

# Detection of *Toxoplasma gondii* in a Eurasian Badger (*Meles meles*) Living in Wildlife Areas of Izmir, Turkey

İzmir Doğal Hayatında Yaşayan bir Avrasya Porsuğunda (*Meles meles*) *Toxoplasma gondii*'nin Saptanması

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## ABSTRACT

*Toxoplasma gondii* is an obligatory intracellular protozoan parasite that causes toxoplasmosis in humans and all warm-blooded animals. In this study, we aimed to investigate the presence of *T. gondii* DNA in a Eurasian badger (*Meles meles*) that was found dead in the wildlife area of Izmir. According to the results of real time polymerase chain reaction, *T. gondii* REP gene was found to be positive in the Eurasian badger brain homogenate. In conclusion, Eurasian badger, a known carnivore, can be a potential source of toxoplasmosis in the natural settings of İzmir, Turkey.

**Keywords:** Eurasian badger, *Meles meles*, PCR, *Toxoplasma gondii*, Turkey

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## ÖZ

*Toxoplasma gondii*, insanlarda ve tüm sıcakkanlı hayvanlarda toksoplazmozise neden olan zorunlu hücre içi protozoan bir parazittir. Bu çalışmada, İzmir'in yaban hayatında ölü olarak bulunan bir Avrasya porsuğunda (*Meles meles*) *T. gondii* DNA varlığının araştırılması amaçlanmıştır. Real time Polimeraz Zincir Reaksiyonu (PZR) sonucuna göre *T. gondii* REP geni Avrasya porsuğunun beyin homojenatında pozitif bulunmuştur. Sonuçta önemli bir etobur olan Avrasya porsuğunun İzmir'in doğal çevresinde potansiyel bir toksoplazmoz kaynağı olabileceği gösterilmiştir.

**Anahtar Kelimeler:** Avrasya porsuğu, *Meles meles*, PZR, *Toxoplasma gondii*, Türkiye

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## INTRODUCTION

*Toxoplasma gondii* is an obligatory intracellular protozoan parasite that causes toxoplasmosis in humans and all warm-blooded animals (1). *T. gondii* has a complex life cycle including sexual and asexual reproductive phases. Felines are the definitive host that oocyst formation occurs through sexual reproduction. Also, tachyzoite and tissue cyst (bradyzoites) production occurs through asexual reproduction in felines and other intermediate hosts such as mammals and birds (1). Oocysts which are highly resistant to

environmental conditions and meat containing tissue cysts are the main factors that spread the infection among wild animals (2, 3). The Eurasian badger (*Meles meles*) belonging to the family *Mustelidae* lives in Europe and Eurasia located in the northern hemisphere. Although the Eurasian badger is classified as a carnivore; these animals also feed on a wide variety of plants (4). In some previous studies conducted in Europe, toxoplasmosis was detected in Eurasian badger using serological methods and/or conventional or nested polymerase chain reaction (PCR) methods (5-10). In this study,

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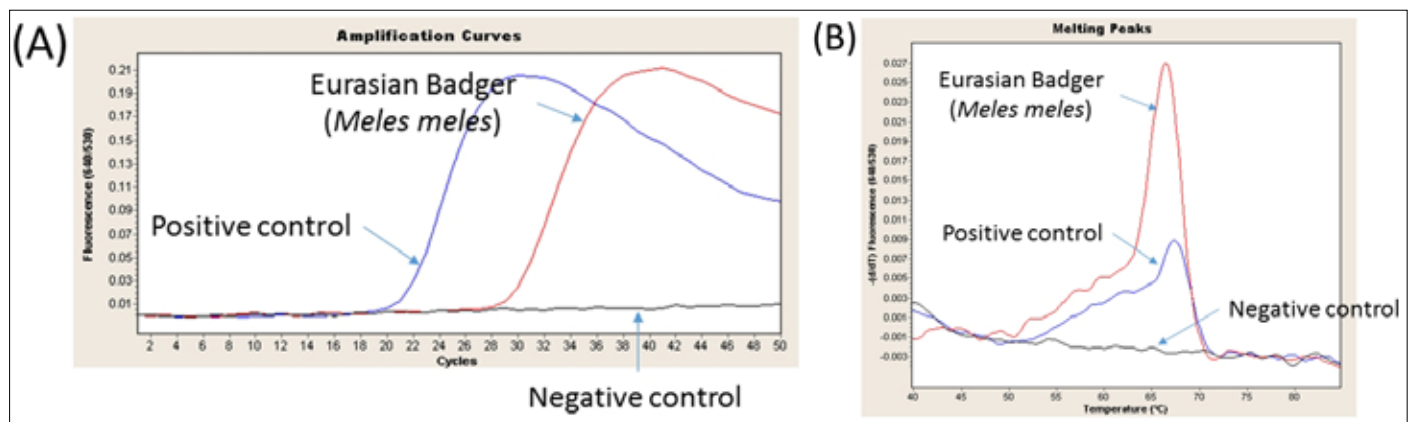
*Bu çalışma 16-20 Kasım 2016 tarihleri arasında Türkiye, Antalya'da düzenlenen International Symposium on Parasitic Zoonoses'da sunulmuştur.*

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**Figure 1. a, b.** Results of real-time PCR targeting *T. gondii* REP gene (a) Quantification and (b) Melting Curve Analysis

using real-time PCR, we aimed to investigate the presence of *T. gondii* in a Eurasian badger that was found dead in the wildlife area of İzmir located in the Aegean region of Turkey.

