Original Article

Molecular Characterization and Phylogenetic Analysis of Pathogenic *Theileria* spp. Isolated from Cattle and Sheep based on Cytochrome *b* Gene in Iran

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Abstract

The present study investigated the phylogenetic relationship based on cytochrome *b* gene sequences among pathogenic *Theileria* species (spp.) in Iran, including *Theileria annulata* and *Theileria lestoquardi*, along with other data available in GenBank. A total of 136 (cattle) and 80 (sheep) blood samples suspected of piroplasm infection were obtained from six different provinces of Iran. Both microscopic and molecular methods using species-specific primers were used for screening *T. annulata* and *T. lestoquardi* positive samples. Finally, the partial cytochrome *b* gene of 30 *T. annulata* and 5 *T. lestoquardi* were amplified, sequenced, and deposited in GenBank. The results indicated that there were 12 different genotypes among *T. annulata* isolates, while only one genotype was observed among *T. lestoquardi* isolates. *T. lestoquardi* infection in cattle was detected in one sample, and no *T. annulata* and *T. lestoquardi* coinfection were detected in sheep and cattle. In the phylogenetic tree, different *Theileria* spp. were placed in separate clades, and the reliability of depicted tree and monophyly of *T. annulata* and *T. lestoquardi* ingroups were supported by the bootstrap value of 94% which significantly indicated that these two species evolved from a common ancestor. The tree also showed that these two pathogenic spp. shared a more recent common ancestor, compared to another species of *Theileria* parasites. To the best of our knowledge, this study is the first phylogenic analysis of pathogenic *Theileria* spp. in Iran based on the cytochrome *b* gene sequences. In addition, the first *T. lestoquardi* cytochrome *b* gene was sequenced and deposited in GenBank.

Keywords: Cytochrome *b*, Iran, Multiplex PCR, Phylogeny, Theileria

Caractérisation Moléculaire et Analyse Phylogénétique de *Theileria* spp. Isolé des Bovins et des Moutons sur la base du Gène du Cytochrome *b* en Iran

Résumé: La présente étude a examiné la relation phylogénétique basée sur les séquences du gène du cytochrome *b* parmi les espèces pathogènes de *Theileria* (spp.) en Iran, y compris *Theileria annulata* et *Theileria lestoquardi*, ainsi que d'autres données disponibles dans la GenBank. Les échantillons de sang de 136 bovins et 80 moutons suspects d'infection par le piroplasme ont été prélevés dans six provinces différentes d'Iran. Des méthodes microscopiques et moléculaires utilisant des amorces spécifiques à l'espèce ont été utilisées pour cibler des échantillons positifs pour *T. annulata* et *T. lestoquardi*. Enfin, le gène partiel du cytochrome *b* de 30 *T. annulata* et 5 *T. lestoquardi* ont été amplifiés, séquencés et déposés dans la GenBank. Les résultats ont indiqué qu'il y avait 12 génotypes différents parmi les isolats de *T. annulata*, alors qu'un seul génotype a été observé parmi les isolats de *T. lestoquardi*. Une infection à *T. lestoquardi* chez les bovins a été détectée dans un échantillon, et aucune co-infection à *T. annulata* et *T. lestoquardi* n'a été détectée chez les moutons et les bovins.
Introduction

Theileriosis is a tick-borne disease of ruminants caused by apicomplexan parasites belonging to the genus *Theileria*. The disease is transmitted by ixodid ticks and distributed in endemic regions from tropical and subtropical countries, including Iran (Hashemi-Fesharki, 1988). The life cycle of *Theileria* is complex and involves asexual and sexual replications in both vertebrate and invertebrate hosts. *Theileria* parasites replicate asexually in three different stages (sporogony in the tick vector, as well as schizogony and merogony in bovine host cells). Sexual reproduction of the parasite occurs within the intestinal lumen of the tick vector (Conrad et al., 1985; Sivakumar et al., 2014). Despite several *Theileria* spp. which infect ruminants in the endemic area, only two *Theileria* spp. are highly pathogenic in Iran, including *T. annulata* and *T. lestoquardi* that infect large and small ruminants, respectively (Hooshmand-Rad and Hawa, 1973; Hashemi-Fesharki, 1988). However, there is also a report of cattle being infected with *T. lestoquardi* (Namavari et al., 2015). Although indigenous cattle are resistant to tropical theileriosis, exotic and crossbred cattle are very susceptible to disease; moreover, the mortality rate can reach up to 40%-60% if untreated (Habibi, 2012).

