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Innovative semi-quantitative multiplex single primer pair PCR method for simultaneous detecting some obligate intracellular piroplasms

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Abstract

The causative agents of piroplasmosis, *Babesia* spp. and *Theileria* spp. infect ruminants in the livestock industry with high economical loses worldwide. The early diagnosis of the disease can help manage the successful treatment. Giemsa staining is a common method used worldwide for diagnosis of piroplasmosis which unfortunately can be accompanied with some serious problems like suitable blood smears and quality of staining and needs high experience. Serological methods like ELISA or IFAT was also used in some laboratories. The molecular methods based on DNA analysis preferably used in many laboratories are accurate and reliable. The aim of the present study was to introduce a simple and innovative semi-quantitative multiplex PCR method which can be helpful for simultaneously detection of *Babesia spp.* and *Theileria spp*. For this aim, we used primers derived from 18S rRNA genes which can amplify simultaneously the DNA from hosts (ruminants and ticks) and also from *Babesia* spp., and *Theileria* spp with different easily distinguishable PCR products. Our results showed that, as was expected, the different length of ampilicons generated from *Theileria spp*. and *Babesia spp*. make it possible to differentiate these parasites from each other on the same agarose gel. Interestingly, since the same primer pair can also amplify the 18S rRNA from host cells with larger amplicon compared to the parasites, this property can be used for semi-quantitative analysis of the parasite burden in the samples. It is necessary to mention that the introduced primer pair (VetUTPiro) can be used for differential diagnosis of babesiosis and theileriosis as well as for semi-quantitative multiplex PCR analysis of the parasite burden consequently for controlled therapy management.

Keywords: *Theileria spp., Babesia spp*., Semi-quantitative multiplex PCR, Therapy management, Sheep

Introduction

Piroplasmosis is a significant hemoparasitic disease that primarily affects livestock, particularly ruminants. This disease is predominantly caused by protozoan parasites from the genera *Babesia* and *Theileria*. The economic impact on the livestock industry is substantial, leading to considerable global losses due to reduced productivity, including weight loss, decreased milk yield,

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increased susceptibility to secondary infections, and higher mortality rates among infected animals. The high costs associated with veterinary care and management further exacerbate this economic burden (Shayan et al, 2007; Hossein et al, 2024; Modirrousta et al, 2019).

The widespread prevalence of piroplasmosis poses serious implications for animal welfare and food security, particularly in regions with heavy tick infestations. In some areas, it has been reported that up to 20% of animals may carry *Babesia* or *Theileria*, often remaining asymptomatic (Onyiche et al, 2019; Onyiche, 2020; Jouglin, 2025). Therefore, early and accurate diagnosis is crucial for effective management of the disease. Traditional diagnostic methods, such as blood smears, frequently yield false negatives, particularly in carrier animals. This limitation highlights the necessity for more sensitive molecular techniques, such as polymerase chain reaction (PCR), which can detect low parasitic loads and improve early diagnosis and treatment outcomes (Tian et al, 2015; El-Ashker, 2015; Shayan et al, 2007). Accurate and early diagnosis of piroplasmosis is essential for effective treatment and management. Traditional diagnostic techniques, including Giemsa-stained blood smears, have been the standard practice in clinical settings; however, they present significant limitations. One major drawback is that these methods require highly trained personnel to correctly identify the parasites under a microscope, because some *Theileria sp.* and *Babesia sp.* resemble each other in stained blood smears. Additionally, the quality of blood smears and variability in staining can lead to inconsistent results, potentially resulting in misdiagnosis or delayed treatment, which can worsen the impact of the disease on livestock (Malekifard et al, 2014; Riaz et al, 2023).

Moreover, Giemsa staining exhibits low sensitivity, particularly in cases with low parasitic loads, and may fail to detect subclinical infections. This limitation is especially problematic in regions where the disease is endemic, as animals may serve as asymptomatic carriers. Misdiagnosis can result in unnecessary treatments, escalating costs and potentially contributing to drug resistance. Therefore, there is an increasing demand for more accurate and sensitive diagnostic techniques (Mendoza et al, 2024; Rosenblatt et al, 2009).

