Original Article

Detection of *Borrelia* DNA in *Ornithodoros tholozani* Ticks and Their Eggs

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Introduction: Spirochetes of *Borrelia* can be visualized directly in infected ticks by dark-field microscopy. Inoculation of in phosphate buffered saline (PBS) suspension of ground Argasid soft ticks to susceptible animals or allowing the ticks to feed on the same species followed by microscopic examination of the animals' blood have also been practiced. With the advent of molecular methods and introduction of various gene markers, *Borrelia persica* DNA was detected in *Ornithodoros tholozani* ticks by using several gene markers, but the data on transovarial transmission of *Borrelia* in this tick by these methods is very scarce. **Methods:** In this study we tried to detect *Borrelia* in field collected *O. tholozani* ticks by allowing them to feed on guinea pigs and then to examine the animals' blood for spirochetes by microscopy. We also used two PCR methods targeting highly repetitive regions of *rrs* gene to detect *Borrelia* DNA in adult ticks, larvae, and eggs**. Results:** All the guinea pig blood samples were negative for spirochetes by microscopy. However, out of the 17 adult ticks, 2 males and 5 females were positive for *Borrelia* DNA. None of the larvae was positive, but two batches of eggs yielded the expected 540 bp amplicon by nested PCR. **Conclusion:** Presence of *Borrelia* DNA in adult *O. tholozani* ticks and their eggs is an indication for transovarial transmission of relapsing fever agent in this tick. *J Med Microbiol Infec Dis, 2014, 2 (3): 118-120.*

Keywords: *Borrelia persica*, *Ornithodoros tholozani*, Transovarial transmission, PCR, Iran.

INTRODUCTION

The genus *Borrelia* comprises 37 species including the causative agents for Lyme disease and tick-borne relapsing fever (TBRF), the two different types of borreliosis transmitted by hard ticks (Family: Ixodidae) and soft ticks (Family: Argasidae), respectively. Louse adapted *Borrelia recurrentis* is the only exception which is transmitted by human body lice [1]. TBRF is an endemic disease in Iran, with more than 140 annual cases throughout the country [2]. In the western, northwestern, and foothill regions of the Alborz Mountains, the Argasid soft tick *Ornithodoros tholozani* is commonplace in animal shelters and adjacent human dwellings and accounts for most of TBRF cases attributed to *Borrelia persica* infection [2-4]. However, in central and western Iran *O. tholozani* coexist with *Borrelia microti*-infected *O. erraticus* ticks [2, 5, 6]. Two other *Borrelia* species, *Borrelia latyshywii* and *Borrelia baltazardi*, were also described in Iran. *B. latyshywii* was isolated from *Ornithodorod tartakowskyi* ticks in northeast [7] and the *B. baltazardi* from a relapsing fever patient with thrombocytopenic purpura in Ardebil, an endemic area for TBRF *B. persica* in northwestern of the country [8]. *B. baltazardi* was discriminated from *B. persica* based on electron microscopy findings, *i.e.*, the number of flagellates, and experimental pathogenicity. The spirochete *B. baltazardi* was isolated only once and attempts to maintain it in laboratory animals failed [8].

Spirochetes of *Borrelia* can be visualized directly in infected ticks by dark-field microscopy. Inoculation of in phosphate buffered saline (PBS) suspension of ground Argasid soft ticks to susceptible animals or allowing the ticks to feed on the same species followed by microscopic examination of the animals' blood have been frequently practiced in Iranian studies. With the advent of molecular biology, various gene markers were used as reliable tools for detection and identification of relapsing agent borreliae in humans and tick vectors. In Iran, some studies reported presence of *B. persica* DNA in *O. tholozani* ticks [1, 3, 9], but the data on transovarial transmission of *Borrelia* in this tick by molecular markers is very scarce. In this study we tried to detect *Borrelia* in field- collected *O. tholozani* ticks by allowing them to feed on guinea pigs followed by examination of the animals' blood for spirochetes by microscopy. We also used two PCR methods to target highly repetitive regions of *rrs* gene to detect *Borrelia* DNA in ticks, and their eggs and larvae.

MATERIAL AND METHODS

Sample collection. Ticks were collected from crevices and cracks of animal dwellings from a rural area in Ardebil Province.

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Among collected samples, 17 *O. tholozani* ticks were identified based on morphological features. The ticks included 9 males and 8 females. The samples were kept in jars in an incubator at 30°C and 70% humidity for approximately two months; over this period, the engorged females laid eggs and some eggs hatched into larva. Six unfed ticks were allowed to feed on two guinea pigs and after five days the animals' blood were examined daily for presence of spirochetes by dark-field microscopy and microscopic examination of Giemsa-stained thin and thick smears for 14 days. DNA extraction was performed from 17 ticks, 4 larvae and 2 pools of 30 eggs by phenol/chloroform extraction method followed by ethanol precipitation as described by others [10].