*T. lestoquardi* (formerly *T. hirci*) is the most virulent species in sheep and goats, followed by a mortality rate up to 90% (Hooshmand-Rad and Hawa, 1973). These high rates of mortality demonstrate the economic impact of theileriosis in the livestock industry.

Traditionally, *Theileria* parasites are diagnosed by the microscopic inspection of Giemsa stained blood smears. However, differentiation of some *Theileria* parasites, such as *T. annulata* and *T. lestoquardi*, is difficult using this method. Different types of molecular markers have been used for diagnostic and phylogenetic studies previously, some of them are 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), sporozoite *T. lestoquardi* antigen-1 (Slag-1) (Skilton et al., 2000), cytochrome *b* gene (*Cytb*) (Sharifiyazdi et al., 2012; Mhadhbi et al., 2015), and rRNA internal transcribed spacers (ITS1 and 2) (Aktas et al., 2007). Among these molecular markers, the *Cytb* gene, due to its sequence variability, is commonly used as a region of mitochondrial DNA for determining phylogenetic relationships between organisms within families and genera levels (Castresana, 2001). Additionally, the role of mutation in *Cytb* ubiquinol/ubiquinone binding sites (Qo1, Qo2) associated with drug resistance has been shown in some studies (Sharifiyazdi et al., 2012; Mhadhbi et al., 2015). Moreover, polymorphism of the *Cytb* gene of *T. annulata* has been studied previously, and the utility of this genetic marker has been proposed as a tool to discriminate between different *T. annulata* genotypes (Mhadhbi et al., 2015). Theileriosis is endemic in Iran due to two highly pathogenic *Theileria* spp.; accordingly, different genotypes are required to be sequenced and identified based on cytochrome *b* gene sequences in order to conduct phylogenetic analysis.
and drug resistance studies. This study aimed at setting up a new multiplex polymerase chain reaction (mPCR) method to simultaneously detect the pathogenic *Theileria* spp. Additionally, sequenced cytochrome *b* gene sequences derived from two pathogenic *T. annulata* and *T. lestoquardi* from different areas in Iran were used for molecular phylogenetic analysis based on both nucleotide and amino acid sequences of the *Cytb* gene.

**Material and Methods**

**Blood Samples.** A total of 216 EDTA whole blood samples suspected to piroplasmosis were obtained from cattle (n=136), sheep (n=69), and goats (n=11) of six different provinces in Iran, including, East Azerbaijan, Khorasan Razavi, Fars, Khuzestan, Alborz, and Qazvin during 2017-2019. Furthermore, the *T. annulata* and *T. lestoquardi* cell lines and Babesia sp. positive blood were obtained from the Department of Parasitic Vaccines Research and Production, Razi Vaccine and Serum Research Institute and were used as positive controls in all PCR reactions.

**Screening for Piroplasms.** Initially, all the samples were screened for the presence of piroplasms by a microscopic examination of prepared (Barcia, 2007) thin blood smears using the mPCR method for simultaneous detection and differentiation of *T. annulata* and *T. lestoquardi* from other piroplasms.

**DNA Extraction.** The phenol-chloroform method was used for genomic DNA extraction from blood and cell line samples. Following that, the extracted DNAs were kept at -20°C till further use in PCR (Sambrook, 1989).

**Primer Design.** This study designed four sets of primers and utilized them in the PCR and mPCR. Species-specific Tams and Slag primers were also used for specific detection of *T. annulata* and *T. lestoquardi*, respectively. Furthermore, a common 18S rRNA primer pair was also designed to increase the specificity of mPCR. It should be noted that the common 18S rRNA primers produce 256 and 266 bp fragment lengths on *Theileria* and Babesia positive specimens, respectively. Finally, a pair of primers (TcytbF, R) was used to amplify the partial sequence of cytochrome *b* gene for two above-mentioned *Theileria* spp. All the primers were designed using Gene Runner and Oligo 7 software and checked for complementarity and primer dimers. The specificity of primers was checked by the Basic Local Alignment Search Toll (BLAST) of the national center for biotechnology information (NCBI) before primer synthesis. Table 1 summarizes some characteristics of the primers.

