ORIGINAL RESEARCH ORIJINAL ARAŞTIRMA

# Investigation of Parasitic Agents in Soil Samples Collected from Picnic Areas in Iğdır/Türkiye Province: A Descriptive Research

## Türkiye'nin Iğdır İlindeki Piknik Alanlarından Toplanan Toprak Örneklerinde Paraziter Etkenlerin Araştırılması: Tanımlayıcı Araştırma

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ABSTRACT Objective: The uncontrolled access of stray cats and dogs to public areas increases the likelihood of transmitting parasitic infections to humans. These animals can be carriers of zoonotic pathogens and the parasite eggs they spread into the environment pose a serious health risk to humans. Children and individuals with weak immune systems are particularly at risk of contracting these agents. The present study aimed to investigate parasitic agents in soil samples collected from picnic areas of Iğdır region. Material and Methods: For this purpose, 10 picnic areas close to the center of Iğdır and highly preferred by the public were determined and a total of 300 soil samples were collected, 30 from each location. The Kazakos method was applied to all samples and examined under a microscope. Results: As a result, 36 (12%) Toxocara spp. and 11 (3.6%) Taenia spp. eggs were found. Here, the highest contamination was found in the picnic area closest to the center and 30/7 (23.33%) Toxocara spp., 30/2 (6.6%) Taenia spp. Conclusion: The reason for the difference in the proportion of these 10 regions is thought to be due to the difference in distance, presence of fences, and animal densities. It was concluded that Iğdır picnic areas are common in terms of parasitic agents and the public should be made aware of this issue. It is considered important for public health to initiate the necessary protection and controls in this regard.

açık alanlara girişi, paraziter enfeksiyonların insanlara bulaşma olasılığını artırmaktadır. Bu hayvanlar, zoonotik patojenlerin taşıyıcısı olabilmekte ve çevreye yaydıkları parazit yumurtaları, insanlar için ciddi bir sağlık riski oluşturmaktadır. Özellikle çocuklar ve immun sistemi zayıf olan bireyler bu etkenlere yakalanma konusunda risk grubunu oluşturmaktadırlar. Sunulan bu çalışmada, Iğdır bölgesi piknik alanlarından toplanan toprak örneklerinde paraziter etkenlerin araştırılması amaçlanmıştır. Gereç ve Yöntemler: Bunun için Iğdır merkeze yakın ve halkın yoğun tercih ettiği 10 tane piknik alanı belirlenmiş, her alandan 30 adet olmak üzere toplamda 300 adet toprak örneği toplanmıştır. Örneklerin tümüne Kazakos yöntemi uygulanarak mikroskop altında incelenmiştir. Bulgular: Sonuç olarak 36 (%12) Toxocara spp. ve 11 (%3,6) tane de Taenia spp. yumurtasına benzer vumurtalara rastlanmıştır. Burada en fazla kontaminasyonunun merkeze en yakın olan piknik bölgesinde olduğu ve oran olarak 30/7 (%23,33) Toxocara spp., 30/2 (%6,6) Taenia spp. olarak bulunmuştur. Sonuç: Bu 10 tane bölgenin oran olarak farklılık göstermesinin nedeninin mesafe, çit varlığı ve hayvan yoğunluklarının farklı olmasına bağlı olduğu düşünülmüştür. İğdır piknik alanlarının paraziter etkenler açısından yaygın olduğu ve bu konuda halkın bilinçlendirilmesi gerektiği kanısına varılmıştır. Bu konuda gerekli koruma ve kontrollerin başlatılmasının halk sağlığı açısından önemli olduğu değerlendirilmiştir.

