, respectively. Positives of PCR assay is considered as the gold-standard for comparison between infected and non-infected animals. Concerned to the animal risk factors (gender, age and breed), significant increases ($P<0.05$) were observed among the groups of females, 1-4 years and mix breed. Clinically, depression, lymph node enlargement, paleness of mucous membranes, decreasing of milk production and emaciation were the most detected symptoms. For vital signs, significant elevation ($P<0.05$) was in temperature, pulse and respiratory rates. Among hematological findings, significant decreases ($P<0.05$) in values of RBCs indices were reported in total RBCs, PCV, and Hb, whereas, no significant variations ($P \geq 0.05$) were showed in values of MCV, MCH and MCHC. For WBCs, significant decreases ($P<0.05$) were found in total WBCs and lymphocytes and significant increases ($P<0.05$) in neutrophils, but in values of monocytes, eosinophils, and basophils ($P \geq 0.05$). Phylogenetic analysis of six PCR-positives was revealed on two trypanosomes, *T. theileri* and *Trypanosoma cf. cervi*, species that detected for the first time among cattle of Iraq.

Key words: *Trypanosoma*, Cattle, Molecular, Phylogeny, Microscopy, Iraq.

Introduction

Trypanosomiasis or trypanosomosis is one of the most important vector-borne diseases in tropical and subtropical countries; which caused by a microscopic unicellular protozoan flagellate belongs to *Trypansoma* genus of Trypanosomatidae family (Hamilton *et al.,* 2004). Among cattle population, the major causes of the disease are *T. congolense* in addition to *T. vivax* and *T. evansi* (Gillingwater *et al.,* 2010). Other species as *T. theileri*, *T. simiae*, *T. godfreyi* and rarely *T. suis* have been diagnosed in livestock but their precise role in clinical disease in cattle remains unclear (NyentiLum, 2000). The disease represents a major threat to farm animals as well as humans worldwide particularly in Africa, Asia and South America (Fèvre *et al.,* 2005, Jackson *et al.,* 2009). A wide range of domestic and wild hosts can be affected by trypanosomiasis including equines, cattle, buffaloes, sheep, goats, pigs, dogs, deer, gazelles and elephants (Pathak and Singh, 2005, Desquesnes *et al.,* 2013). Although, biological mode by tsetse flies is the most route of transmission for *Trypanosoma*, mechanical mode by biting flies as Tabanids and Stomoxyines is the second alternative way (Van den Bossche *et al.,* 2010). However, direct vertical oral or venereal and iatrogenic transmission may have an impact role in transmission (Desquesnes *et al.,* 2009). In general, trypanosomiasis is characterized by severe anemia, weight loss, reduced productivity, infertility and abortion, as well as death that occur in some animals during acute phase of disease (Osório *et al.,* 2008). Also, infections may cause anemia, leucopenia and thrombocytopenia in addition to inflammatory and degenerative lesions in heart, liver, lymph nodes, testes, brain, conjunctiva, cornea, spleen, kidney and some endocrine glands (O'Gorman *et al.,*

2006; Osório *et al.,* 2008). The disease may be fatal in sub-clinical or chronic infection due to absence of treatment, complicating diagnosis and control the disease (Majekodunmi *et al.,* 2013). Nonetheless, animals that survive from the disease are often remain infected for several months or years, exhibiting a low level of fluctuating parasitemia and serves as a reservoir for the disease (Desquesnes *et al.,* 2013). Previously, the presumptive diagnosis can be made based on suspected clinical signs and confirmed by direct microscopic examination of blood smears which considered as cheap, easy and fast (Radwanska *et al.,* 2002). However, diagnosis is difficult to perform at the low levels of parasitemia during the prepatent period or chronic phase, and almost never seen in healthy carriers (Kirchhoff *et al.,* 1996). Currently, polymerase chain reaction (PCR) assays on blood or tissue samples can identify trypanosomes at the genus, species or subspecies level. PCR technique can amplify multiple species of trypanosomes and recognize infections with less commonly identified species (Desquesnes and Davila, 2002, Schijman *et al.,* 2011). Therefore, main objective of present study was to investigate the prevalence of bovine trypanosomiosis among cattle of Wasit province / Iraq, using the microscopy and molecular PCR assay, with detection the association of PCR-positivity with animal risk factors, clinical and hematological data. Also, molecular phylogeny for a part of PCR-positives was aimed.