<table>
<thead>
<tr>
<th>Primer sets</th>
<th>Accession number</th>
<th>Length and position</th>
<th>Sequence (5' to 3')</th>
<th>PCR product(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18SF</td>
<td>KF429795</td>
<td>17nt(1151-1167)</td>
<td>TAATTTGACTCAACACCG</td>
<td>256-266</td>
</tr>
<tr>
<td>18SR</td>
<td></td>
<td>20nt(1416-1439)</td>
<td>ATCACAGACCTGTATTGCC</td>
<td></td>
</tr>
<tr>
<td>TamsF</td>
<td>Z48739</td>
<td>23nt(407-429)</td>
<td>GAGACAAGGAATATTCTGAGCC</td>
<td></td>
</tr>
<tr>
<td>TamsR</td>
<td></td>
<td>25nt(1003-1029)</td>
<td>TTAAGTGGCATATAATGACTTAAGC</td>
<td></td>
</tr>
<tr>
<td>SlagF</td>
<td>AF128526</td>
<td>18nt(138-155)</td>
<td>ATCAGCGGCAACACACC</td>
<td>597</td>
</tr>
<tr>
<td>SlagR</td>
<td></td>
<td>19nt(537-556)</td>
<td>TTCCTGTCATGAAACCC</td>
<td>400</td>
</tr>
<tr>
<td>TcytbF</td>
<td>M63015</td>
<td>20nt(118-137)</td>
<td>GTGCCAGCATATAATGACTTAAGC</td>
<td>983</td>
</tr>
<tr>
<td>TcytbR</td>
<td></td>
<td>20nt(1100-1081)</td>
<td>ATCAGCGGCAACACACC</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Characteristics of the PCR primers used in this study.
PCR and mPCR. Single PCR was carried out using Tams and Slag primers in a final volume of 20 μl, which included 10μl of master mix (Yekta Tajhiz, IRAN) 2X, 7 μl of DEPC water, 1μl of each primer (10 pmol/ μl), and 1μl of DNA template. The PCR conditions were performed as follows: Initial denaturation for 3 min at 95°C, followed by 33 cycles of denaturation (10S at 95°C), annealing (40S at 54°C), and extension (40S at 72°C), followed by final extension for 5 min at 72°C. In addition, the PCR was performed using a Gradient Palm-Cycler™ (Corbett Research, Sydney, Australia), and 10 μl of each PCR product was electrophoresed on 1.5% agarose gel contained GelRed™ (Biotium, USA) and visualized under UV light. The mPCR was carried out in final volume of 25 μl containing 11 μl of master mix 2X, 7.5 μl of nuclease-free DEPC water, 0.5μl (10 pmol/μl) of each Slag F/R and 18S F/R, 1.5 μl (10 pmol/μl) of Tams F/R primers, and 1.5 μl of template DNA. The mPCR conditions were performed as described in the case of single PCR.

Cytb Gene Amplification and Sequencing. T. annulata and T. lestoquardi positive samples obtained from mPCR reaction of different specimens were used for the amplification of partial Cytb gene sequences of two aforementioned pathogenic Theileria spp. Each reaction was performed as the same condition of single PCR that mentioned above, except for annealing (45S at 61°C) and final volume of 50 μl containing 25 μl of master mix 2X, 17.5 μl of nuclease-free DEPC water, 2.5 μl of each Tcytb F/R primers (10 pmol/μl), and 2.5 μl of template DNA. The expected 983 bp amplicons of the Cytb gene were analyzed by electrophoresis on 1.5% agarose gel. Afterward, the amplicons were purified using Gel extraction and PCR clean-up kit (Roche, USA) according to the manufacturer’s instruction. Subsequently, the purified PCR products along with forward and reverse primers were sent for both directional DNA sequencing by the Sanger method on an ABI 3730 sequencer (Bioneer, Daejeon, South Korea).

Sequence Assembly, Alignment, and Analysis. The consensus partial Cytb gene nucleotide and predicted amino acid sequences were generated by editing and alignment of their forward and reverse reads using Mega 7 software; moreover, the similarity of sequences was compared using the BLAST program (NCBI). Multiple alignments were performed using the Clustal W program of Mega 7 software, and the resulted sequences were submitted to the NCBI GenBank database. The phylogenetic tree based on the Cytb gene was constructed with the Neighbor-Joining method using Mega 7 software (Kumar et al., 2016).