ÖZET Amac: Basıbos kedi ve köpeklerin kontrolsüz bir sekilde halka

Keywords: Cat; dog Iğdır; picnic area; Taenia spp; Toxocara spp Anahtar Kelimeler: Kedi; köpek Iğdır; piknik alanı; Taenia spp; Toxocara spp

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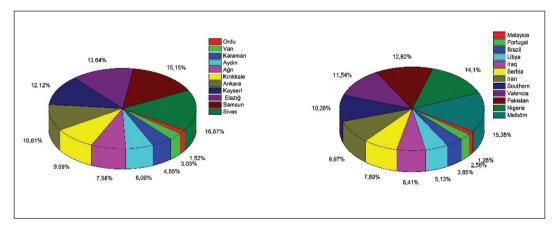
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Free entry of stray cats and dogs into public places is known to increase the risk of zoonotic transmission of parasitic diseases.<sup>1-3</sup> The ease with which they enter and defecate in these areas constitutes the starting point of soil contamination. In addition, owned dogs that are taken outside for walking or defecation have an important contribution to transmission.<sup>1,3,4</sup> Parasitic agents contaminating soil have been noted as a major public health problem. Helminths and protozoa are among the leading causes of these problems. Their transmission, particularly among children, leads to severe health issues.<sup>5</sup> These agents are widely reported worldwide, although they are particularly prevalent in less developed and developing countries.<sup>6-8</sup> Hundreds of millions of people around the world are already infected by parasites transmitted through contact with soil, with significant morbidity and mortality.9 According to studies, the soil contamination rate in developing countries ranges from 20-64%.10 Endoparasite outbreaks from domestic animals are frequently reported in Türkiye.7 Although these zoonotic outbreaks are a threat for all age groups, they are known to cause more serious health problems in children. These problems are symptoms such as diarrhea, abdominal pain, and bloating.8,11 These parasitic agents are on the list of agents that are the source of neglected infections worldwide in tropical regions.<sup>12</sup> The eggs of zoonotic helminths such as Toxocara canis and Toxocara cati, Echinococcus granulosus, E. multilocularis, Ancylostoma caninum, and Trichuris vulpis are a potential risk. It poses a

serious danger especially for young children and officials, as well as for people who come into contact with this soil.<sup>12,13</sup> It has been reported that children infected with these soil-borne parasites have reduced physical activity, impaired learning ability and poor growth.<sup>12</sup> Additionally, Toxocara spp. known as Toxascaris, Ascariasis have been reported to cause visceral and ocular (eye) larval migrans in humans.<sup>2,4,14</sup> Moreover *Toxocara spp.* eggs are highly resistant to environmental conditions and can remain infectious for many years under suitable conditions. Therefore, young children who spend time in such environments form the most vulnerable group for Toxocariasis due to behaviors like soil ingestion, nailbiting, and poor hygiene practices.<sup>2</sup> In addition, Ancylostoma spp. and Echinococcus spp. eggs cause cutaneous larva migrans and echinococcosis (cystic and alveolar types) diseases in humans, respectively.<sup>13</sup> Furthermore Ancylostoma spp. eggs are less resistant to environmental conditions compared to taeniid eggs, which may result in lower prevalence.<sup>4</sup>

In studies investigating soil contamination, it was reported that the prevalence of *Toxocara spp.* in Türkiye ranged between 5.9-59.3%.<sup>15,16</sup> This worldwide research shows that this rate is between 0.55-95.7%.<sup>12,17</sup> Graph 1 shows A the prevalence of Toxocara spp analyzed by Kazakos method in Türkiye and B the prevalence of Toxocara spp analyzed by Kazakos method in Türkiye

In the light of this information, it was aimed to investigate parasitic agents in soil samples collected



**GRAPHIC 1: A:** Prevalence of Toxocara spp. in Türkiye analyzed by Kazakos method.<sup>1,4,13,15,16,24-28</sup> **B:** Prevalence of Toxocara spp. in Türkiye analyzed by Kazakos method.<sup>8,12,17,23,29-34</sup>

from picnic areas in Iğdır region. The number of parks and urban recreation areas in Iğdır province is quite low. For this reason, the people of the Iğdır mostly prefer picnic and recreation areas. Children's contact with soil increases even more in this way. The free roaming of stray cats and dogs makes it quite possible for picnic areas to be polluted. It is thought that the inadequacy of shelters throughout the province and the lack of awareness of the public on this issue maximize the incidence of zoonotic infections. For this reason, the examination of soil samples collected from picnic areas, which have not been brought to the agenda in Türkiye, is very important in terms of public health and serves as a guide for the evaluation of other provinces in this respect.