Material and methods

Samples and data collection

Of 150 cattle selected randomly for the present study from different areas at Wasit province, Iraq during of October 2018 to March 2019, 10ml of jugular blood sample were collected from each animal under aseptic conditions. Post direct preparation of thin and thick slide-smears, each blood sample was divided into two an EDTA tubes to be used for hematology and molecular assay. Direct clinical examination to measurement of vital signs (temperature, pulse and respiratory rates) and to detect of other bodily abnormalities was aimed in this study. In addition, case history data were collected in based on information of the animal owners.

Microscopy

Two slides of blood smears, thick and thin, were prepared directly post-blood draining, labeled with the number of animal, leave dried, fixed with absolute methyl alcohol (*Chemanol, Saudi Arabia*), dried and saved into a plastic box-slide. At laboratory, the slides were stained following the protocol of Giemsa kit (*Syrbio, Syria*) and then examined under light microscopy (*Meija, Japan*) using 1000× power to detect of parasite (Bowman, 2014).

Hematology

EDTA-blood samples were analyzed, automatically, using Mythic 18 Vet (*Orphëe/ Switzerland*) blood hemolyzer to measurement the parameters of red blood corpuscles (RBCs) including total RBCs count, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) and parameters of white blood cells (WBCs) including WBCs count, lymphocytes, monocytes, neutrophils, eosnophils, and basophils.

Molecular Assay

According to manufacturer instructions of G-spin™ Total DNA Extraction Kit (*Bioneer, South Korea*), DNA of whole blood samples were extracted. Purity of extracted DNA was measured using a nanodrop spectrophotometer (*Thermo-Scientific,UK*). Internal transcribed spacer (ITS) based primers was used in present study for detection of *Trypanosoma* DNA. The set of primers provided by (*Bioneer, South Korea*) to amplify of PCR products at 518bp is composed of (ITS1-CF (5´CCGGAAGTTCACCGATATTG-3´) and ITS1-BR (5´TTGCTGCGTTCTTCAACGAA-3´)). PCR mastermix was prepare using a ready to use PCR kit (*Bioneer, South Korea*) at a final volume of 20ml. PCR reaction was performed into thermal cycler (*Bio-Rad, USA*) involving an initial denaturation step of 72ºC for 5 minutes followed by 35 cycles of 94ºC for 40 seconds, 58ºC for 40 seconds, 72ºC for 90 seconds and final extension step of 72ºC for 5 minutes. The final PCR products was analysed in 1.5% agarose gel using (100-1500bp) of DNA ladder (*Qiagen, Germany*), stained with ethidium bromide, separated by electrophoresis and visualized under Ultra-violet transilluminator (*Clinex, China*).

Phylogenetic analysis

Pure PCR products were sent to (*Bioneer*, *South Korea*) for sequencing. Phylogenetic tree analysis between local trypanosomes species isolates and NCBI-Blast submission trypanosome species was performed by MEGA-6 software and identification of species isolates were submitted into of NCBI-GenBank.

Statistical analysis

Statistically, all data were tabled and analysed by using two computerized programs, Microsoft office Excel (2013) and SPSS (*v.23*). ANOVA test used for statistical analysis of study results and to detect significant

Table 1: Total results of microscopic and molecular PCR assays.

