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EXPLORING THE FREQUENT OCCURRENCE AND CHARACTERIZATION OF CYTOCHROME B GENE IN *THEILERIA ANNULATA* IN CATTLE

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ABSTRACT

Bovine theileriosis, caused by *T. annulata*, results in high morbidity and mortality rates, as well as severe financial losses for the livestock industry in Egypt. In this study, fifty cattle were utilized. Whole blood samples were collected for laboratory analysis. Giemsa-stained blood films were employed to detect *Theileria* infection. PCR was used to evaluate various target genes, like *30-kDa* and *Cyto B* of *T. annulata*. Nine (18%) samples tested positive for piroplasm of *Theileria* by microscopic examination of blood film. Twenty-one (42%) of the analyzed samples tested molecularly positive based on the *30-kDa* gene (N516/N517), while 10 (20%) samples were positive based on the *Cyto B* gene. In our study, we carried out DNA sequencing and phylogenetic analysis of *T. annulata* using the *Cyto B* gene. Phylogenetic analysis of the *Cyto B* gene of the Egyptian strain of *T. annulata* (Assiut) revealed a nucleotide identity ranging from 96.16% to 98.92% with *T. annulata* strains from various Egyptian governorates (Sharkia and Qalyubia), as well as from Sudan, Tunisia, Turkey, Iran, and India. The obtained isolates were closely clustered with an isolate from Sudan (accession number LC431533). We identified thirty-point changes at the amino acid sequences. There was substantial variance ($P<0.05$ and $P<0.01$) between age and sex of tested cattle, respectively, and percentages of *T. annulata* infection. The data obtained from our study on the characterization of the *Cyto B* gene of *T. annulata* in Assiut Governorate suggest that the *Cyto B* gene may be used as a genetic marker to identify resistant isolates of *T. annulata*.

Keywords: *Theileria annulata*, PCR, *Cyto B* gene, sequencing, Phylogeny, Assiut, Cattle

INTRODUCTION

Ticks are bloodsuckers that spread various pathogens, can lead to several dangerous diseases in cattle, and cause physical harm for them by damaging their skin (Parveen *et al*., 2021). Bovine theileriosis is one of the most prevalent diseases spread by ticks. Tropical theileriosis in cattle is caused by the apicomplexan protozoa. *Theileria annulata* (*T. annulata)* is among the most financially important species of hemo-protozoan infection in cattle transmitted by several species of *Hyalomma* spp ticks (Sharifiyazdi *et al.,* 2012). According to the life cycle of the *Theileria* species, the sporozoites produced during the ticks'

