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The situation of echinococcosis in stray dogs in Turkey: the first finding of *Echinococcus multilocularis* and *Echinococcus ortleppi*

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Research Article

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Abstract

Echinococcosis, caused by larval stage of the genus *Echinococcus*, is one of the most important zoonotic diseases worldwide. The purpose of this study was to determine the presence and prevalence of *Echinococcus* species in stray dogs of Erzurum, a highly endemic region for cystic echinococcosis (CE) and alveolar echinococcosis (AE) in Turkey. The study samples consisted of 446 stray dog faecal specimens collected from an animal shelter in Erzurum, Turkey, between October 2015 and February 2016. The faecal samples were collected from individual dogs for the isolation of taeniid eggs using the sequential sieving and flotation method (SSFM). Molecular analyses and sequencing revealed the prevalence of *Echinococcus* spp. as 14.13% (63/446) in faecal samples. The stray dogs harboured five different *Echinococcus* spp.: *E. granulosus s.s.* (G1/G3) ($n = 41$), *E. equinus* (G4) ($n = 3$), *E. ortleppi* (G5) ($n = 1$), *E. canadensis* (G6/G7) ($n = 3$) and *E. multilocularis* ($n = 16$). *E. granulosus s.s.* was the most abundant species. Surprisingly, the occurrence of *E. multilocularis* in dogs was revealed for the first time in Turkey. *E. ortleppi* was also reported for the first time in Turkey. These findings highlight a significant public health risk for human AE and CE, presenting useful baseline data on *Echinococcus* spp. infection in dogs for designing control strategies.

Introduction

Echinococcosis, caused by larval stage of the genus *Echinococcus*, is one of the most important zoonotic diseases worldwide (Alvarez Rojas *et al.*, 2014). *Echinococcus granulosus sensu lato* (*s.l.*) and *Echinococcus multilocularis* are the most prevalent species and causal agents of human cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively (Eckert *et al.*, 2001).

Echinococcus granulosus s.l. life cycle involves a carnivorous definitive host, the domestic dog in general and livestock as the intermediate host (Thompson, 2017). CE is a zoonotic disease of global importance and has socio-economic significance in communities where livestock farming is the main source of living. *Echinococcus multilocularis* has a predominantly sylvatic life cycle, with carnivorous species, such as foxes, wolves and coyotes, and to a lesser extent dogs, as definitive hosts and small rodent species serving as intermediate hosts (Vuitton *et al.*, 2003). AE is a severe zoonotic disease that can lead to the death of the patient if left untreated or inadequately treated. Humans can be accidental dead-end intermediate hosts for both species. Human infection occurs through the ingestion of *Echinococcus* eggs with contaminated water or food or after direct contact with definitive hosts (Moro and Schantz, 2009).

E. granulosus s.l. is known to be endemic in all continents. However, *E. multilocularis* is mainly found in the northern hemisphere (Eckert *et al.*, 2001). Both AE and CE are considered neglected zoonotic diseases. Whereas CE is globally distributed and highly prevalent, AE is more pathogenic, often resulting in mortality (Deplazes *et al.*, 2017).

E. granulosus was previously considered a single species, but it is now recognized as an assemblage of cryptic species that have differences in morphology, development and host specificity, including infectivity and pathogenicity for humans (Romig *et al.*, 2017). *E. granulosus s. l.* currently includes five species, namely *E. granulosus sensu stricto* (*s.s.*) (G1/G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* (G6/7, G8 and G10) and *E. felidis* (Romig *et al.*, 2017; Vuitton *et al.*, 2020). Phylogenetic studies based on sequencing of mitochondrial genes and microsatellite analysis in recent years have been successfully used to investigate the polymorphism within the *E. multilocularis*, which was previously considered to have relatively low genetic diversity (Knapp *et al.*, 2009; Vuitton *et al.*, 2020).

Diagnosis of *Echinococcus* infection in dogs is challenging in that the tapeworm eggs are shed irregularly and are indistinguishable from the eggs of other taeniids. The diagnosis of *Echinococcus* species in definitive hosts relies on techniques such as necropsy, arecoline purgation, copro-antigen ELISA and copro-PCR with faecal matter or isolated eggs (Craig *et al.*, 2015).