## MATERIAL AND METHODS

### ETHICAL

All work was carried out in accordance with the principles of the Declaration of Helsinki 2008 (http://www.wma.net/en/30publications/10policies/b 3/index.html) Informed consent was obtained from private picnic owners and actions were taken in accordance with animal rights protection laws.

### SAMPLE COLLECTION AREAS OF THE STUDY

The province of Iğdır, which has an interesting geographical location, offers a significant climatic diversity. In addition to its impressive natural beauties, it is also home to many different types of animals such as cattle, sheep and goats, cats, dogs and single-hoofed animals.<sup>18</sup> There are a limited number of parking areas in the center of the Iğdır. However,

picnic areas near the center of Iğdır or near the towns are at a level to meet the needs of the public. Therefore, for this study, samples were planned to be collected from 10 picnic areas near the center of the Iğdır, which are highly preferred by the public. A total of 300 samples, 30 samples from each picnic area, were collected and records were kept about these areas. The location of the Iğdır province on the map of Türkiye is given in Figure 1.

### SAMPLE COLLECTION

First of all, the necessary materials were provided. 10 picnic areas near the center, which are highly preferred by the public, were selected and visited according to the plan. It was planned to collect a total of 300 soil samples, 30 from each area. In order to collect samples, we went to the areas close to and in the same direction. Then the distant picnic areas were visited. After putting on gloves, soil samples weighing at least 250-300 grams were taken from a depth of 10 cm using small garden trowels. The trowels were cleaned and disinfected before use at each sampling site. The collected samples were placed in plastic bags and assigned a protocol number. The samples were brought to the Iğdır University Animal Hospital laboratory on the same day. The sample were stored at (+4 °C) until complete count. Sample collection was carried out within 1 week. Samples were also collected during the summer season (July) when picnics were popular. In the last week of this month, the air temperature ranged between 28-30 °C, with a humidity level of around 46%, and the weather was dry. Mo Sample collection stages are given in Figure 2.



FIGURE 1: Location of Iğdır province on the map of Türkiye.7

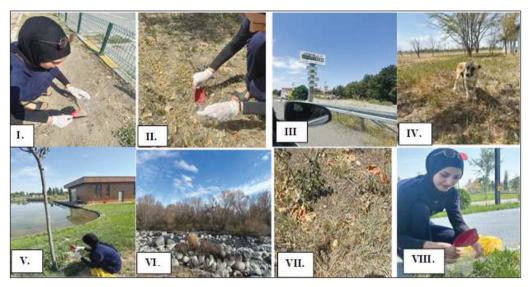


FIGURE 2: Sample collection sites; I: Keyfi Bahçe Picnic Place, II: Tatlı Bağ Picnic, III: Iğdır Urban Forest, IV: Iğdır Tuzluca Picnic Place, V: Iğdır National Garden, VI: Tuzluca Trout Facility, VII: Iğdır Tuzluca Picnic Place, VIII: Halfeli City Forest

#### PREPARATION AND ANALYSIS OF SAMPLES

Soil and samples collected from the picnic area were analyzed using a modified version of the method proposed by Kazacos.<sup>19</sup> Firstly, this study was investigated by the project coordinator and the supervisor. Repeated analysis was also performed by the expert parasitologist. In the laboratory, each sample was thoroughly mixed and homogenized, 50 g was taken, 60 ml of distilled water and 0.5 ml of Tween 40 (Lp Italiana) were added and transferred to a 250 ml beaker. This mixture was shaken well with the help of glass drumsticks and then passed through a 300 µm mesh sieve. This filtrate was transferred to 50 ml capped falcon (Lp Italiana) tubes, distilled water was added to the surface of the tube and the mixture was centrifuged at 1500-2000 rpm for 3 minutes. The top liquid was poured off, distilled water was added again up to the surface of the tube and centrifugation was repeated twice. After the last centrifugation, the top liquid was poured off and 50 ml of saturated zinc sulfate (ZnSO4) (specific gravity: 1.364) was added to the sediment to the surface of the tube and the tube was thoroughly mixed in a vortex. The mixture was transferred into 4 of 15 millimeter centrifuge tubes and centrifuged at 2000 rpm for 10 minutes. Then, saturated ZnSO4 solution was added on the removed tubes again to

form a bump on the surface and covered with coverslips. After 15 minutes of waiting time, the coverslips were placed on slides and examined for helminth eggs under a light microscope at 10x and 40x magnification. After the coverslips were removed, each tube was covered with coverslips again and examined 3 times in this way. The results of the study were statistically analyzed by descriptive statistics method.

