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Copro-PCR prevalence of *Echinococcus granulosus* infection in dogs in Kerman, south-eastern Iran

S.R. Mirbadie[1], H. Kamyabi[2], M.A. Mohammadi[3], S. Shamsaddini[2] and M.F. Harandi[2]*

[1]School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran: [2]Research Center for Hydatid Disease in Iran, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran: [3]Department of Medical Laboratory Sciences, Sirjan School of Medical Sciences, Kerman University of Medical Sciences, Kerman, Iran

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Abstract

The main objective of this study was to determine the prevalence of taeniid parasites and the specific detection of *Echinococcus granulosus* using copro-DNA polymerase chain reaction (PCR) analysis in the stray dogs of Kerman, south-eastern Iran. From September 2013 to May 2014, faecal samples of stray dogs were collected from different parts of the city of Kerman and its suburbs. Faecal samples from dogs were collected randomly within 24 h of defecation. All samples were transferred to the research lab and coprological examinations were conducted by the formalin–ether concentration method. In the microscopically positive samples, mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) specific primers were used to determine the taeniid identity of the infection. In addition, another set of primers was used for the specific diagnosis of *E. granulosus* sensu lato. In total, 307 faecal samples from stray dogs were examined for the presence of the parasites. Taeniidae eggs were detected in 34 dogs (11.07%). All 34 taeniid-positive specimens were PCR positive for *cox1* (444 bp). Of all taeniid-positive specimens, 21 samples (6.8% of all dog specimens) were positive according to primers specific for *E. granulosus*. The findings of the present study revealed that canine echinococcosis is prevalent in the stray dogs in Kerman. The findings of the present study have important implications for hydatid control programmes in the area.

Introduction

Cystic echinococcosis (CE) is a human zoonotic infection caused by the small tapeworm, *Echinococcus granulosus*, with a life cycle involving dogs and livestock animals as definitive and intermediate hosts, respectively. Human CE is a cosmopolitan infection found in different parts of the world, including Europe, Central Asia, China, Africa, America and the Middle East, including Iran. CE is endemic throughout Iran, causing significant medical and economic losses, and relatively high prevalence rates have been reported in dogs, livestock and humans (Lahmar *et al*., 2007). The annual surgical incidence of CE is estimated at 0.8–1.73 per 100,000 in Iran (Harandi *et al*., 2012), and 1% of admissions to surgical wards in the country are believed to be attributed to human CE (Rokni, 2008). Sheep, goats, cattle and camels are the major intermediate host species of the parasite in Iran and CE prevalence rates of 8–32% have been reported from different regions of the country (Harandi *et al*., 2012).

*Fax: +98-34-332 57478
E-mail: fasihi@kmu.ac.ir

Dogs have been considered to be key players in the epidemiology and transmission of CE. The dog population in Iran is estimated at 3.5–11.5 million, most of them being unowned/stray dogs (Harandi *et al.*, 2011). A common source of infection for dogs is offal from infected livestock. Knowledge of the prevalence of echinococcosis in dogs is an important part of hydatid control programmes (Budke *et al.*, 2013).

Various methods have been applied for the specific identification of *E. granulosus* in dogs. Among them, intestinal scraping, copro-ELISA (enzyme-linked immunosorbent assay) and copro-PCR (polymerase chain reaction) are the most reliable techniques. Various studies have used copro-PCR as a reliable method for specific identification of taeniid eggs recovered from faecal specimens and/or environmental samples (Abbasi *et al.*, 2003; Lahmar *et al.*, 2007).

Several studies have demonstrated the prevalence of canine echinococcosis in Iran. Studies conducted in the north-eastern parts of the country showed high rates of *E. granulosus* infection in stray dogs using copro-PCR analysis (Beiromvand *et al.*, 2011; Borji *et al.*, 2013). In Kerman city the prevalence of *E. granulosus* infection in stray dogs was recorded as 7.4%; however, the study was conducted in the 1990s (Sharifi & Zia-Ali, 1996) and no recent data are available for stray dogs in this region.

The purpose of the present study was to determine the prevalence of *E. granulosus* infection among canine definitive hosts, using copro-PCR, in Kerman, south-eastern Iran.