PCR amplification. Detection of *Borrelia* DNA in extracted samples was performed by two PCR methods targeting highly recitative fragments of *rrs* genes using the primers and reagents described by others [11, 12]. The first amplification was performed with primers Rec4 (5'-ATG CTA GAA ACT GCA TGA-3') and Rec9 (5'-TCG TCT GAG TCC CAT CT-3') targeting a 523 bp fragment. For PCR negative samples, a nested PCR was applied with primers Fd3 (5'-AGA GTT TGA TCC TGG CTT AG-3') and 595R (5' CTT GCA TAT CCG CCT ACT CA-3') at the first stage and primers Fd4 (5'-GGC TTA GAA CTA ACG CTG GCA-3') and 500R (5'-CTG CTG GCA CGT AAT TAG CC-3') at second stage. All the amplifications were performed in 25 µl reaction mixtures containing 20 pmol of each primer, 2.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl, 200 µM of dNTPs, 1 U of *Taq* polymerase, and 3 µl of DNA (expect for second stage of the nested-PCR in which 3 µl of 1:10 dilution of the amplicon of the first stage was used as template). All amplifications included negative controls containing all reagents expect DNA template, and positive controls containing *B. persica* DNA as template. The PCR products were run on 2% agarose gel and visualized under UV light.

RESULTS

All the guinea pig blood samples were negative for spirochetes by dark-field microscopy and microscopic examination of Giemsa-stained smears. Out of 17 adult ticks, 2 males and 5 females were positive for *Borrelia* DNA. None of the larvae was positive, but one batches of eggs yielded the expected ≈ 520 bp amplicon by the PCR targeting 16 S rRNA (Figures 1 and 2).

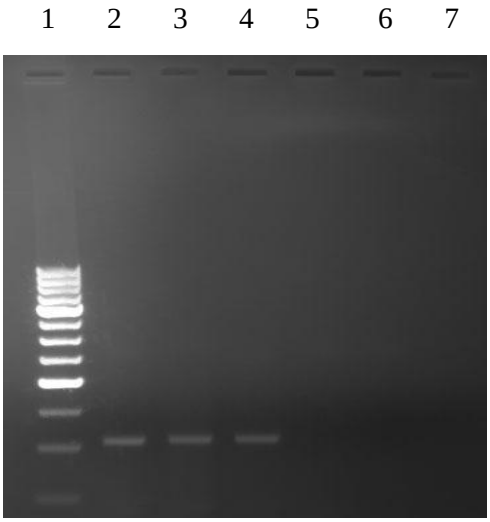

Fig. 1. Detection of *Borrlia* DNA in *O. tholozan*i ticks by PCR targeting *rrs* gene. Lane1, DNA molecular weight marker (250 bp ladder); lanes 2 and 3, adult female ticks; lane 4, pool of 30 eggs of ticks; lane 5, adult female tick; lane 6, adult male tick; lane 7, negative control.

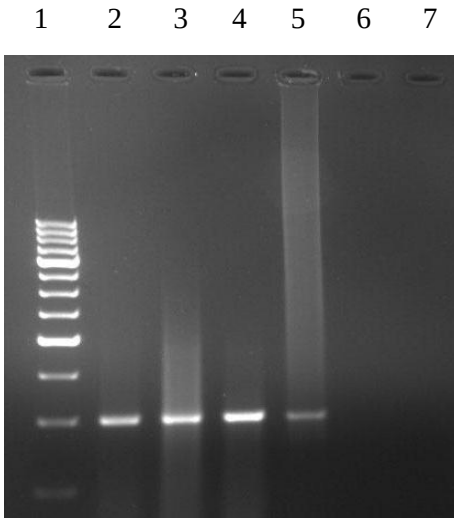

Fig. 2. Detection of *Borrlia* DNA in *O. tholozan*i ticks by nested PCR targeting *rrs* gene. Lane1, DNA molecular weight marker (250 bp ladder); lanes 2-5 female ticks; lane 6, tick larva; lane 7, negative control.

DISCUSSION

The association of *B. persica*, the agent for most relapsing fever cases in Iran, with *O. tholozani* ticks has been known since early 20th century [13]. Presence of the spirochetes in *O. tholozani* ticks has been demonstrated by allowing ticks to feed on susceptible animals like guinea pigs or small white laboratory mice. Inoculation of in-PBS suspension of ground ticks to the same animals followed by microscopic examination of animals' blood for spirochetes has also been practiced [2]. The advent of molecular methods allowed identification and characterization of *Borrelia* spp. in infected ticks. Lyme *Borrelia* DNA have been frequently detected in engorged female *Ixodes persulcatus* ticks and their eggs by PCR methodology targeting *rrs* gene [14, 15]. *B. persica* was detected in about 98% of laboratory-infected *O. tholozani* ticks by the same gene marker [9]. In a study on soft ticks collected from Takistan County in Qazvin Province, west Iran, *B. persica* DNA and unspecified *Borrelia* DNA were detected in *O. tholozani* and in *Orithodoros. lahorensis* ticks, respectively[3]. In this study we could detect *Borrelial* DNA in adult ticks and in their eggs as well. This is an indication for transovarial transmission of the *Borrelia*

spirochetes, an important mechanism for maintaining and distributing tick-borne pathogens in nature; ticks hatch out of the egg already infected, and don't require a reservoir host meal during larval stage. The flaw with this assay was lack of sequencing amplicons. There were chances that apart from *B. persica*, other borrelial species could be present in *O. tholozani* ticks; the importance of this issue is more magnified when we consider the fact that *B. baltazardi* was isolated from a patient in an area where *O. tholozani* ticks were prevalent and *B. persica* relapsing fever was endemic [8]. Further studies with much more tick samples from relapsing fever endemic areas in Iran and other countries are required to elucidate the role of *O. tholozani* ticks as potential vector and reservoir of other *Borrelia* species.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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