Test	Total No.	Positive	Negative
Microscopy	150	5 (3.33%)	145 (96.67%)
PCR assay	150	14 (9.33%) *	136 (90.67%)
Significant increases * (P<0.05)			

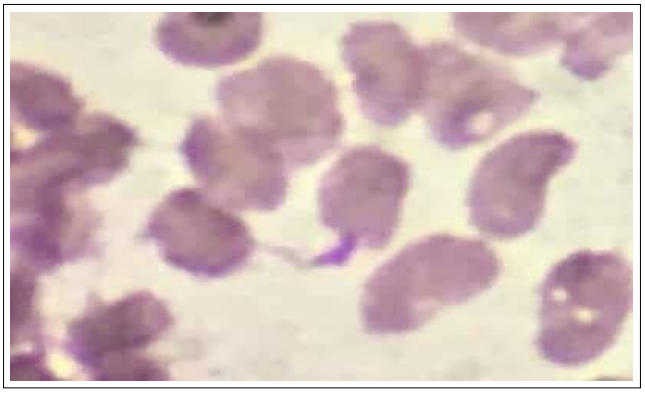

Fig. 1: Positive blood film stained with Giemsa and examined by microscopy under oil immersion (1000×)

differences between positives and negatives and the association of seropositivity with the data of risk factors and values of hematological parameters at a level of P≤0.05, (Petrie and Watson, 2006). Molecular PCR assay is considered as a gold-standard test in this study.

Results

Of 150 blood samples examined by microscopy and conventional PCR assays, the total results were revealed, respectively, on 3.33% (5/150) and 9.33% (14/150) positive cattle (Table 1, Fig. 1).

Association of PCR-positivity with the animal risk factors (gender, age and breed) reported significant increases (P>0.05) in groups of females (10.8%), 1-4 (15.52%) years, and mix breed (12.87%) respectively, (Table 2).

Based on data of clinical examination and case history, many clinical symptoms were detected among PCR-positive cattle, however, depression (64.29%), lymph node enlargement (64.29%), paleness of mucous membranes

Table 2: Association of PCR-positivity with animal risk factors.

Factor	Group	Total No.	Positives by PCR (No=14)
Gender	Female	129	13 (10.8%) *
	Male	21	1 (4.76%)
Age /Year	<1	34	0 (0%)
	1-4	58	9 (15.52%) *
	>4	58	5 (8.62%)
Breed	Pure	26	0 (0%)
	Mix	101	13 (12.87%) *
	Local	23	1 (4.35%)
Significant increases * (P<0.05)			

Table 3: Results of clinical examination among 14 PCR-infected cattle.

Clinical symptom	Positive	Negative
Anorexia	4 (28.57%)	10 (71.43%) *
Depression	9 (64.29%) *	5 (35.71%)
Lymph node Enlargement	9 (64.29%) *	5 (35.71%)
Paleness of mucous membrane	11 (78.57%) *	3 (21.43%)
Nasal / ocular discharges	6 (42.86%)	8 (57.14%) *
Diarrhea	5 (35.71%)	9 (64.29%) *
Reproductive disorders	1 (7.14%)	13 (92.86%) *
Decreasing in milk production	8 (57.14%) *	6 (42.86%)
Emaciation	8 (57.14%) *	6 (42.86%)
Mortality	1 (7.14%)	13 (92.86%) *
Significant increases * (P<0.05)		

(78.57%), decreasing of milk production (57.14%) and emaciation (57.14%) were showed significant increases (P<0.05), (Table 3).

Regarding to vital signs of PCR- positive cattle, significant increases (P<0.05) were detected in temperature (40.05±0.38), pulse (84.45±2.66) and respiratory (39.18±1.5) rates, (Table 4).

Measurement of RBCs parameters of PCR-positive cattle reported that there were significant decreases (P<0.05) in values of total RBCs (4.56 ± 0.79), PCV (26.35 ± 0.35),and Hb (8.76 ± 0.3), whereas, no significant differences were detected in values of MCV, MCH and MCHC, (Table 5).

Table 4: Results of vital signs among infected and non-infected Cattle by PCR.