Serological techniques, such as enzyme-linked immunosorbent assay (ELISA) and the indirect fluorescent antibody test (IFAT), have emerged as valuable alternatives for diagnosing piroplasmosis. These methods detect antibodies against protozoan parasites like *Babesia* and *Theileria*, offering insights into the immune response of infected animals. Recent studies underscore the utility of these serological tests; however, they often face challenges related to specificity and sensitivity when compared to molecular methods like PCR (Jongejan et al, 2024; Jaffer et al, 2010; Tirosh-Levy et al, 2020). PCR has gained prominence in piroplasmosis diagnosis due to its high accuracy and reliability. It is capable of detecting low parasitic loads in blood samples, making it especially useful during the early stages of infection when antibody responses may not yet be detectable. For example, a comparative study demonstrated that PCR could identify piroplasm DNA in horses that were serologically negative, indicating that molecular methods can uncover active infections that serological tests might miss (Jaffer et al, 2010).

While serological methods effectively detect past infections and assess herd immunity, their inability to differentiate between active and passive infections can lead to misdiagnosis. Thus, the integration of PCR with serological techniques is increasingly recommended to enhance diagnostic accuracy and facilitate timely intervention (Jaffer et al, 2010).

In this study, we present a novel semi-quantitative multiplex PCR method

designed for the simultaneous detection of *Babesia spp*. and *Theileria spp*. This innovative approach employs primers derived from the highly conserved 18S rRNA genes, allowing for the amplification of DNA from both the target pathogens and their hosts (ruminants and ticks). The generated PCR products exhibit distinct fragment lengths, which can be effectively analyzed through agarose gel electrophoresis. This dual amplification capability facilitates the differential diagnosis of babesiosis and theileriosis, enabling prompt and accurate identification of the causative agents.

Furthermore, considering the semi-quantitative multiplex nature of this PCR method, it holds significant promise for therapeutic management. It allows veterinarians to monitor the effectiveness of treatment regimens and adjust strategies as necessary based on the parasitic load in the host. The development of this method represents a critical advancement in the diagnostic tool for managing piroplasmosis, with potential implications for enhancing livestock health and productivity.

Materials and methods

Morphologic characterization of ticks

Hard ticks were collected from sheep in different places of Tehran province. All specimens were stored in tubes containing 70% ethanol. All ticks were identified morphologically based on identification keys described by Walker et al. 2003 and Estrada-Penna et al. 2017, using stereomicroscope. Different characteristic factors such as: the length of capitulum, Basis capituli, presence or absence of eye, the number of festoons, leg coloration, were noticed for identification of genus and the other characteristicss like cornua length, the form of cocxae in different legs, lateral groove, the kind of punctuation distribution were used for species identification.

Isolation of salivary gland of ticks

In order to remove the tick's salivary glands for DNA extraction, ticks were first placed on a glass slide from the dorsal surface and their scutum was cut from the posterior margin and set aside.

Then the salivary glands of the ticks located on both sides of the tick's body under the mouthparts were taken out by forceps and kept in 70% ethanol, until used.

DNA extraction

Hyalomma anatolicum anatolicum, *Rhipicephalus turanicus* and blood from sheep infected with *Theileria spp.* were provided by National Parasitology Museum of Iran (Faculty of Veterinary Medicine, University of Tehran). DNA was extracted from the salivary glands of mentioned ticks and blood of sheep infected with *Theileria spp.* using DNA isolation kit (MBST, Iran) according to the manufacturer's protocol. In brief, the salivary gland or 100 µl of blood were first lysed in 180 µl of lysis buffer, and the proteins were digested by adding 20 µl of proteinase K and incubating for 10 minutes at 55°C. Afterward, 360 µl of binding buffer was added, followed by a 10-minute incubation at 70°C. Then, 270 µl of 100% ethanol (Merck, Germany) was added to the mixture. The solution was vortexed and transferred to the MBST column, which was centrifuged, and then washed twice with 500 µl of washing buffer. Finally, DNA was extracted from the column using 70 µl of elution buffer.

PCR and nested PCR

DNA extracted from salivary glands of ticks and blood sample was amplified using primer pair (All sense 5` CACAGGGAGGTAGTGACAAG 3`, All antisense 5` AAGAATTTCACCTCTGACAG 3`) derived from 18S rRNA gene of *Babesia spp*. and *Theileria spp*., which can simultaneously amplify the corresponding DNA region in both piroplasms. This primer pair cannot amplify the DNA

derived from ticks and ruminants (Shayan and Rahbari, 2005).