To date, molecular studies on *E. granulosus* carried out in Turkey have reported several genotypes (G1–G3, G4, G6 and G7) in domestic livestock (Bowles *et al.*, 1992; Vural *et al.*, 2008; Snabel *et al.*, 2009; Simsek *et al.*, 2010, 2015; Simsek and Cevik, 2014; Erdogan *et al.*, 2017) as well as in humans (G1–G3, G6 and G7) (Snabel *et al.*, 2009; Eryildiz and Sakru, 2012) from different endemic foci of Turkey. Only one genotype (G1) has been reported in dogs from different parts of Turkey (Utuk *et al.*, 2008; Kuru *et al.*, 2013; Oge *et al.*, 2017; Oguz *et al.*, 2018).

In Turkey, a highly endemic region for AE and CE (Deplazes *et al.*, 2017), echinococcosis is a major public health problem, especially in the rural areas of eastern regions (Altintas, 2008). Erzurum province in the northeastern part of Turkey is a hyperendemic area for both human AE and CE, and the largest number of AE and CE patients nationwide was reported in the region (Altintas, 2008). Similarly, high prevalence rates of CE in cattle (Simsek *et al.*, 2010) and sheep (Arslan and Umur, 1997) in Erzurum province and have been reported. Thus far, metacestodes of *E. multilocularis* in humans (Kurt *et al.*, 2020) and rodents (Avcioglu *et al.*, 2017*a*), and *E. multilocularis* adults in red foxes (Avcioglu *et al.*, 2016, 2021) and lynx (Avcioglu *et al.*, 2018) have been reported in this region.

Data on the prevalence of echinococcosis in intermediate hosts (for CE and AE) and definitive hosts (for AE) is available for this region, which has provided valuable information on the geographical distribution of the parasites and the role of different animal species in parasite transmission. However, there is no available information on the presence and prevalence of *Echinococcus* species in dogs in Erzurum. The infection in dogs is important to estimate the relative infection pressure on intermediate hosts and humans, and to determine their roles in environmental contamination. Therefore, the purpose of the study was to determine the presence and prevalence of *Echinococcus* species in stray dogs in Erzurum province.

Materials and methods

Study area

The study was conducted from October 2015 to February 2016 in Erzurum (39°54′31″N, 41°16′37″E) province. The province is located in the eastern part of Turkey and has the fourth largest surface area (25 066 km^2) in the country, 20 counties and a total population of 762 000 inhabitants. The province has an elevation of 1853 m above sea level. It receives an annual rainfall of 453 mm. The temperature range is −35 to 35°C. Agriculture and livestock raising constitute the principal economic activities of the province.

Sample collection

The animal shelter under the division of Erzurum Metropolitan Municipality regularly collected stray dogs and cats from all counties. Rehabilitation and medical services including sterilization, vaccination against rabies and praziquantel application for tapeworms were provided by the shelter. After the applications, the animals were ear tagged and may then be either set free or adopted. The stray dogs were housed in pens with concrete floor kennels individually during the application of praziquantel and collection of the faecal samples 24 h after the drug application. All investigated dogs were older than one year. None of the dogs were microchipped or wearing an identity tag. Therefore, we could not determine their collection area or medical history.

A total of 446 faecal samples were regularly collected from individual dogs after the praziquantel application. Faecal samples were placed in labelled Ziploc bags, stored at −86°C for at least seven days (Deplazes and Eckert, 1996) to reduce the risk of laboratory infection by inactivating any *Echinococcus* oncospheres, and stored at −20°C until further examination.

Isolation of taeniid eggs and DNA extraction

The sequential sieving and flotation method (SSFM) described by Mathis *et al.* (1996) was used for the concentration of taeniid eggs in the faecal samples. Briefly, flotation with zinc chloride (density 1.45 g mL^{-1}) and sequential sieving through sieves of 40 and 21 μm mesh sizes were performed. The sediment accumulated in the 21 μm sieve was deposited in a flat-sided tube and examined under an inverted microscope to determine the presence of taeniid eggs. Positive samples were centrifuged at 1000 ***g*** for 10 min, and the pellet with taeniid eggs was stored in 2 mL microcentrifuge tubes.

