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Article

Identification and molecular characterization of *Otobius megnini* (Ixodida: Argasidae) seen in humans in Muş province, Turkey

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ABSTRACT
Otobius megnini (Ixodida: Argasidae) is a cosmopolitan soft tick that parasitizes humans as well as domestic and wild animals. The larval and nymph stages of this tick usually feed by parasitizing in the ear canal. The material of this study consists of ticks collected during ear cleaning in approximately 496 people coming with the complaint of ear pain in state hospitals in Bulanik and Malazgirt districts of Muş province, eastern Turkey. As a result of microscopic examination performed on ticks collected from humans, *O. megnini* tick was determined and molecular identification was made for definitive diagnosis. The 16S rRNA gene fragment of the tick was amplified by PCR. Obtained PCR products were 360 bp. The PCR products were analyzed by sequence analysis and compared with the reference sequences in the BLAST and Genbank. A phylogenetic tree was created with MEGA 7 software using Maximum Likelihood model. As a result, the previously identified *Otobius megnini* in Turkey, was confirmed using molecular methods for the first time.

KEY WORDS: 16S rRNA; Acari; ear tick; PCR; soft tick.

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INTRODUCTION

Otobius megnini (Dugès) is a soft tick belonging to Argasidae family; its larvae and nymphs parasitize in the external ear canal of many wild and domestic animals, and sometimes humans. The ear tick was first identified in 1884 from specimens collected in northern Mexico (Eads and Campos 1984). At that time it was only known as a pet parasite in Mexico and Texas. But now records show that infestation with this parasite occurs in both domestic and wild animals in most states of the United States, and many other regions, including south eastern British Columbia, Peru, Chile, Bolivia and Argentina (Rıch 1957). *Otobius megnini* originates from South,West and North of USA. USA, parts of Hawaii and Canada, countries in Central and South America, Caribbean (Cuba), Madagascar Island, South Asia (India, Sri Lanka), East Asia (Korea), six countries in

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Sahara Africa and the Near East (Turkey) have been reported to harbor this tick (Keirans and Pound 2003; Diyes *et al.* 2014). Like other Argasidae species, *O. megnini* is important in public health as it is a vector to zoonotic diseases such as Q and spotted fever (Diyes and Rajakaruna 2016).

Otobius megnini was first reported in Turkey by Ozer and Aydin (1996), and then by Gargili *et al.* (2011) and Gokdogan *et al.* (2016). This study was conducted on ticks collected after ear cleaning in patients who came to state hospitals in Bulanik and Malazgirt districts of Muş province with complaints of persistent ear pain. Morphologically determined *O. megnini* was characterized by using molecular methods for the first time in Turkey.

MATERIALS AND METHODS

Collection of samples

The study material consisted of the larvae and nymphs of 728 ticks collected from 302 females and 194 males who came to the state hospital in Muş province Bulanık and Malazgirt districts with earache complaints between April 2017 and July 2018. These ticks were placed into bottles containing 70% ethyl alcohol and the name and surname of the person and the date the tick was removed from the patient were written on the bottle and brought to the laboratory of the Department of Parasitology, Faculty of Veterinary Medicine, Van Yuzuncu Yıl University.

Morphological examination of samples

In order to see the taxonomic characters easily, ticks were examined under a stereo-microscope (Leica MZ16) after being treated with lactophenol. In order to examine some difficult-to-see structures, they were examined with the help of a fine forceps or a scalpel. The taxonomic key given by Walker *et al.* (2007) was used for the morphological identification of the samples.

DNA isolation

Ten nymphs and 10 larvae were transferred to 2 ml Eppendorf tubes containing 1 mm sterile micro beads and 4 mm steel bead and kept in liquid nitrogen for 1 minute, then crushed in bead beater (MM301, Qiagen) for 1 minute and 30 seconds. The samples were suspended in 180 µL lysis buffer (ATL buffer, Qiagen) and centrifuged at maximum speed (10000 g). Later, DNA was extracted using the Qiaamp DNA extraction kit (Qiagen). The final elution volume was calculated as 200 µL.

PCR amplification of 16S rRNA gene, agarose gel electrophoresis

DNA specific primers used in the diagnosis of arthropods were used to determine the type of nymph obtained. Primers used in PCR reactions were: forward primer 16s rRNA TB1: 5'– AAA CTA GGA TTA GAT ACC CT –3' and reverse primer T2A: 5'– AAT GAG AGC GAC GGG CGA TGT –3' (Beati and Keirans 2001). PCR was performed using Dream Taq Green PCR Master Mix (Thermo Scientific, Waltham, MA). PCR conditions were applied 95 ℃ for 5 minutes, then 60 seconds at 94 ℃, 60 seconds annealing at 57 ℃ and primary extension at 72 ℃ for 45 seconds, followed by 5 minutes at 72 ℃. After the PCR process, PCR products were stored at 4 ℃ until agarose gel electrophoresis.