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cyclical development are injected into the mammalian host by tick saliva. In white blood cells, they transform into schizonts, while in red blood cells, they become piroplasms (Anter *et al.,* 2019). The clinical findings of bovine theileriosis include enlargement of superficial lymph nodes, fever, pale mucous membranes that turn icteric in the late stages, a drop in milk production, uncommon dark tarry diarrhea, dehydration, salivation, lacrimation, nasal discharge, dyspnea, and corneal opacity (Fadel *et al.,* 2023). The disease results in significant financial losses, due to its high rates of morbidity and mortality, with a major effect on the production of milk and meat (Radiostits *et al.* 2007). To learn about this hemoparasitic disease's epidemiology and variety, a precise identification of them is required (Majidiani *et al.,* 2015). There are different methods for diagnosing theileriosis, such as clinical signs, microscopic analysis of Giemsa-stained blood films, lymph node biopsy smears (Nourollahi-Fard *et al.,* 2015), serological tests like the Indirect Fluorescence Antibody Test (IFAT) and Enzyme-Linked Immunosorbent Assay (ELISA) (Omer *et al.*, 2011), and molecular techniques like Polymerase Chain Reaction (PCR) (Nourollahi-Fard *et al.,* 2015; Afshari *et al.,* 2021 and Jassim *et al.,* 2021). But the lymph node biopsy smear method frequently has low sensitivity when diagnosing carrier cows, incorrect diagnoses, and technical issues (Selim *et al.,* 2020). Serological tests are highly uncertain in areas where *Theileria* species coexist beside long-term carriers; antibodies typically wane while *Theileria* piroplasms remain (Nourollahi-Fard *et al.,* 2015). A molecular technique like PCR is more accurate and precise than other methods, and it has been recorded to be the most sensitive technique for identifying *T. annulata* in clinically and subclinically infected animals (Jassim *et al.,* 2021). Numerous molecular markers have been employed in the past for both diagnostic and phylogenetic purposes. These include small subunit ribosomal RNA (18S rRNA) (Habibi, 2012), large subunit ribosomal RNA (28S rRNA) (Gou *et al.,* 2013), *T. annulata* merozoite surface antigen 1 (Tams1) (Kirvar *et al.,* 2000), N516/N517 (D'Oliveira *et al.,* 1995) and the *Cytochrome B* (*Cyto B*) gene (Sharifiyazdi *et al.,* 2012). The N516/N517 primer set is specific to the gene encoding the *30-kDa* major merozoite surface antigen (*30-kDa*) of *T. annulata* (D'Oliveira *et al.,* 1995). Each parasite genome contains over 100 copies of the *Cyto B* gene, an extra-chromosomal DNA molecule that changes quickly, but is not subject to selection pressure to accumulate polymorphism (Edith *et al.,* 2018). The disease has been alleviated with the use of antiprotozoal medications like buparvaquone, and this drug has been prescribed to treat this disease since the 1990s in order to achieve its antiprotozoal effects towards *T. annulata* (Ali *et al.,* 2022). This drug attaches to the parasite *Cyto B*, inhibiting its mitochondrial respiration (Nehra *et al.,* 2024). Some studies have found a connection between the presence of two (Sharifiyazdi *et al.,* 2012), two (Mhadhbi *et al.,* 2015), eleven (Chatanga *et al.,* 2019), two (Yousef *et al.,* 2020), and twelve (Hacilarlioglu *et al.,* 2023) mutations in the *Cyto B* gene and a rapid increase in treatment failure. However, it remains uncertain which *Cyto B* gene mutation is strongly linked to buparvaquone resistance (Hacılarlıoglu *et al.,* 2023). The objective of this study was to utilize two genes (*30-kDa* and *Cyto B*) to molecularly estimate the prevalence of *T. annulata* among cattle within Assiut Governorate. Additionally, phylogenetic analysis was conducted using partial *Cyto B* gene sequence, and various epidemiological parameters were examined along with the infection rate of *T. annulata* in Assiut Governorate.

MATERIALS AND METHODS

1. Ethical approval

All diseased animals examined in this study were handled according to ethical guidelines. The study was approved by the

Ethical Committee in the Faculty of Veterinary Medicine at Assiut University, Assiut, Egypt, with the approval number 06/2024/0184. Samples were collected from the animals under study, with procedures designed to minimize pain or distress during sample collection, which was done under appropriate sterile conditions.

2. Animals

Between May 2023 and August 2023, fifty cattle with varied breeds, ages, and sexes from several villages in Assiut Governorate were examined in the veterinary teaching hospital at the Faculty of Veterinary Medicine, Assiut University. All cattle under investigation showed fever, swelling of one or more peripheral lymph nodes, diarrhea, respiratory disorders (cough and nasal discharge), tick infestation, and corneal opacity.

3. Clinical assessment

The diseased cattle under study underwent clinical investigation in compliance with Jackson and Cockcroft (2002).

4. Sampling

While the cattle were under the proper constraint, two milliliters of whole blood samples were taken through the jugular vein and placed into sterile Ethylene Diamine Tetra Acetic Acid (EDTA) vacutainer tubes. These blood samples were divided into two parts: one part for making blood films and the other part preserved at -20°C for later extraction of DNA.

5. Conventional diagnosis by blood films

Thin blood films were performed, according to Coles (1986).

6. Molecular diagnosis

6.1. Extraction of DNA

Fifty frozen whole blood samples were used to extract the protozoa DNA using the ABT genomic DNA mini extraction kit (Cat. No. EX01, Applied Biotechnology, Egypt) in accordance with the manufacturer's instructions. DNA extracted was kept at -20°C until use.

6.2. Primers

The particulars of the selected primers (Metabion International AG, Germany) employed in the current investigation for the *30-kDa* and *Cyto B* genes of *T. annulata* had been studied previously (D'Oliveira *et al.,* 1995 and Mhadhbi *et al.,* 2015). Sequences of used primers are illustrated in Table 1.