## RESULTS

In the present study, result 36 (12%) *Toxocara spp.* and 11 (3.6%) *Taenia spp.* eggs were found in 300 soil samples examined. In these picnic areas ranked according to their distance from the center, the highest number of eggs thought to be *Toxocara spp.* 30/7 (23.33%) *Toxocara spp.* 30/2 (6.6%) *Taenia spp.* eggs were found in the national garden. Positive numbers in other picnic areas were near to each other. Microscopic images of the detected parasite eggs are given in Figure 3.

## DISCUSSION

Soil and sand with cat and dog feces have been reported to endanger children's health seriously.<sup>5,8,20</sup> Graphic 2, which was created by compiling studies on soil pollution in Türkiye and in the world, explains the

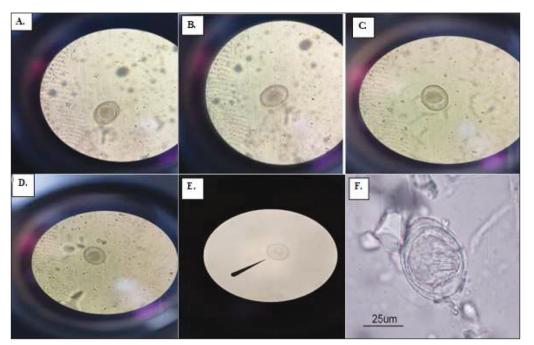
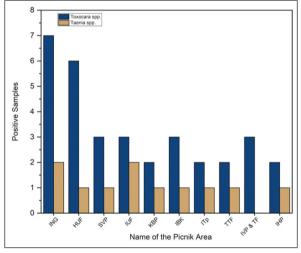


FIGURE 3: Microscopic images; A,B,C,D,E: Toxocara spp.; F: Taenia sp.

prevalence of the soil parasite Toxocara spp. According to this graphic, the prevalence of Toxocara spp. in Türkiye is between 5.9-59.3%, while in other countries this average is between 0.55-95.7%. In parallel, the present study investigated soil contamination with the parasite. As a result of the examination, the rate of Toxocara spp. was found to be 12%. This value is within the percentage averages of studies conducted in Türkiye and around the world. As indicated in Graphic 2, the prevalence of Toxocara spp. recorded in Iğdır region is higher than in Sivas and in Samsun provinces but lower than all other provinces. The reason for this situation is that the area where the research was conducted is a park and children's playgrounds. Indeed, these areas are less distant from the city center than picnic areas. Moreover, the density of stray animals is higher in these regions. In addition, the close proximity of parking areas makes them preferred areas for owner dogs to defecate and walk. Unlike the studies in Graphic 2, this study was conducted in picnic areas far from the city center. Soil contamination in the picnic area of the Iğdır nation garden in this study was found to be higher than in other picnic areas. This result proves that parks are likely to be more contaminated.



GRAPHIC 2: Picnic areas where samples were collected in Iğdır province and the parasite-positive ones

ING: Iğdır nation garden; HUF: Halfeli urban forest; SVP: Sweet vineyard picnic place; IUF: Iğdır urban forest, KBP: Keyfi Bahçe picnic area; IBK: Iğdır Bulakbaşı Karasu picnic area; ITP: Iğdır Tuzluca picnic area; TTF: Tuzluca trout facility; IVP&TF: İnce Village picnic trout facility; IHP: Iğdır Hamurkesen picnic area

Aydın who investigated helminth contamination in soil, took 26 soil samples from parking areas in Karaman province and recorded that *Taenia spp*. was found at a rate of 2/26 (7.69%) by applying the Kazacos method.<sup>4</sup> In parallel with this study, Bozkurt et al. examined 248 soil samples in Kayseri province using the same method and reported that they found 0.8% Taenia spp.1 As presented in Graphic 2, in this study, 300 soil samples were examined by the same method and 11 (3.6%) Taenia spp. were found. This result is lower in Karaman but higher in Kayseri. The difference in the prevalence value obtained in the study was thought to be due to the socioeconomic status in the region, the stray cat and dog population in the province and the different studies of the municipalities on stray animals. In addition, these different values are attributed to the average temperature and humidity values of the region, the rainfall regime, and the various characteristics of the soil and sand in the area where the samples were collected.