Taeniid eggs were subjected to DNA extraction using a Qiamp DNA Mini Kit (DNeasy Tissue kit; Qiagen, Hilden, Germany) according to the manufacturer's instructions. The concentration of extracted DNA was measured with a spectrophotometer (NanoDrop One; Thermo Fisher Scientific, WI) and stored at −20°C until further step.

Molecular analyses and sequencing

Three sets of primers were used to amplify partial sequences of two mitochondrial genes, 12S rRNA and cytochrome c oxidase subunit 1 (COI), to detect *E. multilocularis*, *E. granulosus s.s.* and *E. granulosus s.l.* with conventional PCRs. Two PCR protocols with specific primers Egss1F/1R (254 bp, Dinkel *et al.*, 2004) and Emnestfor/rev (204 bp, Dyachenko *et al.*, 2008), amplifying partial sequences of the 12S rRNA gene of *E. granulosus s.s.* and *E. multilocularis*, respectively, were performed. Only second step of the nested PCR protocol (Dyachenko *et al.*, 2008) was performed in *E. multilocularis* PCR. For all the samples, JB3/4.5 primers (446 bp), which amplified a part of the COI gene, were also used to amplify *E. granulosus s. l.* (Bowles *et al.*, 1992). DNA of *E. granulosus s.s.* and *E. multilocularis,* which were previously confirmed by molecular analysis as positive control and distilled water as negative control, were included in each PCR run. The PCR products were analysed using 1.5% agarose gel electrophoresis, stained with SYBR Safe (Invitrogen, USA) and visualized using UV transillumination (Vilbert Lourmat, Quantum ST4, 1100/20M, France).

Bidirectional sequencing of all amplicons obtained in three PCRs was performed commercially with an ABI PRISM 310 genetic analyser (Applied Biosystems, Foster City, CA). Sequences were checked by eye (Finch TV), aligned using BioEdit 7.0 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and compared with those on the GenBank database through the use of BLAST algorithms (http://www.ncbi.nlm.nih.gov/BLAST/) to determine the species. Pairwise calculations were obtained using BioEdit software.

Ethical approval

Ethical approval was obtained from the Atatürk University Animal Research Local Ethics Committee (Approval no: 2015/27).

Results

Among the 446 faecal samples, 119 (26.68%, Table 1) were positive for taeniid eggs by microscopy. The positive samples were subjected to molecular analysis, and *Echinococcus* spp. were detected in 63/446 (14.13%) of the faecal samples. *E. granulosus s.s.* was obtained in 41 (9.19%) of the samples, whereas *E. multilocularis* was found in 16 (3.58%) samples by 12S rRNA-PCRs.

All the taeniid egg positive samples subjected to COI PCR were also positive. Sequence analysis of the COI PCR amplicons

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Table 1. *Echinococcus* species in stray dogs in Erzurum.

	No. of examined fecal samples	Fecal samples with taeniid eggs	Samples positive with PCR for					
			Echinococcus spp.	E.g.s.s.	E.e	E.o.	E.c.	E.m.
Total	446	119 (26.7%)	63 (14.1%)	41 (9.2%)	3 (0.7%)	1 (0.2)	3 (0.7%)	16 (3.6%)

E.g.s.s.: E. granulosus s.s.
E.e.: E. equinus
E.o.: E. ortleppi
E.c.: E. canadensis
E.m.: E. multilocularis

confirmed 28 of the 41 *E. granulosus s.s.* PCR positive samples and 10 of the 16 *E. multilocularis* PCR positive samples to be *E. granulosus s.s.* and *E. multilocularis*, respectively. To obtain longer sequences for *E. multilocularis*, we repeated the egg isolation and DNA extraction section for 6 of the 16 *E. multilocularis* PCR positive samples and performed COI PCR and sequencing until we succeeded.

Thereafter, BLAST analysis of the sequences revealed that the stray dogs harboured five different *Echinococcus* spp., namely *E. granulosus s.s.* (G1/G3) ($n = 41$), *E. equinus* (G4) ($n = 3$), *E. ortleppi* (G5) ($n = 1$), *E. canadensis* (G6/G7) ($n = 3$) and *E. multilocularis* ($n = 16$) (Table 1). *E. granulosus s.s.* was the most abundant species in stray dogs. *Taenia* spp. identified in 64 samples (details not reported here). DNA sequencing was not achieved in 12/119 of the samples due to the low yield of PCR products.