Table 1: Sequence of nucleotides of utilized primers of *T. annulata* and the PCR product size

Primer	Sequences of nucleotides	Size of product (bp)
N516 (F)	5′- GTA ACC TTT AAA AAC GT -3′	721
N517 (R)	5′- GTT ACG AAC ATG GGT TT -3′	
CytoF	5′- CAG GGC TTT AAC CTA CAA ATT AAC -3′	1092
CytoR	5′- CCC CTC CAC TAA GCG TCT TTC GAC AC -3′	

6.3. Detection of *T. annulata* by PCR

Potential for a PCR designed specifically to amplify *30-kDa* and *Cyto B* genes of *T. annulata*. Using primer sets N516 forward and N517 reverse, DNA fragments of length 721 bp were amplified, while primer sets Cyto forward and Cyto reverse were employed for amplification of DNA fragments of length 1092 bp (D'Oliveira *et al.,* 1995 and Mhadhbi *et al.,* 2015). ABT red master mix (2X) (Cat. No. AMP01, Applied Biotechnology, Egypt) was used in this work. PCR were carried out using a PCR thermocycler (Primus 25, Peqlab, Germany), and the PCR reaction mixture included 10 pmol of each primer, 4 µl of DNA template, 10 µl of master mix, and 4 µl of PCR grade water to obtain a total reaction volume of 20 µl: In conclusion, the primer set (N516/ N517) underwent thermal cycling conditions. First, denaturation was carried out for five minutes at 94°C. There were forty cycles of denaturation at 94°C for one minute, 41°C for one minute for the

annealing step, and 72°C for one minute for extension. Finally, the final extension was carried out for ten minutes at 72°C. The primer set (CytoF/CytoR) was subjected to thermal cycling conditions like the primer set (N516/N517), except the annealing step was 56°C for one minute.

6.4. PCR product analysis and detection

Five microliters of the amplified DNA product were loaded for visualization using gel electrophoresis. The amplicons were analyzed in a 1.5% agarose gel stained with ethidium bromide (10 mg/ml) for 75 minutes at 90 V and 155 mA. The amplicons size was determined using a 100 bp DNA ladder and a gel UV transilluminator (GVM20, Syngene, United Kingdom).

7. Sequencing of PCR products of the *Cyto B* gene of *T. annulata*

DNA sequencing was performed on two PCR products (1,092 bp amplified) to characterize the PCR results. The Cyto forward and Cyto reverse primers were used in the sequencing process. The sequencing of the PCR products was carried out at Macrogen in South Korea. The nucleotide sequence data used in this study were registered in the National Center for Biotechnology Information (NCBI) GenBank database under the following accession numbers: PP465044 and PP465045.

8. Phylogenetic analysis

In order to determine sequence similarity with the GenBank database, the nucleotide sequences were subjected to Basic Local Alignment Search Tool (BLAST) software (http://www.ncbi.nlm.nih.gov/BLAST) analysis. Reference sequences downloaded from GenBank were used for phylogenetic analysis. The query and reference sequences were input into MEGA-X (version 11.0.13) software. Multiple alignments were performed with MEGA-X using the ClustalW program. Genetic distances in MEGA-X were calculated with the Kimura-2 parameter model. The confidence values of internal nodes in phylogenetic trees were calculated using 1000 bootstrap replicates of sequence alignment datasets, and phylograms were produced with MEGA-X utilizing the maximum likelihood method.

9. Statistical evaluation

Using the statistical package for the social sciences (SPSS) version 16 software, the Chi-square of independence test (2007) was employed to analyze the clinical findings and laboratory results. Relative risk and the chi-square test were calculated in SPSS to assess the effect of each risk factor separately on the molecular detection of *T. annulata* in the diseased cattle under examination (i.e., sex, age, and breed). To obtain and evaluate data, odds ratios and 95% confidence intervals (95% CI) were used. Statistical significance was defined as a probability value (P-value) of $P<0.05$.

RESULTS

1. Clinical findings of examined cases

Theileriosis in cattle employed in this investigation displayed the usual clinical manifestations like fever, swelling of peripheral lymph nodes, diarrhea, respiratory symptoms (nasal discharge and cough), tick infestation, and corneal opacity (Table 2).