Results

Microscopic Examination of Blood Films. The presence of piroplasms was assessed by the observation of 50 microscopic fields under a light microscope. Totally, 216 smears were examined, 155 of which were positive for piroplasms (Figure 1 and Table 2).

![Figure 1. Piroplasm of Theileria annulata in red blood cells](image-url)
Single and Multiplex PCR Optimization. The expected PCR products corresponding to Tams (597 bp) and Slag (400 bp) genes were separately amplified using *T. annulata* and *T. lestoquardi* DNA derived from available schizont infected cell lines as positive controls in the PCR reaction (Figure 2a). Furthermore, the 983 bp PCR product resultant to the partial Cytb gene sequences of *T. annulata* and *T. lestoquardi* was successfully amplified (Figure 2b). The optimization of mPCR was successfully performed using species-specific (Tams and Slag) primers along with a common 18S rRNA primer as well as DNAs of *T. annulata*, *T. lestoquardi*, and Babesia sp. as positive controls (Figure 3).

### Table 2. Results of microscopy and multiplex PCR screening

<table>
<thead>
<tr>
<th>Method</th>
<th>Cattle</th>
<th>Sheep and Goat</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Sample Examinned</td>
<td>136</td>
<td>80</td>
<td>216</td>
</tr>
<tr>
<td>Microscopy</td>
<td>+98</td>
<td>+57</td>
<td>+155</td>
</tr>
<tr>
<td></td>
<td>-38</td>
<td>-23</td>
<td>-61</td>
</tr>
<tr>
<td></td>
<td>T. annulata</td>
<td>104</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>T. lestoquardi</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Other piroplasms</td>
<td>10</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>Negative</td>
<td>25</td>
<td>13</td>
<td>38</td>
</tr>
</tbody>
</table>

mPCR Screening. Out of 216 examined samples, 104, 10, and 64 positive samples were found for *T. annulata*, *T. lestoquardi*, and other piroplasms spp., respectively. Neither *T. annulata* and *T. lestoquardi* coinfection nor sheep and goat *T. annulata* infection was detected. More details of microscopy and mPCR screening results are described in Table 2.

Cytochrome b Gene Amplification, Sequencing, and Analysis. The partial 983bp fragment of the Cytb gene was subjected to sequence analysis. The fragments of the mitochondrial Cytb gene were amplified and sequenced from 30 *T. annulata* isolates (from Alborz, Qazvin, Fars, Khuzestan, East Azerbaijan, and Khorasan provinces along with *T. annulata* vaccine strain) and five *T. lestoquardi* isolates (from Qazvin, Fars, and Khuzestan provinces) using

![Figure 2](image1.png)

**Figure 2.** Agarose gel electrophoresis of single PCR products. a) lane 1: Tams, lane 2: DNA size marker (100bp), lane 3: Slag, and lane 4: negative control. b) lane 1: negative control, lanes 2-3: *T. annulata* and *T. lestoquardi* partial Cytb gene, and lane 4: DNA size marker (1kb).

![Figure 3](image2.png)

**Figure 3.** Agarose gel electrophoresis of the multiplex PCR amplification products. Lane 1: DNA size marker 100 bp, lane 2: *T. annulata* (597 and 256 bp), lane 3: *T. lestoquardi* (400 and 256 bp), lane 4: Babesia sp. (266 bp), and lane 5: no DNA as a negative control.
PCR. After sequence editing and assembly, the sequences between 918 and 936 bp in size of the Cytb gene were obtained and submitted to GenBank for sequence deposition (Accession numbers: MN422301-5, MN432494-518, and MN481235-9).