Multiple infections were recorded in some faecal samples: *E. canadensis* (G6/G7) and *E. multilocularis* in one sample, *E. granulosus s.s.* and *Taenia* spp. in 13 samples and *E. multilocularis* and *Taenia* spp. in five samples.

Isolated partial sequences were then deposited into GenBank with the following accession numbers: *E. granulosus s.s.* (G1/G3): MN732801-MN732821, *E. equinus* (G4): MN737094-MN737096, *E. ortleppi* (G5): MN737097, *E. canadensis* (G6/G7): MN737098-MN737100, *E. multilocularis*: MN732822-MN732837.

The COI nucleotide sequences of Erzurum isolates were compared with those of the references. Erzurum *E. granulosus s.s.* isolates showed 99.7–100% identity with each other and 100% identity with those reported from Turkey (MN990735, HM598451, KM100574, KX874711, EU178104), Brazil (KT382540, HF947571), Iran (MW350099, KJ162568, MT786855) and India (JX854029). Erzurum *E. equinus* isolates showed 100% identity with each other and were identical with those reported from Turkey (MK616473, KC953029, KM525658), Namibia (KP161210) and Uzbekistan (MK975893). Erzurum *E. ortleppi* isolates had 100% identity with the isolates reported from Japan (AB235846), Namibia (KU743926), Brazil (KT382535), Bosnia and Herzegovina (MG976769), France (KC430087) and Egypt (MK492625). Erzurum *E. canadensis* isolates were 100% identical with each other, and also had 100% identity with isolates from Iran (KU359038, KU220241), Sudan (MH300947), Nigeria (MN025264, KY996491) and France (MH823709). Erzurum *E. multilocularis* showed 99.5–100% identity with each other, also 100% identical with the isolates from Switzerland (MT461411), Canada (MK843308, MT461409, KC550004), USA (LC380931), China (MN251849, MH259774), South Korea (AB780998), Poland (KY205679, MW255909), Slovakia (DQ979365), Kyrgyzstan (MN829539), Russia (AB777915, AB688134) and Japan (AB385610).

Discussion

Echinococcus granulosus s.l. and *E. multilocularis* are two of the most widespread zoonoses as they cause disease in both humans and animals which are responsible for serious health and economic problems. In Turkey, a highly endemic region for AE and CE (Deplazes *et al.*, 2017), echinococcosis is a major public health problem, especially in the rural areas of eastern regions (Altintas, 2008). Erzurum province in the northeastern part of Turkey is a hyperendemic area for both human AE and CE. However, to date, there have been no data available on the presence and prevalence of *Echinococcus* spp. in the dogs of this province. This study reports the prevalence of *Echinococcus* spp. (14.1%) in dogs based on faecal samples in Erzurum province. The prevalence of *E. granulosus s.l.* and *E. multilocularis* was 10.8 and 3.6%, respectively. The presence of *E. granulosus s.s.* (G1/G3), *E. equinus* (G4), *E. ortleppi* (G5) and *E. canadensis* (G6/G7) was reported. Notably, *E. multilocularis* from dogs and *E. ortleppi* (G5) in Turkey were identified for the first time.

Most studies in Turkey have been performed on CE in humans and livestock animals but limited in dogs (Altintas, 2008; Simsek *et al.*, 2010; Deplazes *et al.*, 2017; Avcioglu *et al.*, 2017*b*; Kurt *et al.*, 2020). This is thought to be due to the difficulties in fieldwork of definitive hosts, such as obtaining dogs and wild canids, and contamination risk with zoonotic infections such as rabies and echinococcosis.

In Turkey, several studies on *E. granulosus* infection in dogs indicated endemicity across the country, ranging from 0.8 to 40.5%, and varying according to the geographical location and diagnostic methods (Umur and Arslan, 1998; Oter *et al.*, 2011; Kuru *et al.*, 2013; Oge *et al.*, 2017). Determination of the prevalence of *Echinoccoccus* spp. in definitive hosts in an endemic area is essential to understand the transmission dynamics of the parasite and to design effective control programmes. Although there is a lack of data on the presence and prevalence of *E. granulosus* in dogs, Erzurum is known as an endemic region for CE based on human cases and the high prevalence in livestock animals. The study reports the overall prevalence of *E. granulosus s.l.* as 10.8% in dogs based on faecal samples in Erzurum province. The results of the study determined the presence and prevalence of *E. granulosus s.l.* in the stray dogs in the province for the first time. This information will serve as a source for control strategies in the region.