2. Giemsa-stained blood smear

The microscopic examination of the Giemsa-stained blood smear showed piroplasm of *Theileria* spp. in the infected cattle. 9/50 (18%) of whole blood samples tested conventionally positive using Giemsa-stained blood smear (Table 3).

3. Molecular detection of *T. annulata* infection by PCR

PCR was used to test DNA samples to generate the required bands at 721 bp of the *30-kDa* gene of *T. annulata* (Figure 1), and to produce the specific diagnostic bands at 1092 bp of the *Cyto B* gene of *T. annulata* (Figure 2). Twenty-one (42%) and ten (20%) of 50 whole blood samples tested molecularly positive for the *30-kDa* gene and *Cyto B* gene using PCR, respectively (Table 3).

Table 2: Clinical signs of theileriosis in diseased cattle (No. = 50)

Clinical findings	No. of assessed diseased cattle	No. of *30-kDa* gene positive cattle (%)	P-value
Fever and tick infestation	8	5 (62.50%)	0.286
Fever, enlarged peripheral lymph nodes and respiratory symptoms	11	2 (18.18%)	
Fever, respiratory symptoms and tick infestation	12	6 (50%)	
Fever, enlarged peripheral lymph nodes and tick infestation	7	2 (28.57%)	
Enlarged peripheral lymph nodes, diarrhea, respiratory symptoms and tick infestation	11	5 (45.45%)	
Fever, enlarged peripheral lymph nodes, respiratory symptoms, tick infestation and corneal opacity	1	1 (100%)	
Total	50	21 (42%)	

No significant variation at $p<0.05$.

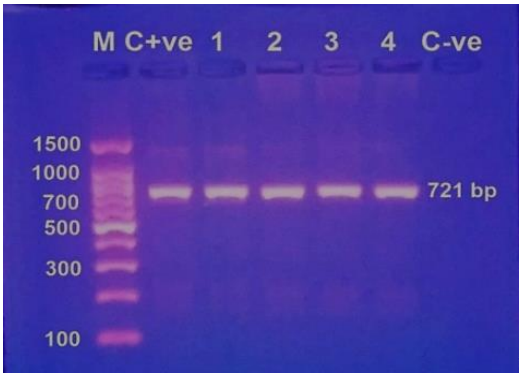

Figure 1: Agarose gel electrophoresis of PCR following *30-kDa* gene amplification of *T. annulata* infection in diseased cattle. Line M: DNA ladder 100 bp, line C+ve: control positive sample; lines 1, 2, 3, and 4: positive samples, and line C-ve: control negative.

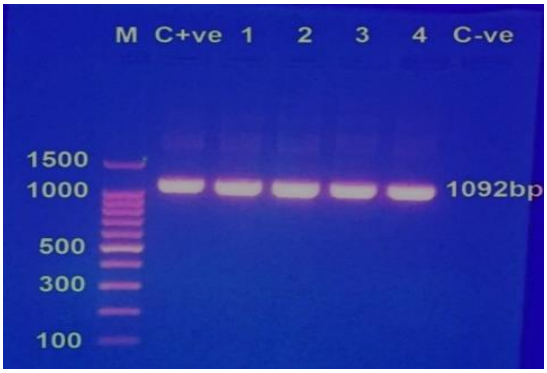

Figure 2: Agarose gel electrophoresis of PCR following *Cyto B* gene amplification of *T. annulata* infection in diseased cattle. Line M: DNA ladder 100 bp, line C+ve: control positive sample; lines 1, 2, 3, and 4: positive samples, and line C-ve: control negative.

Table 3: Prevalence of *T. annulata* infection in investigated cattle by Giemsa-stained blood film and molecular method

No. of examined cattle	Giemsa-stained blood film		PCR				P-value
			30-kDa gene		*Cyto B* gene		
	No. of positive	%	No. of positive	%	No. of positive	%	
50	9	18	21	42*	10	20	0.011

Significant variation at $p<0.05$.

4. Phylogenetic evaluation

Phylogenetic analysis of *Cyto B* gene of the Egyptian isolates of *T. annulata* (Assiut, Accession numbers: PP465044 and PP465045), Figure 3 showed that 96.16%-98.92% nucleotide identity with *T. annulata* isolates of different governorates (Sharkia and Qulyubia) of Egypt, Sudan, Tunisia, Turkey, Iran, and India. The obtained isolates' phylogenetic analysis revealed a close cluster with an isolate from Sudan (accession number LC43153).3).