Phylogenetic analysis

The study was carried out with a PCR method targeting the *O. megnini* 16S rRNA gene. Sequencing was performed on the PCR products with ABI 3130 (Applied Biosystems) genetic analyzer with the bidirectional Big DyeTM terminator sequence kit (Ver 3.1 Applied Biosystems, Forster City, CA) and primers TB1 and T2A. As a result of the sequencing procedure, 360 bp 16S rRNA sequence was obtained. Sequence analysis and alignment of sequences were performed with Bioedit7 software (Hall 1999). NCBI (http://blast.ncbi.nlm.nih.gov/BLAST.cgi) was used to

determine the similarity of the obtained sequences to the database entries. The smallest BIC (Bayes Information Criteria) value was modelled in MEGA 7 (Kumar *et al*. 2016) software to determine the relationship between sequences, and determined by the T92 + G (Tamura 3-Parameter + Gamma distribution) method.

RESULTS

Ticks were found on the camera during inner ear cleaning process in state hospitals in Bulanik and Malazgirt districts of Muş province. The obtained tick larvae and nymphs were made transparent and examined under a stereo-microscope. It was determined by morphological identification that the examined tick larvae and nymphs belonged to *O. megnini.* As a result of ear examinations, nymphs and larvae of *O. megnini* were detected in a total of 496 humans, consisting of 302 (60.8%) females and 194 (39.12%) males. When the distribution was made according to age, ticks were detected in 248 women and 156 men between the ages of 20–30, and in 54 women and 38 men over the age of 30. After the PCR amplicons obtained were run on a 1% agarose gel for about 30 minutes, the presence of a target band of 360 bp for the 16S rRNA gene was determined using an imaging system (UVP) and photographed (Fig. 1).

A phylogenetic tree was drawn using the Maximum Likelihood method to observe the phylogenetic relationship between the sequences obtained from the tick species and those obtained in Genbank (Fig. 2). In this study, it was observed that the sequence obtained from *O. meginini* and registered in GenBank with the number MK951983.1 was 100% similar to EF120989.1, L34325.1, DQ159447.1, KJ133592.1, KC769589.1 obtained from NCBI.

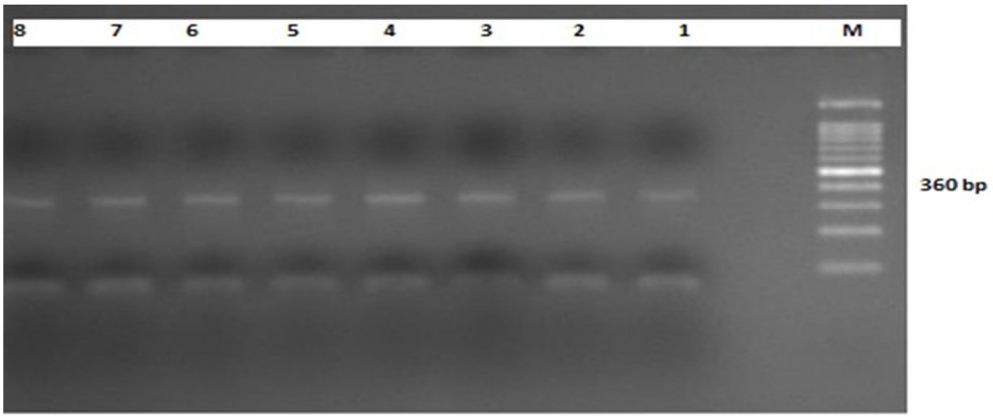

Figure 1. PCR amplification of 16S rRNA gene region in *Otobius megnini* (Amplicon length 360 bp).

DISCUSSION

Argasidae ticks fall into *Argas, Ornithodoros* and *Otobius* genera and are important for medical and veterinary purposes. Argasidae live and parasitize close to the host, but feed for a short time in the host and then return to their hiding place. *Otobius megnini* larval and nymph forms can remain parasitic in the host's external ear canals for a long time (Schwan *et al*. 1992). *Otobius megnini* is more commonly known as a cattle parasite, but it can also be parasitic in other organisms such as cats, dogs and humans (Nava *et al*. 2009; Skvarla *et al*. 2015; Lindström and Lindström 2017).