The same DNA probes were also amplified using primer pair (VetUTPiro-Forward primer 5` TGCAGTTAAAAAGCTCGTAG 3` and VetUTPiro-Reverse primer 5` GAGCACTCTAATTTTCTCAAAGTA 3`) which can amplify simultaneously not only *Babesia spp*. and *Theileria spp*., but also can amplify DNA from ticks and ruminants with different PCR products in length, to be used for semi-quantitative multiplex PCR analysis. The PCR reaction mixture consisted of 20 µM of each primer, 1 µl of extracted DNA, 9.5 µl of double distilled water, and 12.5 µl of Taq PCR Master Mix (SinaClon), for a final volume of 25 µl. In all experiments, DNA from *Theileria*-infected blood was used as positive control and H_2O as negative control. The PCR was performed in Thermo cycler (MWG/Germany) under following conditions: initial denaturation of 5 minutes at 95°C, 38 cycles of 45 seconds at 94 °C (denaturation), 45 seconds at 52 °C (annealing), 45 seconds at 72 °C (extension) and final 10 minutes at 72 °C. This condition was used for each primer pair. The amplified DNA was then analyzed on agarose gel under UV condition using Gel-Documentation-system (Pouiesh Teb Adak, Theran, Iran).

Results

Identification of ticks

The morphometric characterization of ticks was performed using identification keys described above. Among hard Ticks collected from sheep and goat, two different genera including Rhipicephalus *turanicus* (Figure 1 A1) and *Hyalomma anatolicum* (Figue 1A2) were detected. The *Hyalomma anatolicum* was characterized according to the length of capitulum, the presence of typical oval and darkness colour parma cell, separation of paracentral festoons and the presence of pale rings in leg coloration (Figure 1 A2). *Rhipicephalus turanicus* was characterized according to having hexagonal base of capitulum and festoons, narrow Adanal plates, spiracle plate arias having sparse setae and broad spiracle plate tails. Figure 1B showed the extracted mentioned tick's salivary glands. In order to extraction of pure salivary glands, it was noticed that they were without contaminated materials from midgut as can be seen in Figure 1B.

The Figure of the tick's salivary glands which were taken out, is shown in Figure 1. Regarding our experience, it should be mentioned that the color of infected salivary glands to *Babesia* and *Theileria* piroplasms usually is brown, while in the uninfected salivary glands, it turns transparent milky color (our observations).

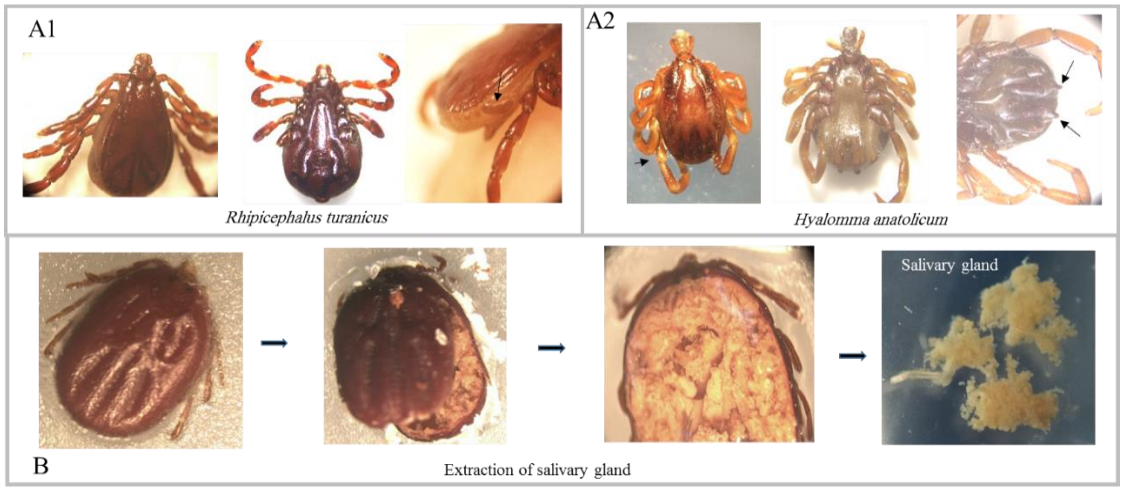

Figure 1: Upper figures are morphological characteristics of *Rhipicephalus turanicus* **and** *Hyalomma anatolicum* **and lower figures are the process of tick`s salivary glands extraction.**