The prevalence in stray dogs reported here was lower than some studies and higher than others from different parts of the country. The lower prevalence in this study can be explained by the lower sensitivity of our detection method (eggs in faeces) compared to the previous studies that used copro-antigen, arecoline purgation and necropsy. Prevalence rates between these studies are therefore not comparable. The low prevalence could also be explained by the prepatent infections and periodic shedding of *Echinococcus* eggs during patent infections (Trachsel *et al.*, 2007; Huttner *et al.*, 2009). However, for about two decades, antiparasitic applications on stray dogs periodically collected by the local government in Erzurum are thought to reduce the prevalence of the parasite. In connection with the results of this practice, three studies conducted approximately 10 years apart on the prevalence of CE in cattle in Erzurum province reported 46.4% (Arslan and Umur, 1997), 34.3% (Simsek *et al.*, 2010) and 24%

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(Avcioglu *et al*., 2017*b*) prevalence rates. It has been observed that the prevalence decreases by about 10% every 10 years in cattle. The fact that the prevalence of CE in intermediate hosts has decreased in recent years compared to previous years undoubtedly has a negative effect on the prevalence in final host dogs.

Determination of the species and genotypes of *Echinoccoccus* in definitive and intermediate hosts in an endemic area is essential to understanding the transmission dynamics of the parasite and designing effective control programmes (Alvarez Rojas *et al*., 2014; Romig *et al*., 2015). To date, molecular studies on *E. granulosus* carried out in Turkey have reported several genotypes (G1/G3, G4, G6 and G7) in livestock (Bowles *et al*., 1992; Vural *et al*., 2008; Snabel *et al*., 2009; Simsek *et al*., 2010, 2015; Simsek and Cevik, 2014; Erdogan *et al*., 2017) and humans (G1/G3, G6 and G7) (Snabel *et al*., 2009; Eryildiz and Sakru, 2012; Kurt *et al*., 2020) from different endemic foci. Among these, G1 has been considered the most prevalent in animals and human CE cases in Turkey. The G1 genotype was first reported in a dog by Utuk *et al*. (2008) and later among 100 dogs by three other studies (Kuru *et al*., 2013; Oge *et al*., 2017; Oguz *et al*., 2018) from different parts of Turkey using limited molecular studies.

Based on COI sequence analysis, the majority of Erzurum samples ($n = 41$) were identified as G1 genotype (*E. granulosus s.s.*); three were G4 genotype (*E. equinus*), one was G5 (*E. ortleppi*) and three were G6/G7 genotypes (*E. canadensis*). It was found that *E. granulosus s.s.* was the most abundant species in the study, confirming the results of the studies conducted in humans and livestock in Turkey, as indicated earlier. In Erzurum, traditional animal husbandry practices are typically used. Poor abattoir conditions and home slaughtering (especially sheep) are common in rural regions of the city, and there is a high population of stray dogs with insufficient public health education. The metacestodes of *E. granulosus s.s.* are able to reach fertility in sheep, which increases the infection risk of dogs. The combination of home slaughter, lack of thorough meat inspection, poor abattoir conditions, high cyst fertility and the high numbers of roaming dogs may explain the abundance of *E. granulosus s.s.* identified in the present study.

Apart from *E. granulosus s.s.*, *E. canadensis* (G6/G7) has been estimated to be responsible for 12.2 and 9.6% of global human CE infections, respectively (Cucher *et al*., 2016). The presence of G6 and G7 genotypes in livestock and humans was reported in Turkey (Snabel *et al*., 2009; Simsek *et al*., 2011; Eryildiz and Sakru, 2012). The G6/G7 genotypic cluster was first reported in cyst sample of a sheep in Elazig province of Turkey by Mehmood *et al*. (2020). The camel population is fairly lower in Turkey than in southeast neighbour countries, which have a large camel population. In Erzurum, camel breeding is not carried out or even available. Pig production is very limited due to religious reasons but wild boar population is very common in the country. In rural areas of the Erzurum, unofficial wild boar hunting is practiced because of their harmful effects on farming activities. Illegal dog transport from the border or access of stray dogs to dead wild boars can explain the existence of G6 and G7 genotypes. Reported G6/G7 genotype both from sheep in Elazig and from stray dogs in Erzurum province indicate that this genotype may have a wider distribution than previously thought in Turkey.