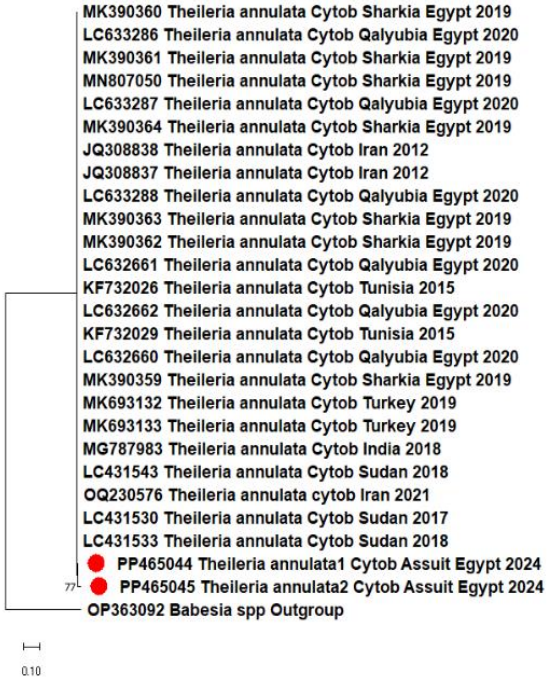

Figure 3: Phylogenetic tree with 1092 bp amplicon with Egyptian (Assiut, accession numbers: PP465044 and PP465045) *Cyto B* gene of *T. annulata*. The tree was made using maximum likelihood and kimura two-parameter as a nucleotide substitution model. The Egyptian (Assiut) *T. annulata* is marked with a filled circle. *Babesia* spp. was used as an outgroup (OP363092).

5. Amino acid comparison between detected *T. annulata* isolates with references isolates of Egypt

In the present investigation, the *Cyto B* gene (1092 bp) was sequenced from two isolates of *T. annulata*. This was carried out in order to study the changes in the parasite's gene. After that, the obtained *Cyto B* amino acid sequences were aligned to and compared with the *Cyto B* gene amino acid sequences of the Egyptian *T. annulata* references. The obtained result showed 94.82%-96.73% amino acid identity with *T. annulata* isolates of different governorates (Sharkia and Qulyubia) of Egypt. Although both Assiut *T. annulata* isolates (WWZ83999 and WWZ84000) were obtained from the same region, there were sixteen change positions observed between them (Figure 4). Additionally, thirty positions with changes were noted between the recorded Assiut *T. annulata* isolate (WWZ83999) and other compared isolates (Figure 4).

6. Potential risk factors

This study dealt with some risk factors (sex, age, and breed) of investigated cattle that influence the prevalence of *T. annulata* infection (Table 4). The studied female cattle had a significantly higher infection rate of *T. annulata* than male cattle, while the age group >2–5 years possessed a significantly greater rate of *T. annulata* infection than the 45 days–2 years age group, but there was no statistically significant variation in the prevalence of *T. annulata* infection between native and mixed breeds (Table 4).

Table 4: Association between *T. annulata* infection in examined diseased cattle and potential risk factors according to PCR result (relied on *30-kDa* gene).

	Variable	No. of examined animals	PCR		Odds ratio	95% CI	P-value
			Positive n (%)	Negative n (%)			
Sex	Male	25	7 (28)	18 (72)	0.31	0.09 - 0.99	0.045
	Female	25	14 (56)*	11 (44)			
	Total	50	21 (42)	29 (58)			
Age	45 days - 2 years	22	4 (18.18)	18 (81.82)	0.14	0.04 - 0.54	0.002
	> 2-5 years	28	17 (60.71)**	11 (39.29)			
	Total	50	21 (42)	29 (58)			
Breed	Native	33	13 (39.39)	20 (60.61)	0.73	0.22 - 2.38	0.603
	Mixed	17	8 (47.06)	9 (52.94)			
	Total	50	21 (42)	29 (58)			

*Significant differences at $p<0.05$ **Highly significant variation at $p<0.01$
No significant variation at $p<0.05$.