DNA extraction from salivary gland and PCR

DNA was extracted from salivary glands of *Hyalomma anatolicum* and *Rhipicephalus turanicus* and amplified using common specific primer pair amplifying simultaneously *Babesia spp*. and *Theileria spp*. resulting in PCR product of approximately 426 bp for *Theileria spp*. and approximately 389 bp for *Babesia spp.* (Figure 2). Figure 2, shows that salivary glands of one *Hyalomma anatolicum* was negative (lane 1) and the second one was positive (lane 2) for *Theileria spp.* resulting PCR product of approximately 426 bp.. Figure 2 also showed the amplification of extracted DNA from salivary glands of *Rhipicephalus turanicus* resulting in PCR product of 389 bp in length, confirming infection with *Babesia spp*. (lane 3 and 4).

PCR using host-parasite common primer pair

Interestingly we could find a small region in 18S rRNA gene, which were found conserved from one site in homo sapiens, mammalians and ticks and from the other site in obligate intracellular protozoan parasites like *Theileria spp.* and *Babesia spp*. Figure 3 shows the analysis of DNA extracted from *Theileria* infected Blood samples using VetUTPiro primer pair by PCR. As it can be seen in Figure 3, lanes 2 and 3, two PCR products were visible. The PCR product with 157 bp in length belong to the host genomic DNA whereas the PCR products with 125-127 bp in length belong to the genomic DNA of *Theileria* species. The results to the amplification of regarding genomic DNA originated from *Babesia* and *Theileria* species with the mentioned primer pair can be confirmed in ticks infected with these piroplasms. Figure 4, lane 6 and 7 shows PCR product generated from host and parasite in ticks *Hyalomma anatolicum* and *Rhipicephalus turanicus*. Interestingly, lane 7 clearly shows the mix infection (*Babesia* and *Theileria*) in *Rhipicephalus turanicus,* since PCR product generated from *Babesia spp*. should be between 91-113 bp. Figure 4, lanes 4 and 5 shows PCR analysis with DNA sample extracted from uninfected salivary gland of *Hyalomma anatolicum* and *Rhipicephalus turanicus* respectively, in which only PCR product generated from salivery glands with 157 bp in length was visible. We believe that the ability to amplify the genomic DNA generated from host and parasite simultaneously can help to analyze the parasite burden while the treatment. For this aim, the ratio between intensity of PCR product of parasite to intensity of PCR product of host cell gives an index for parasite burden in the sample, which can be used for semi-quantitative multiplex analysis. Furthermore, the mentioned index can be also used for determination of drug efficacy.

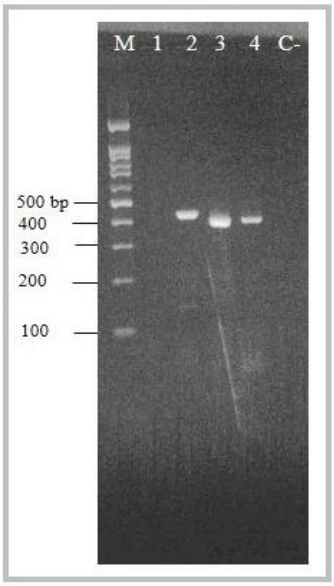

Figure 2: DNA was extracted from salivary glands of *Hyalomma anatolicum* **and** *Rhipicephalus turanicus* **and analyzed using common specific primer pair amplifying simultaneously** *Theileria* **and** *Babesia spp.* **Lane 1 and 2 (PCR analysis of DNA extracted from salivary glands of** *Hyalomma anatolicum***), Lane 3 and 4 (PCR analysis of DNA extracted from salivary glands of** *Rhipicephalus turanicus***). C- = negative control and M = 100 bp DNA marker.**

The VetUTPiro primers were checked by https://www.ncbi.nlm.nih.gov/guide/howto/design-pcr-primers program and could find that they can amplify also *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale* and

Plasmodium malariae generating PCR product of 198, 202, 207 and 236 bp respectively, which could be easily distinguished from the genomic DNA derived from human and other Mammalians (Table 1). It is also necessary to mention that the above introduced primers can also amplify *Toxoplasma gondii* producing PCR products of 86, 157 bp respectively. Interestingly, these primers cannot amplify genomic DNA generated from *Trypanosoma* or *Neospora* (Table 1).