E. equinus (G4) is known to be a specific parasite of equids and non-pathogenic for humans (Romig *et al*., 2006). However, in a study conducted on the molecular characterization of *E. granulosus s.l* in humans, *E. equinus* was reported for the first time (Romig *et al*., 2017). Further, there have been many reports indicating the presence of *E. equinus* in different intermediate hosts worldwide (Thompson and McManus, 2002; Boufana *et al*., 2012). *E. equinus* has been detected in humans, mules and donkeys in Turkey (Simsek and Cevik, 2014; Kesik *et al*., 2019; Macin *et al*., 2021). This study is the first report of *E. equinus* from stray dogs in Turkey.

Echinococcus ortleppi (G5) is particularly well adapted to cattle as intermediate hosts. The morphology and developmental features of *E. ortleppi* show substantial differences compared with those of *E. granulosus s.s.* and other taxa (Romig *et al*., 2015). Although it was formerly known as almost exclusively found in cattle as intermediate hosts, in recent years, many countries have detected infections in sheep (Mbaya *et al*., 2014; Addy *et al*., 2017), pigs (Dinkel *et al*., 2004; Pednekar *et al*., 2009; Tigre *et al*., 2016; Addy *et al*., 2017), goats (Mbaya *et al*., 2014; Addy *et al*., 2017), camels (Ahmed *et al*., 2013; Amer *et al*., 2015; Addy *et al*., 2017; Ebrahimipour *et al*., 2017), monkeys (Pednekar *et al*., 2009), oryx (Addy *et al*., 2017) and spotted deer (Boufana *et al*., 2012). Human infections with *E. ortleppi* occur less frequently than with other species, such as *E. granulosus s.s.* (Alvarez Rojas *et al*., 2014), with only 12 human cases reported in various parts of the world (Alvarez Rojas *et al*., 2014; Shi *et al*., 2019). To our knowledge, only four reports of the presence of *E. ortleppi* in dogs are available, including those from Argentina (Kamenetzky *et al*., 2002; Soriano *et al*., 2010), Brazil (de la Rue *et al*., 2011) and Kenya (Mulinge *et al*., 2018). *E. ortleppi* was detected in one dog faecal sample and was reported for the first time in Turkey by this study. Cattle are most frequently infected with *E. granulosus* (G1/G3), but the majority of the cysts are infertile (Latif *et al*., 2010), which may explain the rare presence of *E. ortleppi* in the study (Avcioglu *et al*., 2017*b*). Home slaughtering is uncommon in cattle, unlike sheep, which restricts the access of the local dog populations to cattle offal.

AE has been recognized as an emerging zoonosis in Turkey, with an annual incidence of 100 cases (Torgerson *et al*., 2010). Most human AE cases have occurred in Eastern Anatolia, particularly in Erzurum (Gurler *et al*., 2019). The occurrence of *E. multilocularis* in definitive hosts is used to describe its endemicity in areas of Europe and North America. However, human cases were considered the most reliable source of data concerning AE in Turkey (Deplazes *et al*., 2017). Until the last few years, the occurrence or prevalence of *E. multilocularis* has not been studied in detail in wild or domestic canids. Recently, *E. multilocularis* was confirmed morphologically and molecularly in red foxes (Avcioglu *et al*., 2016, 2021) and lynx (Avcioglu *et al*., 2018) in Erzurum. *E. multilocularis* in fox faecal samples from Central Anatolia and the European part of Turkey was reported by Gurler *et al*. (2018). Avcioglu *et al*. (2021) reported that the prevalence of adult *E. multilocularis* in the fox intestines and environmental faecal contamination with *E. multilocularis* eggs were 42% (21/50) and 10.5% (63/600), respectively. Additionally, the prevalence of the infection was found to be higher in the urban (32.1%) than the rural (5.5%) in that study.