Vital sign	Unit	Infected (No=14) (M ± SE)	Non-infected (N=136) (M ± SE)
Temperature	℃	40.05 ± 0.38 * (38.64-41.17)	38.17 ± 0.35 (36.23-41.20)
Pulse rate	Per/ Minute	84.45 ± 2.66 * (52-99)	57.14 ± 1.8 (41-112)
Respiratory rate	Per/ Minute	39.18 ± 1.5 * (26-45)	32.32 ± 0.66 (23-54)
Significant increases * (P<0.05)			

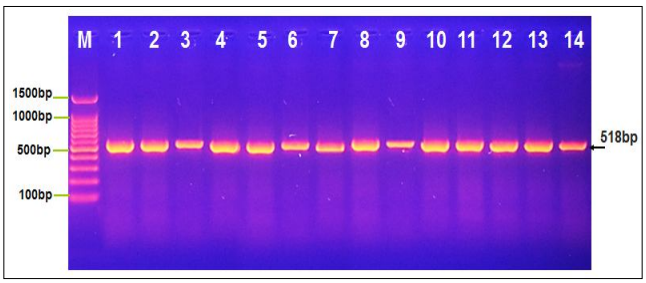

Fig. 2: Total positive blood samples by gel electrophoresis for the primer of *ITS1* gene. M: Ladder, Lanes 1-14: Positive results.

Table 5: Results of RBCs parameters of molecular infected and non-infected Cattle.

Parameter	Unit	Infected M ± SE	Non-infected M ± SE
Total RBCs	× 106/µl	4.56 ± 0.79 * (3.71-7.32)	5.99 ± 0.28 (3.09-8.51)
PCV	%	26.35 ± 0.35 * (21.84-35.07)	30.58 ± 0.64 (19.22-39.26)
Hb	g/dl	8.76 ± 0.3 * (4.85-12.36)	11.24 ± 0.21 (4.15-13.79)
MCV	fl	56.79± 0.63 (44.16-58.41)	52.05 ± 1.16 (38.72-61.13)
MCH	pg	19.52 ± 0.28 (15.87-21.93)	19.06 ± 0.39 (14.83-22.24)
MCHC	g/dl	34.24 ± 0.56 (29.41-37.19)	36.91 ± 0.27 (29.86-38.92)
Significant increases * (P<0.05)			

Table 6: Results of WBCs parameters of molecular infected and non-infected Cattle.

Parameter	Unit	Infected M ± SE	Non-infected M ± SE
Total WBCs	× 10^3/µl	8.32 ± 0.91 (4.12-11.53)	9.65 ± 0.83 * (3.92-12.26)
Lymphocytes	%	38.98 ± 2.48 (29.11-47.46)	44.21 ± 2.44 * (28.27-51.33)
Monocytes	%	5.07 ± 0.68 (3.52-7.06)	5.38 ± 0.51 (3.21-8.18)
Neutrophils	%	52.14 ± 1.82 * (39.92-61.42)	46.37 ± 2.25 (39.92-63.01)
Eosinophils	%	3.01 ± 0.66 (1.12-5.17)	3.45 ± 0.31 (1.07-6.84)
Basophils	%	0.97 ± 0.23 (0.45-1.41)	0.59 ± 0.09 (0.37-1.71)
Significant increases * (P<0.05)			

Measurement of WBCs parameters of infected cattle was revealed on significant increases (P<0.05) in values of neutrophils (52.14 ± 1.82), with significant decreases (P<0.05) in total WBCs (8.32 ± 0.91) and lymphocytes (38.98 ± 2.48). However, no significant differences (P≥0.05) were detected in values of monocytes, eosinophils and basophils, (Table 6).

In this study, six of PCR-positive samples were submitted for sequencing. Multiple sequence alignment analysis of 18S ribosomal RNA gene in local *Trypanosoma* spp. isolates and NCBI-Genbank *Trypanosoma* species isolates. The multiple alignment analysis was constructed using Clustal W alignment tool of MEGA-6 software which showed the nucleotide alignment similarity as (*) and substitution mutations in 18S ribosomal RNA gene, (Fig. 3).

Phylogenetic tree analysis based on 18S ribosomal RNA gene partial sequence in local *Trypanosoma* spp. IQ-Cattle isolates was used for *Trypanosoma* spp. identification. It constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) of MEGA-6 software, (Fig. 4).