Materials and methods

Collection and examination of samples

Kerman, the largest province of Iran, is situated in south-eastern Iran, covering an area of 182,000 km^2. The province lies between latitude 26°29′ and 31°58′N and longitude 54°20′ and 59°34′E. It is located in the arid and semi-arid zones, and the average annual rainfall is low and decreases towards the south-east. Kerman city is the capital of the province, with an estimated population of 722,000 in 2011 and an area of 185 km^2 (Statistical Center of Iran, 2015).

From September 2013 to May 2014, a total of 307 faecal samples of stray dogs were collected from different parts of the city of Kerman and its suburbs. From each of ten regions in the Kerman metropolitan area 30 faecal samples were collected, namely Ekhtiarabad, Zangiabad, Kazemabad, Chatroud, Kouhpayeh, Sarasiab Farsangi, Joupar, Mahyabad, Mahan and the city of Kerman. The coordinates ranged from 30°03′24″ to 30°36′16″N and from 56°50′37″ to 57°17′12″E.

The faecal samples were collected mostly from places where high numbers of stray dogs were frequently observed. Samples from dogs were collected less than 24 h after defecation. Fresh dog faeces were identified by characteristics such as colour, shape, moisture and smell. To avoid duplicate faecal sampling of each individual dog, samples were taken 200 m apart.

The faecal specimens were transferred to the research laboratory of the Department of Parasitology, Kerman University of Medical Sciences. Each sample was stored separately in a plastic bag for at least 8–12 days at −80°C to inactivate *Echinococcus* eggs before processing further (Abbasi *et al.*, 2003). The coprological examination was conducted using the formalin–ether concentration method. The microscopic examination was performed at ×400 magnification. All those samples found positive for taeniid-type eggs were selected for further molecular studies.

Molecular analysis

Microscopically positive specimens were subjected to DNA extraction. Faecal samples (weight 1 g) were freeze-thawed ten times in lysis buffer and DNA extraction was performed using Exgene Stool Mini Kit (GeneAll, South Korea), according to the manufacturer's instructions. The eluted DNA was stored at 4−8°C until used in PCR amplification. Two primers, JB3, 5′-TTTTTTGGGCATCCTGAGGTTTAT-3′ (forward), and JB4.5, 5′-TAAAGAAAGAACATAATGAAAATG-3′ (reverse), were used to amplify a taeniid-specific fragment of 444 bp of the *cox1* gene (Bowles *et al.*, 1992). The PCR was carried out in a volume of 25 µl in a FlexCycler (Analytik Jena, Jena, Germany). The thermal profile included and initial denaturation at 94°C for 5 min; followed by 35 cycles, each of 30 s at 94°C, 45 s at 50°C and 35 s at 72°C; and a final extension of 10 min at 72°C. The amplification products were subjected to electrophoresis on 2% agarose gel in TAE buffer (Tris–acetic acid–EDTA).

The primers Eg1121a, 5′-GAATGCAAGCAGCAGATG-3′ (forward) and Eg1122a, 5′-GAGATGAGTGAGAAGGA GTG-3′ (reverse) were used for specific identification of *E. granulosus* sensu lato. This primer pair amplifies a 133-bp segment from a repeat unit of the parasite genome (Abbasi *et al.*, 2003). The thermal profile was as follows: 5 min at 94°C; followed by 35 cycles, each of 30 s at 94°C, 1 min at 55°C and 1 min at 72°C; and a final extension of 5 min at 72°C. Templates of DNA from previously sequenced taeniid species, i.e. *E. granulosus* sensu lato, *Taenia multiceps*, *T. hydatigena*, *T. ovis* and *T. saginata* (Rostami *et al.*, 2013a, b, 2015a, b), were used as controls to test species specificity of the primer. Seven microscopically negative specimens were randomly selected and subjected to PCR amplification using both taeniid-specific (JB3 and JB4.5) and *E. granulosus*-specific (Eg1121a and Eg1122a) primer pairs, to test the accuracy of microscopic examination.

Results

Of 307 faecal samples examined microscopically, *Taenia*-type eggs were found in 34 (11.1%) of stray dogs. All microscopically positive specimens were subjected to taeniid-specific PCR amplification and a 100% concordance with microscopy was obtained. Of the 34 taeniid isolates identified, 21 samples (6.8%) were shown to be *E. granulosus* sensu lato using a species-specific primer pair. The distribution of taeniid-positive faecal samples in different localities in Kerman and its suburbs showed that the western and eastern regions had the highest proportion of positive specimens (table 1).