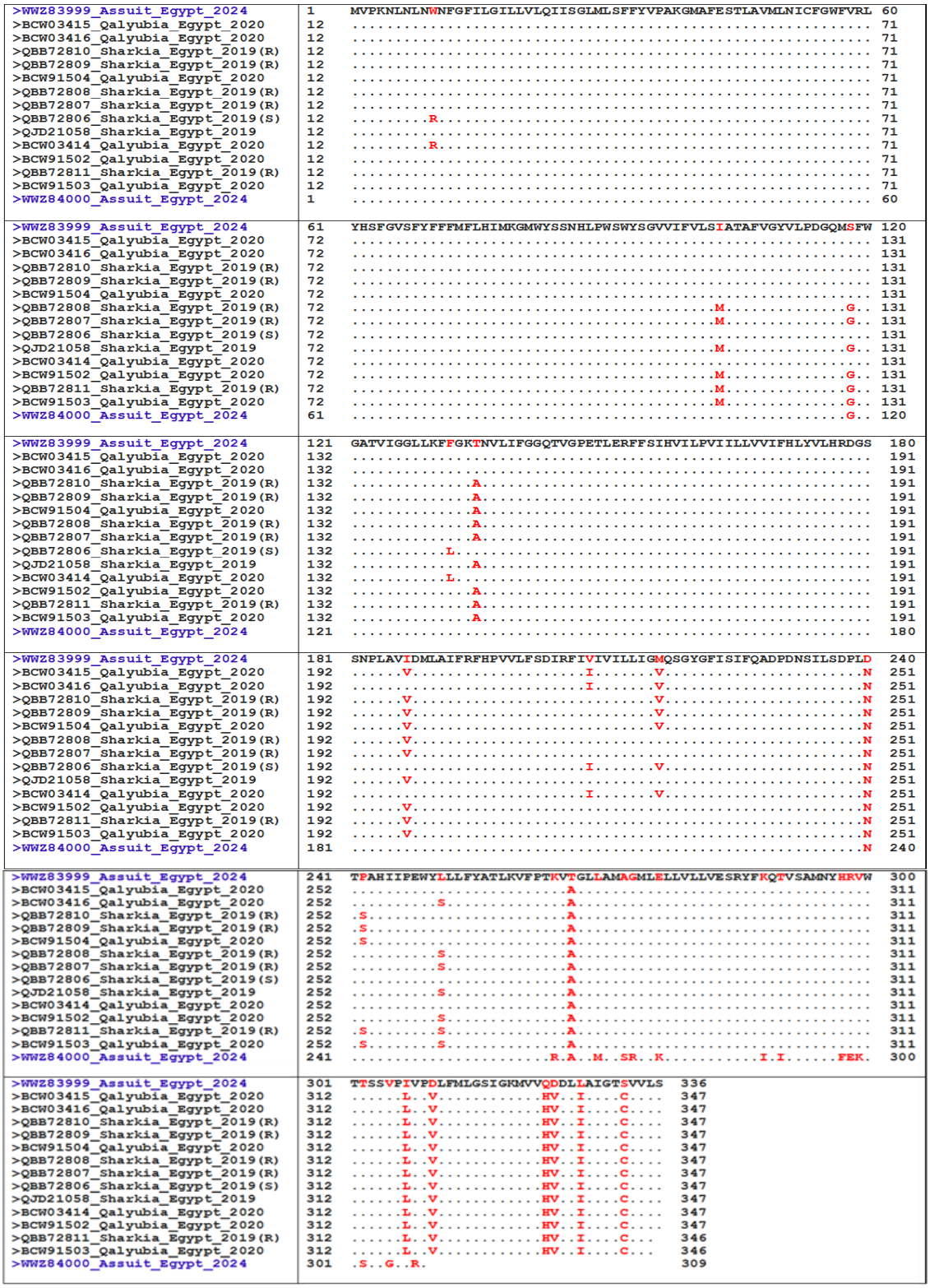

Figure 4: Numerous amino acid sequence alignments of the *Cyto B* gene of the newly produced isolates (WWZ83999 and WWZ84000) in the current study, with the reference sequences of *T. annulata* recorded in Egypt. Dots indicate amino acid identity with the reported Assiut *T. annulata* isolate (WWZ83999). Amino acid variations were observed in several positions in the reference amino acid sequences of *T. annulata* isolates (Sharkia and Qulyubia) and the established Assiut *T. annulata* isolate (WWZ84000), compared to the obtained Assiut *T. annulata* isolate (WWZ83999).