The dog is a suitable host for the development of adult *E. multilocularis* (Kapel *et al*., 2006). In endemic areas, roaming dogs that have access to infected rodents are considered to have a high risk of intestinal *E. multilocularis* infection. They are also a potential source of infection for humans (Gottstein *et al*., 2001). Dogs are also a source of concern for non-endemic areas, as infected companion animals may carry the parasite across country borders (Hojgård *et al*., 2012). These indicate that dogs cannot be ignored as potential sources of AE for humans. Sixteen (3.6%) *E. multilocularis*-positive stray dogs were found in the study, resulting in the report of *E. multilocularis* infection in dogs for the first time in Turkey. There have been several reports of *E. multilocularis* infection in dogs from highly endemic regions in some countries. Many studies in China have reported prevalence rates in the range of 3–36% (Zhang *et al*., 2006; Yang *et al*., 2009). Epidemiological data showed 5% in Kazakhstan (Torgerson

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et al., 2009), 18% in Kyrgyzstan (Ziadinov *et al.*, 2008), 0.2–1.1% in Japan (Morishima *et al.*, 2006; Yamamoto *et al.*, 2006) and 7% in Iran (Beiromvand *et al.*, 2011). Despite the high prevalence of *E. multilocularis* in foxes in European countries, a relatively low prevalence of *E. multilocularis* in dogs was determined (Oksanen *et al.*, 2016), for example, 1.5% in Poland (Karamon *et al.*, 2019), 2.8% in Slovakia (Antolová *et al.*, 2009), Lithuania 0.8% (Bruzinskaite *et al.*, 2009), 0.5% in eastern France (Umhang *et al.*, 2014) and 0.24% in Germany (Dyachenko *et al.*, 2008). The prevalence of *E. multilocularis* (3.6%) in stray dogs reported in Turkey was lower than reports from Asian countries but higher than European countries.

E. multilocularis was once considered a problem unique to rural areas due to the habitat requirements of its hosts. However, Deplazes *et al.* (2004) documented the presence of *E. multilocularis* near urban areas. This situation could be related to the anthropogenic food resources for foxes as they adapt to synanthropic life (Deplazes *et al.*, 2004). Regarding dog infections, the *E. multilocularis* peridomestic cycle is also known to exist through dogs preying on infected rodents in close proximity to human settlements in endemic areas (Kamiya *et al.*, 2006; Vaniscotte *et al.*, 2011). Avcioglu *et al.* (2017*a*) previously reported that rodents captured from Erzurum's urban areas also exhibited *E. multilocularis* positivity. Recently, Avcioglu *et al.* (2021) reported a relatively higher prevalence of *E. multilocularis* in fox carcasses and faecal samples from Erzurum's central district counties, suggesting that the foxes in these counties have adapted to the human environment. The higher prevalence of *E. multilocularis* in intermediated host (rodent) and definitive host (fox) is higher in urban areas close to the settlements and will inevitably be a source of infection for stray dogs in the city. Despite the low prevalence of *E. multilocularis* in stray dogs, they may be an important source of infection for humans due to their close contact. The risk of transmission from infected dogs to humans by shedding parasitic eggs remains a significant concern. In endemic areas, data of the infection prevalence among dogs is essential for understanding the risk for human AE and guiding recommendations for the prevention of infections in dogs.

Conclusion

The study has clarified *Echinococcus* spp. infection in stray dogs, verifying the extensive knowledge of the definitive host in a region endemic for AE and CE. Our study confirmed that both *E. granulosus s.l.* and *E. multilocularis* were present in stray dogs in Erzurum province. The occurrence of *E. multilocularis* in dogs was revealed for the first time in Turkey. Notably, the presence of *E. ortleppi* was reported for the first time in Turkey. Dogs may be regarded as epidemiologically important components of the *E. multilocularis* life cycle because of their closer relationship with humans than sylvatic final hosts. The results of the study indicate a significant public health risk for human AE and CE and provide important baseline data on *Echinococcus* spp. infection in dogs for the design of control strategies.

Author contribution. Conceptualization and methodology: HA, EG; Investigation: HA, EG, IB, RD, MA, MMB, HG, SY; Writing – Original draft: HA, EG; Writing – Review & editing: HA, EG. All authors approved the final version of the manuscript before submission.

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