Of the seven randomly selected microscopically negative specimens tested with both taeniid- and *E. granulosus*-specific primer pairs, none of them showed positive results, indicating the accuracy of microscopic

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Table 1. The prevalence (%) of taeniid/*Echinococcus granulosus* eggs in faecal samples of stray dogs from different localities of Kerman and its suburbs.

Location	No. of samples examined	Taeniidae, number (%)	*E. granulosus*, number (%)	True prevalence* (%)
North	103	15 (14.6)	8 (7.7)	9.9
South	82	6 (7.3)	4 (4.8)	6.2
West	21	4 (19)	3 (14.3)	18.3
East	63	7 (11.1)	6 (9.5)	12.2
Central	38	2 (5.3)	0 (0)	0
Total	307	34 (11.07)	21 (6.8)	8.8

*Based on the estimated sensitivity of copro-PCR by Ziadinov *et al.* (2008).

examination. Furthermore, none of the *Taenia* isolates were amplified when applied as templates for PCR using *E. granulosus*-specific primers.

Discussion

Understanding the epidemiology of canine echinococcosis is essential for the implementation of CE control programmes. The present epidemiological investigation revealed that *E. granulosus* infection is present in 6.8% of dog samples in Kerman. Table 2 summarizes the most recent data on the prevalence of canine echinococcosis around the world. The rate of dog infection ranges from 6% in Kazakhstan to 25.9% in Tunisia (Stefanic *et al.*, 2004; Lahmar *et al.*, 2007). In a recent study in Uganda, 12.2% of household dogs were found to be infected with *E. granulosus* (Oba *et al.*, 2015). In Sichuan province on the Tibetan plateau, 3.6 and 3.2% of dogs were found to be infected in Shiqu and Honglong regions, respectively, using copro-DNA PCR (Moss *et al.*, 2013).

Canine echinococcosis is widespread in Iran and several copro-DNA studies have indicated that dogs are commonly infected with the parasite, especially in north-western and north-eastern parts of the country. In north-western Iran, 23.7% of stray dogs were found to be infected on the Moghan plain (Mobedi *et al.*, 2013). A couple of studies in the north-eastern part of the country, using the copro-PCR technique, revealed that 20 and 16.9% of dogs were infected by *E. granulosus* (Beiromvand *et al.*, 2011; Borji *et al.*, 2013).

Kerman is situated in the south-eastern part of Iran, where the climate is arid and the humidity is very low at most times of the year. Kerman climatic conditions do not favour the survival of parasites in the environment. The annual precipitation in Kerman is 135 mm and the city is located on a semi-arid plateau, with sunny days at most times of the year. In a study in 1996 using necropsy, 7.4% *Echinococcus* infection was recorded in dogs of the Kerman area (Sharifi & Zia-Ali, 1996). According to Mirzaei & Fooladi (2012) 1.4% of owned dogs referred to a veterinary hospital were found to be infected with taeniid eggs. In the present study, *Echinococcus*-positive samples were most frequently found in western (14.3%) and eastern (9.5%) parts of the region (table 1). Fifteen per cent of the samples collected around the city abattoir were found to be infected. Dogs roaming around abattoirs have access to the offal and garbage of the abattoir, and this increases the chances of acquiring *Echinococcus* infection. No dog faeces were found to be infected in the central parts of the city, which are more urbanized and where access to livestock viscera for dogs is less probable.

Three *Taenia* species – T. *hydatigena*, *T. ovis* and *T. pisiformis* – were used as controls by Abbasi *et al.* (2003) to test the species specificity of the primers. Another two species of *Taenia* (*T. multiceps* and *T. saginata*) were used in our study, and negative results confirmed that the primer pair is *E. granulosus*-specific and none of the major taeniid tapeworms of dogs could be amplified in copro-PCR analysis.

Using microscopy, the faecal examination of stray dogs would be expected to underestimate the actual parasitism rate when compared to other methods such as the copro-PCR test (Hartnack *et al.*, 2013). Ziadinov *et al.* (2008) found that egg isolation followed by PCR for diagnosing *E. granulosus* in dogs had a sensitivity of 78%, because some of the parasites are in a prepatent phase and are not detectable by purgation and/or microscopy. In contrast, prepatent infections could be diagnosed accurately by DNA-based methods (Al-Sabi *et al.*, 2007; Lahmar *et al.*, 2007). Therefore molecular methods are more sensitive than conventional intestinal scraping or faecal microscopy. Using the 78% estimated sensitivity of copro-PCR obtained by Ziadinov *et al.* (2008), a prevalence rate of 8.8% could be inferred by adjusting observed prevalence values in the present study (table 1).