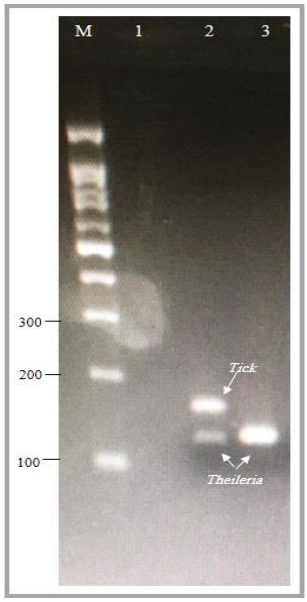

Figure 3: DNA was extracted from salivary glands of ticks, The DNA was amplified using VetUTPiro specific primer pair. Lane 2 was amplification of DNA from *Theileria* infected salivary gland using VetUTPiro primers. Lane 3: amplification of PCR product generated from DNA extracted from infected blood using the first primer pair shown in figure 2 with VetUTPiro primers (Lane 2) and Lane 1 was negative control and M = 100 bp DNA marker.

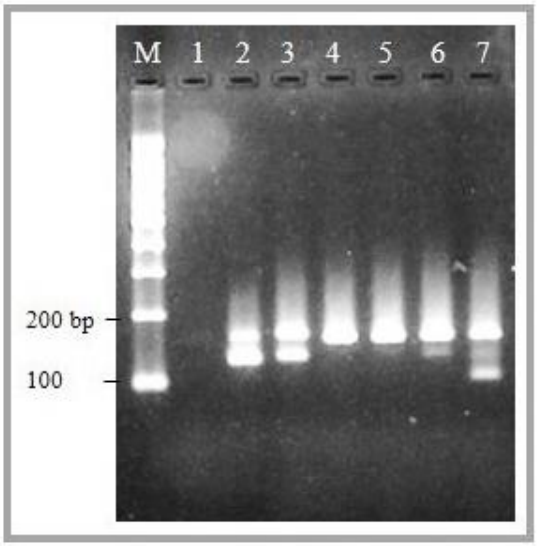

Figure 4: DNA was extracted from *Theileria* infected sheep blood, salivary gland of *Hyalomma anatolicum* and *Rhipicephalus turanicus* and analyzed using VetUTPiro specific primer pair amplifying simultaneously *Theileria* and *Babesia spp*. Lane 2 and 3 are PCR analysis of *Theileria* infected blood samples, Lane 4 and 5 were PCR analysis with DNA sample extracted from uninfected salivary gland of *Hyalomma anatolicum* and *Rhipicephalus turanicus* respectively, Lane 6 was PCR analysis with DNA sample extracted from salivary gland of *Theileria* infected *Hyalomma anatolicum*, Lane 7 was PCR analysis with DNA sample extracted from salivary gland of *Theileria/Babesia* mix infected *Rhipicephalus turanicus*. Lane 1 was negative control and M = 100 bp DNA marker (CinaClone, Iran).

Table 1: Alighnment of the genomic DNA of some hemoparasites and hosts with vetmedTUPiro-primers using https://www.ncbi.nlm.nih.gov/tools/primer-blast program

species	Accession no.	PCR product	species	Accession no.	PCR product
T. lestoquardi *T. ovis*	AF081135.1 AY533144.1	126	*H. anatolicum*, *H. asiaticus*, *Rh. sanguineous*, *Rh. turanicus*, *Rh. haemolyticus*, *Rh.Appendiculatus*, *Rh. bursa*, *D. andersoni*, *D. marginatus*, *D. niveus*, *D. nuttalli*, *I. ricinus*	JX051052.1, JX051026.1, L76342.1, JX987495.1, DQ839552.1, AF018653.1, AJ003816.1, L76340.1, Z74480.1, KR002680.1, KJ410657.1, GU074704.1	157
T. orientalis *T. annulata* *T. equi*	LC576821.1 KF429795.1 JQ390047.1	125			
T. parva *B. equi* (*T. equi*)	L02366.1 AB515315.1	127			
B. bovis *B. ovis*	KF928959.1 AY260178.1	91			
B. bigemina	JQ437261.1	95			
B. motasi	AY260180.1	96			
B. crassa	MK240324.1	102			
B. cabballi	XR_010957797.1	105	*Bos Taurus*, *Ovis aries*, *Equus asi*, *Equus caba*, *Camelus dr*, *Homo sapie*	DQ222453.1, KY129860.1, XR_006524340.1, XR_011434517.1, XR_010379165.1, XR_007090847.1	157
B. divergens *B. canis*	U16370.1 PP574310.1	113			
P. falciparum	CP101634.1	198			
P.vivax	LT635613.2	202			
P. ovale	MN515109.1	207			
P. malariae	XR_003751948.1	236			
Toxoplasma gondii	PQ894615.1 U03070.1	86 157			