A total of 918 positions were used for analysis in the final dataset, and among 30 sequences of the *T. annulata* Cytb gene, 12 different genotypes were detected after multiple alignments using previously registered *T. annulata* Cytb gene sequence in GenBank (accession number CR940346) as a reference sequence. The nucleotide transition substitutions were detected in 14 positions, and nonsynonymous substitutions were detected in five positions and indicated in bold in Figure 4. Among five *T. lestoquardi* Cytb sequences obtained from sheep, goats, and cattle, only one genotype was detected. The phylogenetic tree was constructed by the Neighbor-Joining method using partial Cytb gene of *T. annulata* and *T. lestoquardi* sequences obtained in this study along with the Cytb sequences of *T. annulata*, *T. parva* (AB499089, NC_011005), *T. orientalis* (AB499090), and *Theileria* spp. Sri Lanka and *Babesia bovis* (GQ214235) available in GenBank (Figure 5). As can be observed in Figure 5, different *Theileria* spp. were grouped in separate clades supported by bootstrap values higher than 90%. The studied *T. annulata* isolates were divided into three subclades of A, B, and C. In total, 10 out of 12 different *T. annulata* Cytb genotypes obtained in this study along with *T. annulata* from other countries (Turkey, India, and Sudan) were grouped in subclade A, and two other genotypes (Fars and Azerbaijan genotypes) were grouped in subclades B and C, respectively, which were close to African isolates (Figure 5).

Figure 4. Cytochrome b sequence diversity among Iranian isolates, compared to *T. annulata* strain Ankara (CR940346) as a reference sequence. Totally, 12 different genotypes were characterized from 30 total *T. annulata* samples collected from cattle with theileriosis. Furthermore, 14 nucleotide substitutions were discovered throughout the 918 bp fragment of the Cytb gene, five of which were nonsynonymous (gray shading).

**Abbreviations:**

n: number of sequences with same genotypes
GT: genotype number
Figure 5. Phylogenetic tree based on partial Cytb gene sequences of *Theileria* spp. using the Neighbor-Joining method in MEGA7. Black triangles denote Iranian *T. annulata* (GT1-GT12) and *T. lestoquardi*. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches and those with bootstrap values lower than 90% were eliminated. There were a total of 879 positions in the final dataset. The analysis involved 49 Cytb nucleotide sequences.

Table 3. Nucleotide and amino acid Cytb sequence identity scores (%) shared within and among *T. annulata*, *T. lestoquardi*, and *Theileria* spp. Sri Lanka based on partial (879 bp) Cytb gene sequences

<table>
<thead>
<tr>
<th></th>
<th>Nucleotide sequences</th>
<th>Amino acid sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>T. annulata</em></td>
<td><em>T. lestoquardi</em></td>
</tr>
<tr>
<td><em>T. annulata</em></td>
<td>98.6-100</td>
<td>97.3-100</td>
</tr>
<tr>
<td><em>T. lestoquardi</em></td>
<td>88.6-89.1</td>
<td>87.1-88.8</td>
</tr>
<tr>
<td><em>Theileria</em> sp. Sri Lanka</td>
<td>87.5-87.9</td>
<td>82.5-83.5</td>
</tr>
</tbody>
</table>
Discussion

*T. annulata* and *T. lestoquardi* are the main pathogenic *Theileria* spp. in large and small ruminants in Iran. Although case history, clinical signs, as well as lesions and microscopic examination of thin blood smears can lead to the diagnosis of infection with *Theileria* parasites, there are some difficulties associated with microscopic detection, including morphological similarity of piroplasms, false-negative diagnosis due to the low number of the parasite (particularly in carrier animals), and difficulty in the detection of mixed infections. Therefore, a comprehensive diagnosis and differentiation of various species of piroplasms could be achieved by sensitive and specific diagnostic assays, such as PCR. Although *T. lestoquardi* is the causative agent of malignant theileriosis in sheep and goats, there are some reports of *T. lestoquardi* infection in cattle (Namavari et al., 2015; Jalali et al., 2016). In the present study, an mPCR was designed for simultaneous detection and differentiation of *T. annulata* and *T. lestoquardi* from other piroplasms. Moreover, the species-specific Tams, Slag primers, and common 18S rRNA primers were used to increase the specificity of the mPCR reaction. As indicated in Figure 3, the mPCR method has been successfully utilized for the differentiation of these two pathogenic *Theileria* spp.

In total, among 216 examined blood samples, 155 (71.7%) and 178 (82.5%) samples were positive for piroplasms using microscopy and mPCR method, respectively (Table 2). Only an experimentally infection of sheep and goats with *T. annulata* has already been reported (Brown et al., 1998), and no natural infection by this parasite was reported in small ruminants to date. In the present study, no *T. annulata* and *T. lestoquardi* coinfection was noted in any sample; moreover, *T. annulata* infection was not detected in sheep and goats; however, *T. lestoquardi* infection was detected in cattle from Khuzestan province (Table 2). Since all of *T. lestoquardi* have been previously reported in cattle from Khuzestan province (Namavari et al., 2015; Jalali et al., 2016), cross-infection of this parasite among sheep, goats, and cattle should be considered in the control and prevention of the disease, particularly in this region.