The local *Trypanosoma* spp. IQ-Cattle isolate No.1 was showed a close-related to NCBI-BLAST *Trypanosoma cf. cervi* PJH-2013 isolate WTD A5 clone Cl1-18s ribosomal RNA gene (JX178196.1) and the local *Trypanosoma* spp. IQ-Cattle isolate No.2-No.6 were showed close-related to NCBI-BLAST *Trypanosoma theileri Obihiro* isolate (LC385952.1), at total genetic changes (0.1-0.5%), (Table 7).

Discussion

Trypanosomiasis in cattle represents a serious threat to the agriculture and socioeconomic development of livestock in vast areas worldwide. The impact of bovine trypanosomiasis is not restricted to livestock production alone, but extends to changes in land use and exploitation of natural resources use, access to available and cultivable land, restriction of opportunities for diversification of agriculture production (Swallow, 2000, Dobson *et al.,* 2009). The application of microscopy and PCR assay for the species-specific detection of trypanosomes in bovine blood samples was established in this study and revealed respectively on 3.33% and 9.33% positive

Fig. 3: Multiple sequence alignment of local isolates with NCBI-Genbank isolates.

Table 7: Homology Sequence identity between local and NCBI-BLAST *Trypanosoma* spp. isolates.

Trypanosoma local isolate	Genbank accession No.	NCBI-BLAST Homology Sequence identity (%)			
		NCBI BLAST Identical *Trypanosoma* spp.	Genbank accession No.	Country	Identity(%)
No.1	MN121743.1	*Trypanosoma cf. cervi* PJH-2013 isolate	JX178196.1	USA	98.42%
No.2	MN121744.1	*Trypanosoma theileri Obihiro* isolate	LC385952.1	Japan	99.32%
No.3	MN121745.1	*Trypanosoma theileri Obihiro* isolate	LC385952.1	Japan	98.12%
No.4	MN121746.1	*Trypanosoma theileri Obihiro* isolate	LC385952.1	Japan	99.54%
No.5	MN121747.1	*Trypanosoma theileri Obihiro* isolate	LC385952.1	Japan	98.13%
No.6	MN121748.1	*Trypanosoma theileri Obihiro* isolate	LC385952.1	Japan	99.11%

animals with trypanosomiasis. The central finding of present study is that PCR is significantly more sensitive over microscopy, and the relative higher positivity might be correlated to several factors such as the primers used, type of animal samples, seasonal influences and management-socioeconomic elements. Nonetheless, PCR assay offered no advantage since the parasite can easily seen by microscopic examination of fresh blood smear during acute phase of disease (Uilenberg and Boyt, 1998). In many studies, it is assumed that the microscopy has not standardized since the method must be done on fresh, neither delayed or stored blood and the test is based on experience of observer for detection of parasite and severity of infection (Kirchhoff *et al.,* 1996, Herwaldt, 2001). The greater sensitivity of PCR assay allowed for considerably earlier detection of trypanosomiasis before developing of parasitemia and in chronic phase when the number of parasites is very small (Pizarro *et al.,* 2007). As well as, observer is not an issue in PCR assay and the blood processing by this technique can be interpreted at several points without any deleterious effects (Galvao *et al.,* 2003). False positive findings could be resulted with PCR assay due to either contamination of reaction mixture with amplification products of previous run, or presence DNA fragments of the parasite which could be amplified by the primer (Qvarnstrom *et al.,* 2012). However, primer used in present study to detect of *Trypanosoma* in blood samples was targeted the ITS region of ribosomal DNA. This region is a preferred for universal test because of its capability for detection of all pathogenic trypanosomes in a single PCR, highly conserved flanking regions and size variability among trypanosomes species and subgroups (Njiru *et al.,* 2005, Sidibë *et al.,* 2007).