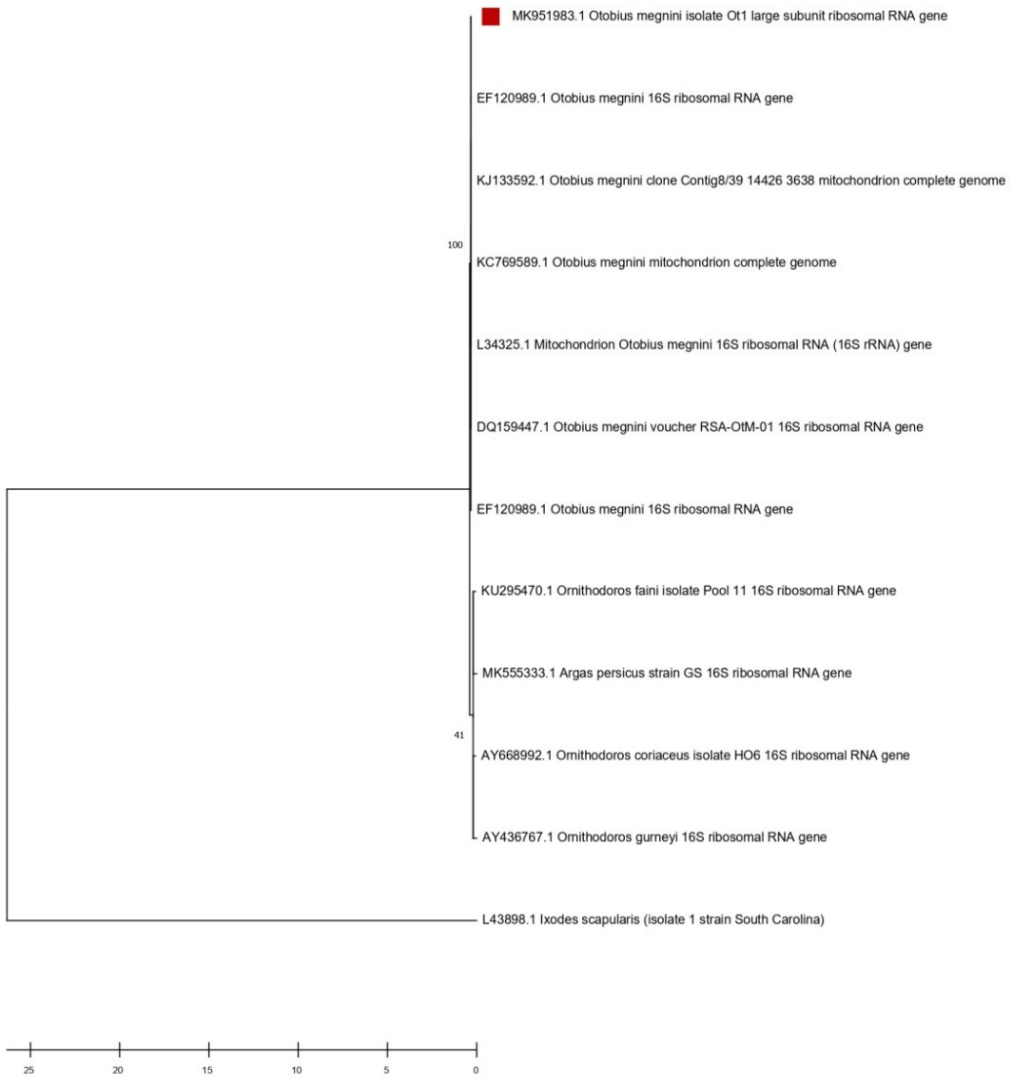

Figure 2. Phylogenetic relationship of partial sequences of *Otobius megnini* 16S rRNA gene isolates obtained in this study with sequences of other tick species from GenBank. Analyzes were performed using Tamura 3-Parameter + Gamma distribution (T92+G+I) and Maximum Likelihood method. *Ixodes scapularis* (L43898.1) was used as the outgroup. Sequences were sorted by Genbank accession number, host, and origin. *Otobius megnini* identified in this study is shown in red square color.

Ear tick in pets is reported to cause various disease symptoms such as allergy, colic, myotonia, muscle spasm, otitis, and paralysis (Zarate-Ramos *et al*. 2014; Diyes and Rajakaruna 2016). It has also been reported to have a possible role in the transmission of infertility and abortion as clinical signs in domestic animals (Woldehiwet 2004), flu-like disease in humans (Angelakis and Raoult 2010), Q fever and *Coxiella burnetti* (USDA 1976).