Nevertheless, as the faecal examinations for taeniid eggs had been carried out prior to the PCR, we are unable to discuss any prepatent infection in dogs. In conclusion, the study showed that canine echinococcosis is widespread in south-eastern areas of the country and copro-DNA techniques can be used as reliable tools for

Table 2. The prevalence (%) of canine echinococcosis in faecal samples of dogs from seven countries, using the copro-PCR method.

Country	Number of faecal samples (%)	Reference
China	276 (3.6)	Moss *et al.*, 2013
Iran	59 (23.7)	Mobedi *et al.*, 2013
Iran	50 (20)	Borji *et al.*, 2013
Iran	307 (6.8)	Present study
Kazakhstan	131 (6)	Stefanic *et al.*, 2004
Lithuania	240 (3.8)	Bruzinskaite *et al.*, 2009
Palestine	93 (18.2)	Al-Jawabreh *et al.*, 2015
Tunisia	58 (25.9)	Lahmar *et al.*, 2007
Uganda	261 (12.2)	Oba *et al.*, 2015

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field surveys and CE surveillance campaigns in endemic areas.

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Conflict of interests

None.

References

Abbasi, I., Branzburg, A., Campos-Ponce, M., Hafez, S. K.A., Raoul, F., Craig, P.S. & Hamburger, J. (2003) Copro-diagnosis of *Echinococcus granulosus* infection in dogs by amplification of a newly identified repeated DNA sequence. *American Journal of Tropical Medicine and Hygiene* **69**, 324–330.

Al-Jawabreh, A., Dumaidi, K., Ereqat, S., Nasereddin, A., Al-Jawabreh, H., Azmi, K., Al-Laham, N. & Abdeen, Z. (2015) Incidence of *Echinococcus granulosus* in domestic dogs in Palestine as revealed by copro-PCR. *PLOS Neglected Tropical Diseases* **9**, 3934.

Al-Sabi, M.N.S., Kapel, C.M.O., Deplazes, P. & Mathis, A. (2007) Comparative copro-diagnosis of *Echinococcus multilocularis* in experimentally infected foxes. *Parasitology Research* **101**, 731–736.

Beiromvand, M., Akhlaghi, L., Massom, S.H.F., Mobedi, I., Meamar, A.R., Oormazdi, H., Motevalian, A. & Razmjou, E. (2011) Detection of *Echinococcus multilocularis* in carnivores in Razavi Khorasan province, Iran using mitochondrial DNA. *PLOS Neglected Tropical Diseases* **5**, 1379.

Borji, H., Naghibi, A. & Jahangiri, F. (2013) Copro-DNA test for diagnosis of canine echinococcosis. *Comparative Clinical Pathology* **22**, 909–912.

Bowles, J., Blair, D. & McManus, D.P. (1992) Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. *Molecular and Biochemical Parasitology* **54**, 165–173.

Bruzinskaite, R., Sarkunas, M., Torgerson, P.R., Mathis, A. & Deplazes, P. (2009) Echinococcosis in pigs and intestinal infection with *Echinococcus* spp. in dogs in southwestern Lithuania. *Veterinary Parasitology* **160**, 237–241.

Budke, C.M., Carabin, H., Ndimubanzi, P.C., Nguyen, H., Rainwater, E., Dickey, M., Bhattarai, R., Zeziulin, O. & Qian, M.B. (2013) A systematic review of the literature on cystic echinococcosis frequency worldwide and its associated clinical manifestations. *American Journal of Tropical Medicine and Hygiene* **88**, 1011–1027.

Harandi, M.F., Moazezi, S.S., Saba, M., Grimm, F., Kamyabi, H., Sheikhzadeh, F., Sharifi, I. & Deplazes, P. (2011) Sonographical and serological survey of human cystic echinococcosis and analysis of risk factors associated with seroconversion in rural communities of Kerman, Iran. *Zoonoses and Public Health* **58**, 582–588.

Harandi, M.F., Budke, C.M. & Rostami, S. (2012) The monetary burden of cystic echinococcosis in Iran. *PLOS Neglected Tropical Diseases* **6**, e1915.