Statistical analysis regarding the association of animal risk factors (gender, age and breed) to PCR-positivity deemed necessary, (Table 2). It was indicated that females were significantly more infected than males and the reasons cannot be explained exactly, but it might be attributed to large number of females subjected to this study compared to males, exposing of females to high stress as a result of frequent pregnancy and milking and neglected attitude of the farmers toward the management of females since many of the farmers focusing on the health of males that used mostly as a meat-source by meatmans or for natural fertilization. The high prevalence of trypanosomiasis in animals of 1-4 years compared to those of <1 year and >4 years was reported in this study. It is thought that presence of maternal immunity during the first 6-12 months of life is play a vital role in protection against different infection and this form of passive immunity is greatly diminished after one year of age. Whereas, active immunity that developed with advanced age due to frequent exposure of the animal to pathogen increases resistant to infection with the same pathogen (Flynn and Sileghem, 1994, Niewiesk, 2014). Variation in PCR-positivity among different breeds of present study might be related to existence of genetic and/or management differences. Worldwide, there continues to be very significant advances in efforts to control diseases in cattle with the potential for significant improvements to both performance and welfare. Concurrently, there have been considerable advances in animal breeding and genetics relevant to animal disease control (Berry *et al.,* 2011). When considering the significance of resistance / tolerance at the breed level, the intrinsic evolutionary advantage of breeds that are adapted to an environment should be taken into account. In tropical areas, where extreme endemic diseases are widespread, due to their evolutionary roots, locally adapted autonomous breeds

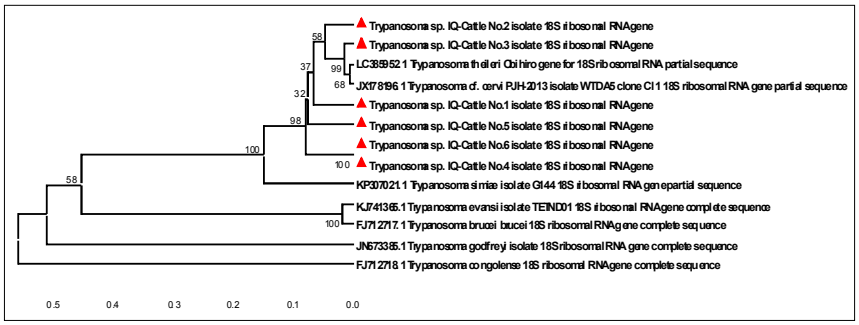

Fig. 4: Phylogenetic tree analysis of local isolates.

display a far greater level of genetic resistance and adaptation as compared to imported breeds (Jovanovië *et al.,* 2009).

In this study, data of clinical examination and case history were revealed on significant increases in depression, L.N. enlargement, paleness of M.M., decreasing in milk production, and emaciation (Table 3). In addition, significant increases were reported in values of temperature, pulse and respiratory rates (Table 4). In cattle, acute episode of disease can be last for a few days to few weeks, during which, the animal either dies or lapses in a subacute to chronic phases. However, the disease might be chronic from the beginning (Tulu, 2019). Globally, limited clinical data are available about bovine trypanosomiasis due to the poorly characterized symptoms. Also, there are no pathognomonic signs that would help in pinpointing a diagnosis because the general clinical picture of infection is characterized by many variations that determined by the level of vector challenge, species and strain of trypanosome, breed and management of the host (Radostits *et al.,* 2006, Jittapalapong *et al.,* 2009).

Significant decreases in total RBCS, PCV and Hb indicated the presence of anemia among PCR-positive cattle of present study, (Table 5). As reported previously, when animal becomes infected with trypanosomiasis, their physiological alters due to the wide range of blood biochemical changes and hematological aberrations that occur (Anosa and Isoun, 1980, Biryomumaisho *et al.,* 2003). The evaluation of blood parameters helps to determine the health status of animal, as well as to establish the degree of damage to host tissues and severity of infection (Coles, 1986, Allam *et al.,* 2011). In most animal species, anemia is the main hematological features which can be detected during the course of disease. Although the etiology of anemia is complex, three mechanisms have been implicated including dyshemopoiesis, hemoldilution, and hemolysis (Amole *et al.,* 1982). Reduction in RBC mass and life-span due to sensitization of erythrocytes during acute phase of disease, in addition to erythrophagocytosis, hemosiderosis and hyperbilirubinemia during chronic trypanosomiasis could be occurred as a result of constant high parasitemia and immunosuppression (Stijlemans *et al.,* 2015, Constable *et al.,* 2016).