DISCUSSION

T. annulata infection is a tick-borne disease and is considered one of the most economically important diseases of cattle in Egypt (Anter *et al.,* 2019). In the present study, the noted clinical findings like fever, swelling of peripheral lymph nodes, diarrhea, respiratory symptoms (nasal discharge and cough), tick infestation, and corneal opacity in examined cattle with theileriosis were analogous to those found in prior studies conducted by Sharifiyazdi *et al.* (2012); Jassim *et al.* (2021) and Albayati *et al.* (2023). By employing blood film analysis, the prevalence of *Theileria* was estimated at 18% (9/50), whereas *30-kDa* gene-based PCR revealed a higher prevalence of 42% (21/50). This variation in outcomes can be attributed to the ability of PCR to detect minimal parasite loads that may be undetected by traditional blood film analysis. Some negative blood films were positive for two *T. annulata* primer genes, indicating the superiority of PCR over the commonly used conventional method, and that PCR has a higher sensitivity and specificity than blood film (Anter *et al.,* 2019). Variations in sample size, sampling duration, ecological agriculture, environment, immune condition of the host, tick frequency, breed, and differences in handling and sanitation procedures may contribute to the variations observed in the prevalence rates of *Theileria* across prior investigations (Abdel-Rady *et al.,* 2010; Abd Ellah and Al-Hosary, 2011; Anter *et al.,* 2019 and Zaitoun *et al.,* 2019).

In our study, the prevalence of positive cases found by the *Cyto B* gene was 20% (10/50). The lower distribution of the *Cyto B* gene of *T. annulata* might be explained by the fact that the *Cyto B* gene has increased copy number, rapid evolution, and a high mutation rate (Edith *et al.,* 2018). According to our findings, all cases that tested positive for blood film also tested positive for two *T. annulata* primer genes.

The present study described the identification and characterization of the *Cyto B* gene of *T. annulata* in the Assiut governorate. The phylogenetic evaluation of the PCR products of the *Cyto B* gene of the Egyptian isolates (Assiut) of *T. annulata* proved 96.16%-98.92% nucleotide identity with *T. annulata* isolates of different governorates (Sharkia and Qulyubia) of Egypt, Sudan, Tunisia, Turkey, Iran, and India, beside the nearby cluster with the isolate of Sudan (accession number LC431533).

This result may be attributed to the large-scale legal and illegal importation of cattle from Sudan. In addition to the absence of natural barriers, the large import of cattle from Sudan and the spread of illegal trade by peoples in both countries all increase the chances of various pathogens being transmitted and present. The obtained amino acid result displayed 94.82%-96.73% amino acid identity with isolates of *T. annulata* from two different Egyptian governorates: Qulyubia and Sharkia. Sixteen mutation positions were noted between the two Assiut *T. annulata* isolates (WWZ83999 and WWZ84000), although both were obtained from the same region, and thirty mutation positions were reported between the recorded Assiut *T. annulata* isolate (WWZ83999) and other compared isolates. Mutations in multiple regions confer resistance to buparvaquone that increases treatment failure in theileriosis, because buparvaquone's action of competitively inhibiting ubiquinone (coenzyme Q) binding to *Cyto B*'s mitochondria, inhibits the electron transport chain and ultimately collapses the membrane potential at the two drug binding sites, Q01 (130–148) and Q02 (244–266), stopping pyrimidine biosynthesis and parasite respiration (Chatanga *et al.,* 2019 and Nehra *et al.,* 2024). It has been proposed that multiple changes that replace hydrophobic amino acids (proline and leucine) with hydrophilic amino acids (serine) reduce buparvaquone's affinity for *Cyto B* and provide resistance (Hacilarlioglu *et al.,* 2023 and Nehra *et al.,* 2024). The