Discussion

Among the hard Ticks collected from sheep and goat, two different genera including *Hyalomma anatolicum* and *Rhipicephalus turanicus* were detected based on characterization keys (Walker, 2003). *Hyalomma anatolicum* is an important three-host hard ticks which is distributed widely from North Africa to India and its more importance is for transmission of some hemoparasites (Walker, 2003). *Rhipicephalus turanicus* is also a three-host hard tick whose information about geographical distribution, ecology and genetic patterns and diversity is very contradictory and unreliable. So, its vectorial capacity is also under discussion. Therefore, its role as a vector of human pathogens, including *Rickettsia massiliae, R. conorii* and other microorganisms, needs confirmation based on molecular detection (Estrada-Peña et al, 2018).

It is worthy of mention that these tick species are distributed across various provinces of Iran, suggesting a high level of adaptability to the country`s diverse climatic and environmental conditions. Studies indicate that both tick species can be considered as potential vectors of hemoprotozoan such as *Babesia* and *Theileria*. In a study conducted by Abdallah et al, 2017. *Hyalomma anatolicum* was reported to be infected with protozoan parasites from both *Theileria* and *Babesia* genera, indicating the potential role of this tick species in the simultaneous transmission of these two pathogenic agents. Furthermore, findings from a study by Shayan et al, 2007, using PCR, confirmed infection in *Rhipicephalus turanicus* by *Babesia ovis*, highlighting the significant role of this species in the transmission of ovine babesiosis in Iran. In addition, a more recent study conducted in

2024 in the Xinjiang Autonomous Region of China identified *R. turanicus* and *H. anatolicum* among ticks collected from sheep and confirmed that they were infected with *Theileria* parasites.

Theileria and *Babesia* species play an important role in the animal and public health with economical losses. The early diagnosis of malignant piroplasmosis is of great importance for both disease control and controlling the economic losses worldwide. Shayan and Rahbari (2005), introduced an easy PCR method for simultaneous differential diagnosis of *Theileria* and *Babesia* infections on DNA extracted from stained blood smears. In that study, they showed that the amplification of the DNA generated from *Theileria* spp. using common primer pair by PCR resulting in PCR product of ca. 426-430 bp in length, whereas for *Babesia* genus the PCR product was 389-402 bp in length. Although the difference of PCR products between these two genera was approximately 30 bp, but on the 2% agarose gel was this difference distinguishable. Therefore, in lesser agarose gel concentration, the differentiation between both PCR products could be accompanied with some problems. To resolve this problem, a new innovative primer pair was designed, with which interestingly, the genomic DNA from *Theileria* or *Babesia spp*. as well as host (tick or mammalian) can be simultaneously amplified. Since the PCR products generated from genomic DNA of *Theileria, Babesia spp*. and host are 125-127 bp and 91-113 and 157 bp respectively, this extraordinary property of primers makes them useful for molecular semi-quantitative multiplex analysis and detection of mix-infections. Another method, in which the simultaneously detection of *Theileria* and *Babesia spp.* at genus and species level was previously described, was based on the species-specific probes linked to the sufficient membrane for detecting biotin marked PCR products by streptavidin-peroxidase in chemoluminescence (Gubbels et al, 1999; Ranjbar-Bahadori et al, 2012). This method is much expensive and time intensive and can be performed in special studies. For routine diagnosis, the mentioned method in the present study is recommended. Furthermore, since the introduced VetUTPiro-primers can amplify simultaneously the genomic DNA from parasite as well as host, the results achieved by PCR can be used for semi-quantitative multiplex analysis. This possibility can be useful for therapy management and additionally the mentioned method can be used for determination of the efficacy of the drugs. Interestingly, the above mentioned VetUTPiro-primers can also amplify simultaneously the genomic DNA generated from *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Toxoplasma gondii*. Data search in GenBank showed that for some small subunit of rRNA gene generate the mentioned primers 157 bp (accession no. U03070.1), whereas for some others 86 bp (accession no. PQ894615). We believe that the reason of this difference of 71 bp should be answered. Moreover, the genomic DNA derived from genomic DNA of other hemoparasites like *Trypanosoma spp* and *Neospora caninum* cannot be amplified. Since in the subtropical or tropical area, the obligate intracellular parasites can occur simultaneously, so the use of the primers can be performed for broad spectrum of parasite infection.