Among the studies on wildlife, Ayaz et al. stated that Mesocestoides lineatus, Tetratridium, Toxascaris leonina and Crenosoma vulpis were detected in a red fox in Van.<sup>21</sup> In another study, G1c1k et al. in Kars, Alaria alata (30%), *Mesocestoides lineatus* (60%), *Taenia multiceps* (10%), *T. pisiformis* (10%), *T. taeniaeformis* (5%), *Taenia spp.* (10%), *Echinococcus granulosus* (5%), *Toxascaris leonina* (65%), *Toxocara canis* (20%), *Capillaria spp.* (5%) and *Linguatula serrata* (40%).<sup>22</sup> In another study, Avcioğlu et al. found *Echinococcus multilocularis* with 100% similarity with the European isolate in a fox in Erzurum.<sup>23</sup> In this study, *Toxocara spp.* (12%) and *Taenia spp.* (3.6%) were found at rates and species distinction could not be made due to lack of finance.

It has been stated that ascarid eggs require high humidity and temperatures between 25-30°C to develop in the external environment.<sup>24,25</sup> While the temperature during the season and months when samples were collected in the Iğdır region aligns with these conditions, the humidity levels are relatively low, and the area is characterized by dry heat. Despite this, *Toxocara spp.* was detected at a rate of 12%, which aligns with the conditions described by researchers.

Picnic areas where people and cats and dogs have the opportunity to be close are of great importance for public health. Furthermore, especially children's contact with soil, the habit of eating soil in some children due to lack of hygiene and the lack of awareness of families further increase the transmission of these parasites. For this reason, knowing the presence of gastrointestinal parasites in stray cats and dogs is very important in terms of revealing the frequency of parasitic diseases and preventing the spread of parasitic diseases.<sup>3</sup> This study, which is closely related to human health, examined picnic areas for the first time in Türkiye and contributed to the literature.

## CONCLUSION

As a conclusion, 36 (12%) Toxocara spp. and 11 (3.6%) Taenia spp. eggs were found in 300 soil samples in Iğdır province. It was concluded that Iğdır picnic areas are common in terms of parasitic agents. The reason for this is believed to be the free roaming of stray cats and dogs in picnic areas. Additionally, the higher prevalence of Toxocara spp. has been attributed to the resilience of its eggs to weather conditions. The absence of other parasites (such as Strongvlus spp.) can also be attributed to the different intermediate hosts and the difficulty of finding these intermediate hosts in picnic areas. Furthermore, the resilience of other parasites to environmental conditions, season, and weather conditions is believed to have affected their prevalence. As can be seen in Graphic 2 soil contamination was detected mostly in the Iğdır nation garden. The reason for the difference in the ratio of these 10 regions is attributed to their distance from the center and the fact that the picnic areas are open or closed. It is thought that public awareness should be raised on this issue and necessary protection controls should be provided.

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#### **Conflict of Interest**

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

#### Authorship Contributions

Idea/Concept: Fatma Ertaş Oğuz, Pelin Akça; Design: Fatma Ertaş Oğuz, Pelin Akça; Control/Supervision: Ali Haydar Kırmızıgül, Fatma Ertaş Oğuz; Data Collection and/or Processing: Pelin Akça; Analysis and/or Interpretation: Fatma Ertaş Oğuz, Pelin Akça; Literature Review: Pelin Akça; Writing the Article: Fatma Ertaş Oğuz; Critical Review: Ali Haydar Kırmızıgül; References and Fundings: TUBİTAK 2209; Materials: Pelin Akça.

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