Significant elevation in neutrophils, in addition to eventual depression in total WBCs and lymphocytes were reported among infected study animals (Table 6). In cattle, neutrophils have biochemical and functional differences from those of other species and play protective and harmful roles in naturally occurring infectious diseases that occur at times of transition (Bassel and Caswell, 2018). However, chronic inflammations due to infectious (viral, bacterial, protozoal, parasitic and fungal) or non-infectious (thrombosis, injuries and necrosis) in addition to stress are the most common causes of neutrophilia (Roland *et al.,* 2014). The mechanisms underlying leukopenia include decrease production, increase tissue demand and consumption combined with marginalization. In cattle, leucopenia often occurs with metabolic disorders, liver and infectious disease, as well as hematopoietic stem cell disorders (Kraft and Dürr, 2005, Jones and Allison, 2007). Lymphocytes are the dominant subpopulation of WBC which decreases progressively with age (Webb and Latimer, 2011). In general, lymphocytopenia can be seen in association with immunosuppression due to physiologic and pathologic reasons (Jones and Allison, 2007). In cattle infected with trypanosomiasis, there is reduced proliferation of T-cell in response to stimulation by T-cell mitogens such as ConA or PHA and reduced in antibody response (Tabel *et al.,* 2013).

In Iraqi cattle herds, *T. brucei* and *T. congolense* (Al-Badrani, 2012) in addition to *T. vivax* (Rhaymah and Al-Badrani, 2012) were the only Trypanosomes that reported previously. In current study, the results of phylogenetic analysis for a six of PCR-positive samples were revealed on newly two trypanosome spp. (*Trypanosoma theileri* and *Trypanosoma cf. cervi*) that reported for the first time in Iraq, (Fig. 3 and 4, Table 7). According to NVBI, both species are classified under a trypanosome spp. of unspecified subgenus. *T. theileri* is a heterogeneous group of large trypanosomes which share mammalian host restriction, distributed worldwide, contaminative transmission by tabanids, cause chronic and cryptic infection usually and detectable only in blood (Böse and Heister, 1993, Rodrigues *et al.,* 2003). Many cases indicate its potential pathogenicity in concurrent infections with *Anaplasma*, *Babesia* and *Theileria*, or associated with stress, poor nutrition and gestation (Braun *et al.,* 2002, Rodrigues *et al.,* 2006). Also, *T. theileri* has been detected to be related with inflammatory conditions in cattle such as peritonitis and submandibular edema, as well as with concomitant diseases such as bovine leukosis (Matsumoto *et al.,* 2011, Vairamuthu *et al.,* 2011). *Trypanosoma cervi*, first identified in elk in 1975, is a worldwide trypanosome spp. which detected later in different hosts involving cattle and water buffaloes (Fisher *et al.,* 2013). Although, there has little opportunity in nature for cross-transmission of trypanosomes to occur, successful cross-transmission from deer to elk has been accomplished in areas where these hosts occupy the same

territory (Mathews *et al.,* 1977). However, it's observed that there no signs of pathogenicity and no specific of pathology has been attributed to infection of other animals (Kingston *et al.,* 1982). In Iraq, the introduction of susceptible cattle from Brazil, India, Iran, Turkey and other countries might be contributed to increase the prevalence of bovine trypanosomiasis since this country is free of tsetse fly that the most vector capable for transmitting of trypanosomes spp. (Al-Badrani, 2012, Sazmand and Joachim, 2017).

Conclusion

The results of current study confirmed the efficacy of molecular phylogeny in detection of two trypanosome species, *T. theileri* and *T. cervi*, among cattle of Wasit province for the first time in Iraq. In addition, the prevalence of bovine trypanosomiasis was detected using the microscopy and PCR assay, with evaluation the association of the positivity with clinical and hematological findings. Further studies are needed to detect the prevalence of bovine trypanosomiasis in other Iraqi regions as well as other trypanosome species, to focus on vectors, and to evaluate the risk factors for planning future control programs.

Ethical Considerations

Permission for the study and ethical approval was obtained from the department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Baghdad, Iraq.

Conflict of interest

No conflict to interest.

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