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Conflict of Interests

The authors declare no conflict of interest.

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روش نوآورانه نیمه کمی Multiplex Single Primer Pair PCR برای تشخیص همزمان بعضی از پیروپلاسم‌های داخل سلولی اجباری

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چکیده

گونه‌های بابزیا و تیلریا از اجرام پیروپلاسمی هستند که نشخوارکنندگان را در صنعت دامپروری آلوده کرده و خسارات اقتصادی بالایی را در سراسر جهان موجب می‌شوند. تشخیص زود هنگام بیماری می‌تواند به مدیریت موفقیت‌آمیز درمان کمک کند. رنگ‌آمیزی گیمسا روشی رایج در سراسر جهان برای تشخیص پیروپلاسموز است که متأسفانه می‌تواند با برخی مشکلات جدی مانند تهیه‌ی لام‌های خونی مناسب و کیفیت رنگ‌آمیزی همراه باشد و گذشته از آن، آنالیز گسترش‌های تهیه شده نیز نیاز به تجربه‌ی بالایی دارد. روش‌های سرولوژیکی مانند ELISA یا IFAT نیز در برخی آزمایشگاه‌ها مورد استفاده قرار می‌گیرند. همچنین روش‌های مولکولی که مبتنی بر آنالیز DNA بوده و دقیق و قابل اعتماد هستند نیز ترجیحاً در بسیاری از آزمایشگاه‌ها مورد استفاده قرار می‌گیرند. هدف از مطالعه حاضر معرفی یک روش ساده و نوآورانه به نام semi-quantitative multiplex PCR بود که بتواند برای تشخیص همزمان گونه‌های بابزیا و تیلریا مفید واقع شود. برای این منظور، از آغازگرهای مشتق شده از ژن‌های 18S rRNA استفاده گردید که می‌توانند همزمان DNA را از میزبان‌ها (نشخوارکنندگان و کنه‌ها) و همچنین از گونه‌های بابزیا و تیلریا طوری تکثیر کنند که محصولات PCR آن‌ها در اندازه با یکدیگر متفاوت و قابل تشخیص تفریقی باشند. نتایج مطالعه حاضر همان طور که انتظار می‌رفت، نشان داد که طول متفاوت محصولات PCR از گونه‌های تیلریا و بابزیا، تمایز این انگل‌ها را از یکدیگر بر روی ژل آگارز ممکن می‌سازد. جالب توجه این است که این آغازگرها ناحیه‌ای از ژن 18S rRNA از سلول‌های میزبان را نیز به صورت همزمان تکثیر می‌کنند و محصول PCR حاصله بزرگتر از آمپلیکون‌های انگل‌ها می‌باشد. از آن جایی که جفت آغازگر معرفی شده به صورت همزمان DNA میزبان و انگل را تکثیر می‌کند، این ویژگی می‌تواند برای تجزیه و تحلیل نیمه کمی بار انگلی در نمونه‌ها مورد استفاده قرار گیرد. بنابراین می‌توان تاکید کرد که جفت آغازگر معرفی شده (VetUTPiro) می‌تواند به صورت همزمان برای تشخیص تفریقی بابزیوز و تیلریوز و همچنین برای تجزیه و تحلیل نیمه کمی بار انگلی برای مدیریت قابل کنترل درمان مورد استفاده قرار گیرد.

کلمات کلیدی: جنس تیلریا، جنس بابزیا، پی‌سی‌آر چندگانه نیمه کمی، مدیریت درمان، گوسفند

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