Hartnack, S., Budke, C.M., Craig, P.S., Jiamin, Q., Boufana, B., Campos-Ponce, M. & Torgerson, P.R. (2013) Latent-class methods to evaluate diagnostic tests for *Echinococcus* infections in dogs. *PLOS Neglected Tropical Diseases* **7**, e2068.

Lahmar, S., Lahmar, S., Boufana, B., Bradshaw, H. & Craig, P.S. (2007) Screening for *Echinococcus granulosus* in dogs: comparison between arecoline purgation, coproELISA and coproPCR with necropsy in prepatent infections. *Veterinary Parasitology* **144**, 287–292.

Mirzaei, M. & Fooladi, M. (2012) Prevalence of intestinal helminthes in owned dogs in Kerman city, Iran. *Asian Pacific Journal of Tropical Medicine* **5**, 735–737.

Mobedi, I., Zare Bidaki, M., Siavashi, M.R., Naddaf, S. R., Kia, E.B. & Mahmoudi, M. (2013) Differential detection of *Echinococcus* spp. copro-DNA by nested-PCR in domestic and wild definitive hosts in Moghan Plain, Iran. *Iranian Journal of Parasitology* **8**, 107–113.

Moss, J.E., Chen, X., Li, T., Qiu, J., Wang, Q., Giraudoux, P., Ito, A., Torgerson, P.R. & Craig, P. (2013) Reinfection studies of canine echinococcosis and role of dogs in transmission of *Echinococcus multilocularis* in Tibetan communities, Sichuan, China. *Parasitology* **140**, 1685–1692.

Oba, P., Ejobi, F., Omadang, L., Chamai, M., Okwi, A.L., Othieno, E., Inangolet, F.O. & Ocaido, M. (2015) Prevalence and risk factors of *Echinococcus granulosus* infection in dogs in Moroto and Bukedea districts in Uganda. *Tropical Animal Health and Production* **48**, 249–254.

Rokni, M. (2008) The present status of human helminthic diseases in Iran. *Annals of Tropical Medicine and Parasitology* **102**, 283–295.

Rostami, S., Salavati, R., Beech, R.N., Sharbatkhori, M., Babaei, Z., Saedi, S. & Harandi, M.F. (2013a) Cytochrome c oxidase subunit 1 and 12S ribosomal RNA characterization of *Coenurus cerebralis* from sheep in Iran. *Veterinary Parasitology* **197**, 141–151.

Rostami, S., Talebi, S., Babaei, Z., Sharbatkhori, M., Ziaali, N., Rostami, H. & Harandi, M.F. (2013b) High resolution melting technique for molecular epidemiological studies of cystic echinococcosis: differentiating G1, G3, and G6 genotypes of *Echinococcus granulosus* sensu lato. *Parasitology Research* **112**, 3441–3447.

Rostami, S., Salavati, R., Beech, R.N., Babaei, Z., Sharbatkhori, M., Baneshi, M.R., Hajialilo, E., Shad, H. & Harandi, M.F. (2015a) Molecular and morphological characterization of the tapeworm *Taenia hydatigena* (Pallas, 1766) in sheep from Iran. *Journal of Helminthology* **89**, 150–157.

Rostami, S., Salavati, R., Beech, R.N., Babaei, Z., Sharbatkhori, M. & Harandi, M.F. (2015b) Genetic variability of *Taenia saginata* inferred from mitochondrial DNA sequences. *Parasitology Research* **114**, 1365–1376.

Sharifi, I. & Zia-Ali, N. (1996) The present status and intensity of *Echinococcus granulosus* infection in

391 stray dogs in rural and urban areas of the city of Kerman, Iran. *Iranian Journal of Public Health* **25**, 13–20.

Statistical Center of Iran (2015) Available at http://amar.sci.org.ir (accessed 4 June 2015).

Stefanic, S., Shaikenov, B.S., Deplazes, P., Dinkel, A., Torgerson, P.R. & Mathis, A. (2004) Polymerase chain reaction for detection of patent infections of *Echinococcus granulosus* ('sheep strain') in naturally infected dogs. *Parasitology Research* **92**, 347–351.

Ziadinov, I., Mathis, A., Trachsel, D., Rysmukhambetova, A., Abdyjaparov, T.A., Kuttubaev, O.T., Deplazes, P. & Torgerson, P.R. (2008) Canine echinococcosis in Kyrgyzstan: using prevalence data adjusted for measurement error to develop transmission dynamics models. *International Journal for Parasitology* **38**, 1179–1190.

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