Over the past years, several molecular markers, such as 18S rRNA, ITS1, ITS2, and *Cytb* genes have been used to determine the phylogenetic relationship among the piroplasms population (Aktas et al., 2007; Habibi, 2012; Tian et al., 2013). The mitochondrial cytochrome *b* gene is commonly employed for analyzing phylogenetic relationships between organisms within families and genera levels (Castresana, 2001).

In the present study, the cytochrome *b* gene sequence was used for the construction of the phylogenetic tree among different species of *Theileria* parasites. It should be noted that the reliability of the phylogenetic tree is measured by a bootstrap value, whereas bootstrap values higher than 90% are considered highly significant and values between 70% and 89% are slightly significant; moreover, the ones below 70% have less value and consistency (Shaffer et al., 1997). As indicated in Figure 5, different *Theileria* spp. were placed in separate clades, and the reliability of depicted tree and monophyly of *T. annulata* and *T. lestoquardi* ingroups were supported by a bootstrap value of 94%, which significantly indicated that these two species evolved from a common ancestor.

Based on the drawn phylogenetic tree, the main *T. annulata* clade was divided into three subclades of A, B, and C, and the most of Iranian studied isolates were fitted along with *T. annulata* *Cytb* gene sequences from Turkey, India, and Sudan in subclade A. However, the *T. annulata* sequences from Africa were classified in the subclades of B and C, except for the sequences from Fars and Azerbaijan provinces, both of which were placed into the subclades of B and C, respectively. Molecular phylogenetic studies based on the 18S rRNA sequence clearly indicated that *T. lestoquardi* was more closely related to *T. annulata*, compared to *T. parva* (Katzer et al., 1998).

As indicated in Figure 5, similar results were obtained for the differentiation of two species by the *Cytb* gene
sequences in this study. As indicated in Table 3, the degrees of similarities between T. annulata Cytb gene sequences were determined at 98.6% to 100%; however, the similarities between T. annulata and T. lestoquardi isolates were estimated at 88.6% to 89.1%. These values show clearly that the similarity between T. annulata isolates is 10% more than that between two T. annulata and T. lestoquardi species. Nonetheless, the calculated similarity between the new species (Theileria spp. Sri Lanka) (Sivakumar et al., 2019) and T. annulata was obtained at 87%-87.5%, and similarity between new Theileria spp. and T. lestoquardi was determined to be 87.5%-87.9%. These findings show clearly that the similarity value between two Theileria spp., namely T. annulata and T. lestoquardi, is higher than that between the new Theileria spp. or T. annulata or T. lestoquardi. In other words, the results indicate that the T. lestoquardi spp. is more close to T. annulata, compared to Theileria spp. Sri Lanka. Additionally, the phylogenetic tree shows that T. annulata and T. lestoquardi share a more recent common ancestor, compared to the Sri Lankan species of Theileria.

Although most of the phylogenetic analyses have used the 18S rRNA gene in the studies of Theileria, there is a vast region of conserved sequences that do not always differentiate among closely related species (Li et al., 2011); however, our results indicated that the Cytb sequence could distinguish different Theileria spp. in distinct clades effectively (Figure 5).

To the best of our knowledge, this study is the first phylogenetic analysis of pathogenic Theileria spp. in Iran based on cytochrome b gene sequences. In addition, the first T. lestoquardi Cytb gene was sequenced and deposited in GenBank (MN481235-9).

Authors’ Contribution

Study concept and design: M. T., Gh. H., K. E. and A. A.
Acquisition of data: A. A. and B. E.
Analysis and interpretation of data: A. A. and Gh. H.

Drafting of the manuscript: A. A., B. E. and Gh. H.
Critical revision of the manuscript for important intellectual content: M. T., Gh. H. and A. A.
Statistical analysis: A. A.
Administrative, technical, and material support: M. T., Gh. H. and A. A.

Ethics

The authors hereby declare that all ethical standards have been respected in the preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References