#### CASE REPORT

A Eurasian badger was found dead in wild life of İzmir located in the Aegean region of Turkey and was brought to İzmir Natural Life Park. On external examination, no pathological sign was detected on the body, fur, nails, and eyes. Only ticks were detected on the skin of the animal. Furthermore, no macroscopic abnormality was observed on necropsy of the abdomen and chest cavity. All experiments were performed under the instructions and approval of the Institutional Animal Care and Use Committee of Ege University for animal ethical norms (Permit number: 2014-16).

To investigate the presence of *T. gondii* in the Eurasian badger, brain tissue was homogenized as previously described (11). Briefly, the brain was removed by opening the skull using a costatome and was homogenized in sterile 0.9% NaCl using a blender (Waring, USA). During the homogenization process, 125 mL 0.9% NaCl was used for 10 g tissue. After homogenization, 0.5 g of trypsin (BD sciences, USA) was added to the homogenate and incubated at 37°C for 75 min using an incubator shaker (New Brunswick, USA). Later, the homogenate was filtered into a 50 ml falcon tube using two-layered sterile gauze and was centrifuged for 15 min at 910 g. After this process, the supernatant was discarded and the pellet was washed two more times with 0.9% NaCl. Following the last centrifugation, the supernatant was discarded, and DNA extraction from the pellet was performed using QIAamp DNA mini kit (Qiagen, USA).

*T. gondii* REP gene (Genebank Accession no: AF146527) was amplified using real-time PCR as previously described (11). To amplify a 134 bp gene fragment, the primers (F: 5'-AGGCGAGGGTGAGGATGA-3' and R: 5'-TCGTCTCGTCTGGATCGCAT-3') and hybridization probes [5'-GCCGGAACATCTTCTCCCTCTCC-3'-FL and 5'-640-CTCTCGTCTCGCTCCCAACCACG-3' (Metabion, Germany)] were used. Real-time PCR was performed using LightCycler 1.5 Real Time instrument with LightCycler software, version 4.1 (Roche, Germany). *T. gondii* genomic DNA, which was diluted from 10<sup>6</sup>/mL to 10<sup>1</sup>/mL, and distilled water were used as positive

and negative controls, respectively. According to the quantification and melting curve analysis (Figure 1), *T. gondii* REP gene was positive in the Eurasian badger brain homogenate.

#### DISCUSSION

In Turkey, it is well known that badgers particularly inhabit the regions of Adana, Adapazarı, Ankara, Aydın, Balıkesir, Bingöl, Burdur, Denizli, Eskişehir, Isparta, Kahramanmaraş, Konya, Mersin, Muğla, Ordu, Trabzon, and Zonguldak. Furthermore, badgers live throughout Anatolia as well as Thrace region of Turkey and can live up to an altitude of 2000 m (12). In this study, a dead Eurasian badger was found in the wildlife area of Bornova-İzmir located in the Aegean region of Turkey. It was thought that investigations on this animal may provide new data for those who work in the field of animal ecology. On the other hand, the presence and/or frequency of *T. gondii* in various wild animals is currently being investigated to reveal the animal species that helps spread the parasite and to determine the potential source of toxoplasmosis in nature. The presence of *T. gondii* has been detected in the Eurasian badger in previous studies conducted in UK, Spain, Portugal, Slovakia, and Poland; these studies have shown the presence of anti-*Toxoplasma* antibodies and/or used conventional or nested PCR methods to show the presence of *T. gondii* (5-10). The presence of *T. gondii* has been reported in various wild animals belonging to the family *Mustelidae*, such as mink (*Mustela vison*), river otters (*Lontra canadensis*), southern sea otters (*Enhydra lutris nereis*), black-footed ferrets (*Mustela nigripes*), stone marten (*Martes foina*), pine marten (*Martes martes*), ferret (*Mustela putorius furo*), Eurasian otter otters (*Lutra lutra*), and polecat (*Mustela putorius*), using microscopy, *in vitro* cultivation, and serological methods (6, 13-16).

#### CONCLUSION

In this study, *T. gondii* REP gene was detected for the first time in a Eurasian badger that is found in Turkey using real-time PCR. This result showed that *T. gondii* is capable of infecting the Eurasian badger and causing chronic infection. Since chronically infected animals are accepted as a potential source of toxoplasmosis, the Eurasian badger can also be a source for the transmission of toxoplasmosis in the natural setting of İzmir, Turkey.

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