Cyto B gene may have undergone spontaneous mutation due to several factors, including multicopy number, inefficient proofreading by mitochondrial DNA polymerase, and heightened genetic mutation of mitochondrial DNA brought on by the mitochondrial respiration chain's formation of hydroxyl radicals (Sharifi-yazdi *et al.,* 2012 and Nehra *et al.,* 2024). Based on the current investigation, certain of the risk factors, such as sex, age, and breed of cattle, were studied for their association with the *T. annulata* infection rate. Regarding sex susceptibility, female cattle under investigation had a higher rate of infection of *T. annulata* than male cattle. This may be attributed to the managemental variation, raising purpose, and different stress factors for female cattle than males. Besides, this could imply that females' higher hormone fluctuations and relatively weaker immune systems raise the risk of infection. Additionally, males housed in indoor feeding systems with restricted grazing are spared from tick infestation and decrease the infection rate of *T. annulata* (Al-Hosary *et al.,* 2018 and Parveen *et al.,* 2021). With respect to age susceptibility, the age group >2–5 years had a significantly higher rate of *T. annulata* infection than the 45 days–2 years age group. Some possible explanations for this finding include declining immunity with age, older animals getting infections multiple times in their lives, and younger animals having low tick infestation rates (Anter *et al.,* 2019 and Selim *et al.,* 2020). In the current study, there was no statistically significant difference in the prevalence of *T. annulata* infection between native and mixed breeds. However, mixed breeds were mathematically more likely to contract the disease than native breeds. Mixed breeds may have had higher numerical values due to their weakened immune systems compared to native breeds.

CONCLUSION

The present study recorded the genetic characterization of *T. annulata* based on the *Cyto B* gene. The data obtained in the present study provided insight into several changes in the *Cyto B* gene of *T. annulata*. Further in vitro and in vivo research is required to determine the precise mechanism(s) underlying particular point mutations in the *Cyto B* gene, and how they contribute to the development of drug-resistant strains of *T. annulata* in Assiut governorate. The detection of resistance markers ought to be useful in assessing how well treatment plans are working to control theileriosis in Assiut governorate, Egypt.

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استكشاف التواجد المتكرر وتوصيف جين السيتوكروم ب في الثيليريا الحلقية فى الأبقار

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داء الثيليريا البقري الناتج عن الثيليريا الحلقية يؤدي إلى ارتفاع معدلات النفوق والخسائر الإقتصادية لصناعة الثروة الحيوانية في مصر. تم إستخدام خمسين رأس بقرى فى هذه الدراسة. تم جمع عينات الدم الكامل للتحليل المختبري. تم إستخدام مسحات الدم المصبوغة بالجيمسا للكشف عن عدوى الثيليريا. تم استخدام تفاعل البلمرة المتسلسل لتقييم مختلف الجينات المستهدفة مثل *30-kDa* و *Cyto B* من الثيليريا الحلقية . تم اختبار تسع عينات (١٨٪) إيجابية لبيروبلازم الثيليريا عن طريق الفحص المجهري للمسحات الدموية. تم اختبار واحد وعشرون (٤٢٪) من العينات التي تم تحليلها إيجابية جزيئيًا بناءً على جين *30-kDa* ، بينما كانت ١٠ عينات (٢٠٪) إيجابية بناءً على جين *Cyto B*. أجرينا في دراستنا تسلسل الحمض النووي والتحليل التطوري للثيلريا الحلقية بإستخدام جين *Cyto B*. أظهر التحليل التطوري لمنتجات تفاعل البلمرة المتسلسل للسلالة المصرية من الثيليريا الحلقية (أسيوط) هوية نيوكليوتيدية تتراوح من ٩٦,١٦٪ إلى ٩٨,٩٢٪ مع سلالات الثيليريا الحلقية من مختلف المحافظات (الشرقية والقليوبية) في مصر, السودان, تونس, تركيا, إيران والهند. ووجد أن العزلات التي تم الحصول عليها كانت متقاربة مع عزلة من السودان (رقم الوصول LC431533). حددنا ثلاثين طفرة نقطية في تسلسل الأحماض الأمينية. كان هناك تباين كبير (P <٠,٠٥ و P <٠,٠١) بين عمر وجنس الأبقار المختبرة على التوالي ونسب الإصابة بالثيلريا الحلقية. تشير البيانات التي تم الحصول عليها من دراستنا في توصيف جين *Cyto B* للثيليريا الحلقية في محافظة أسيوط إلى أنه يمكن استخدام جين *Cyto B* كعلامة وراثية لتحديد العزلات المقاومة للثيليريا الحلقية.