Otobius megnini is the cause of equine otoacariasis or parasitic otitis in horses. This situation can cause serious injuries and sometimes death in horses (Wall and Shearer 2008). Common clinical signs include abnormal head movements, head shaking, and head friction (Perris 1995). Due to the presence of *O. megnini*, horses suffer nerve disease in the ear canal (Ramanujachari and Alwar 1955) and auricular nerve palsy (FAO 1958). In northern Mexico, symptoms of myotonia

and colic are reported as a result of the infestation of *O. megnini* in a two-year-old quarter breed horse (Zarate-Ramos *et al*. 2014).

In humans, there are many reports of *O. megnini* infestation (Keirans and Pound, 2003). Eye conjunctivitis of a child has also been reported in Arizona (Jensen *et al*. 1982). In addition, it has been reported to cause external ear inflammation, rupture of the tympanic membrane in the ear, and facial and body paralysis by invading the ears in humans (Peacock 1958; Marquardt *et al*. 2000). It has been reported that *O. megnini* nymphs and their larvae were found in the ear cleaning of a 15-year-old girl who was admitted to the hospital with a complaint of ear pain in the Pretoria region of South Africa. (Naudé *et al*. 2001). Most cases of autoacariasis have been reported in people interacting with pets or doing outdoor activities (e.g. gardening, firewood picking) (Keirans and Pound 2003; Ariyarathne *et al*. 2016). In this study, *O. megnini* ticks were found in 496 human ears, mostly in milking women. The fact that people with ticks in their ears are generally among those who deal with animal husbandry supports the studies of other researchers; *Otobius megnini*, first reported in Turkey by Ozer and Aydin (1996), in cattle in the province of Malatya; later Gargili *et al*. (2011) and Gokdogan *et al*. (2016) also reported it. With this study, otoacariasis caused by *O. megnini* was detected in people in Muş province. According to the anamnesis taken, it was determined that people with *O. megnini* in their ears generally deal with animal husbandry, and especially milking women. With this study, *O. megnini*'s tick larvae and nymphs were identified morphologically; its molecular characterization was done for the first time in Turkey.

As a result, *O. megnini* infestation has been intensely detected in people who are engaged in animal husbandry and are unaware of the presence of these ticks in the province of Muş, a rural region of Turkey. Since people are unaware of the existence of the *O. megnini* tick, we believe that it can cause serious health problems, so people in this area should be informed about the presence of this tick. People should be warned to close cracks and crevices in barns and houses, especially because ticks are hiding in cracks and crevices, and animal owners should be informed about the use of ectoparasitic drugs on a regular basis.

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شناسایی و ویژگی‌های مولکولی *Otobius megnini* (Ixodida: Argasidae) مشاهده شده در انسان‌ها در استان مُش، ترکیه

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چکیده

گونهٔ *Otobius megnini* (Ixodida: Argasidae) کنهٔ نرم جهان‌وطنی است که انسان و همچنین حیوانات اهلی و وحشی را انگلی می‌کند. مراحل لاروی و پورگی این کنه معمولاً با انگل شدن در مجرای گوش تغذیه می‌کنند. نمونه‌های این مطالعه شامل کنه‌های جمع‌آوری‌شده در حین تمیز کردن گوش در حدود ۴۹۶ نفر است که با شکایت گوش درد به بیمارستان‌های دولتی در مناطق بولانیک و مالازگیرت استان مُش، شرق ترکیه مراجعه کرده‌اند. در نتیجه معاینه میکروسکپی که روی کنه‌های جمع‌آوری شده از انسان انجام شد، کنهٔ *O. megnini* شناسایی شد و برای تشخیص قطعی شناسایی مولکولی انجام شد. قطعه ژن 16S rRNA کنه با روش پی‌سی‌آر تکثیر شد. محصولات پی‌سی‌آر به دست آمده از باندهای ۳۶۰ جفت باز به دست آمدن. محصولات پی‌سی‌آر با آنالیز توالی بررسی و با توالی‌های مرجع در بلاست و بانک‌ژن مقایسه شدند. درخت فیلوژنتیک با نرم افزار MEGA 7 با استفاده از مدل حداکثر درست‌نمایی ایجاد شد. در نتیجه، *Otobius megnini* که پیش‌تر در ترکیه شناسایی شده بود، با استفاده از روش‌های مولکولی برای نخستین بار در این مطالعه تأیید شد.

واژگان کلیدی: 16S rRNA؛ زیردهٔ کنه‌ها؛ کنهٔ گوش؛ پی‌سی‌آر؛ کنهٔ نرم.

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