

Short Communication

Molecular identification of *Echinococcus granulosus* strain in stray dogs from Northeastern Iran

Sara Ghabdian, Hassan Borji*, Abolghasem Naghibi

Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Iran

ARTICLE INFO

Keywords:
Stray dogs
Echinococcus granulosus
Genotype
PCR-RFLP

ABSTRACT

Canine echinococcosis, caused by the adult form of taeniid cestodes of the genus *Echinococcus* is zoonotic and has an epidemiologically worldwide distribution. Dogs infected with *E. granulosus* are the main source of human hydatidosis, however, there is little information on molecular epidemiology of adult *Echinococcus* spp. in stray dogs.

In the present study, 100 stray dogs (48 males and 52 females, 72 adults and 28 juveniles) were collected from Khorasan Razavi province in Northeastern Iran during October 2013 to December 2014. Thirty-eight (21 males and 17 females, 3 juveniles and 35 adults) out of 100 dogs were infected with *E. granulosus*. There were no significant differences in prevalence observed between females (43.5%) and males (34.4%), however, the prevalence of *E. granulosus*, showed a significant increasing trend with increasing host age ($p < 0.05$). Thirty-eight isolated parasites from 38 stray dogs (one parasite per dog) were used for PCR-RFLP analysis of the ITS1 gene. PCR-RFLP analysis showed that all the 38 parasites were *E. granulosus* G1 genotype (common sheep strain). Five PCR products were sent for sequencing. The results of sequencing were similar to those reported by PCR-RFLP analysis and the presence of *E. granulosus* G1 genotype (sheep strain) as dominant genotype in dogs were emphasized. The results of this study suggest that the sheep strain occurs in definitive host in Northeastern Iran. Data presented here are expected to be useful for health and educational authorities responsible for designing and implementing effective measures for disease control.

1. Introduction

Canine echinococcosis (CE) caused by the adult stages of taeniid cestodes of the genus *Echinococcus granulosus* sensu lato is zoonotic and has an epidemiologically worldwide distribution. Dogs infected with *E. granulosus* are the main source of human hydatidosis. However, estimating the prevalence and genotype frequencies of this parasite in definitive host populations is essential not only for measuring the progress of CE control programs, but also for assessing the distribution, host specificity, transmission dynamics, and for risk of infection for humans in a specific area (Barnes et al., 2012; Craig and Larrieu, 2006).

Molecular genetic studies have confirmed that *E. granulosus* is a cryptic species complex including *E. granulosus* sensu stricto (*E. granulosus* s.s., genotypes G1–G3), *E. equinus* (genotype G4), *E. ortleppi* (genotype G5), and *E. canadensis* (genotypes G6–G10) (Thompson and McManus, 2002; Thompson, 2008). There are limited molecular epidemiological surveys aiming to investigate genetic characterization of the *E. granulosus* species complex in stray dogs in Iran and other countries (Stefanic et al., 2004; Zhang et al., 2006; Hüttner et al., 2008;

Parsa et al., 2012; Borji et al., 2013; Boufana et al., 2015; Shariatzadeh et al., 2015). Previous molecular studies on the *E. granulosus* complex have been performed on larval stages of *E. granulosus* isolated from different livestock species including sheep, goat, cattle and camels, revealing the existence of G1 genotypes in the Khorasan Razavi province (Fadakar et al., 2015; Moghaddas et al., 2015). However, in spite of high prevalence of canine echinococcosis in the Khorasan Razavi province (Emampour et al., 2015), there is little information on the molecular epidemiology of adult *Echinococcus* spp. in stray dogs in this region. The present work was therefore undertaken to investigate the molecular characteristics of *Echinococcus* spp. in definitive host in Northeastern Iran.

2. Materials and methods

The present study was conducted in the Khorasan Razavi province, which is located in Northeastern Iran and borders with Afghanistan to the east and Turkmenistan to the north. The region is $> 144,000 \text{ km}^2$ and is situated at 33.30–37.41 north latitude and 56.19–61.18 east

* Corresponding author at: Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, PO Box: 9177948974, Iran.
E-mail address: hborji@um.ac.ir (H. Borji).

longitude.

From October 2013 to September 2014, one hundred stray dogs (48 males and 52 females, 72 adult and 28 juvenile) were randomly selected from different regions of Mashhad and euthanized following ethical approval by the Municipality committee. In order to evaluate the role of the different risk factors for infection of dogs, sex and age of the selected dogs were recorded.

Following necropsy, epithelial scrapings and intestinal contents were passed through 60- and 80-mesh wire sieves. The contents of the sieves were washed into a glass container and examined carefully by microscopy for *E. granulosus*. One worm from each of the 38 parasitized dog was collected, washed three times with physiological saline solution, and stored in 70% ethanol until DNA extraction. Before extracting genomic DNA, the worms were thoroughly washed in distilled water to remove ethanol.

Genomic DNA was extracted using a commercial DNA extraction kit (MBST, Iran) according to the manufacturer's protocol. A 462 bp fragment of the internal transcribed spacer 1 (ITS1) gene was amplified from each isolate and PCR-RFLP was used for genotyping of isolates as described previously (Moghaddas et al., 2015).

The PCR mixture was carried out in a total volume of 25 μ l containing 2.5 mM MgCl₂, 250 μ M dNTP mix, 20 pmol of each primer, 5 μ l of 10 \times PCR buffer, 1.25 U Taq DNA polymerase (MBI, Fermentas) and 2 μ l of template DNA (50 ng DNA) in an automated Thermocycler (Eppendorf, Germany). The following program was used for PCR: an initial denaturation step at 95 °C for 5 min, 35 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C for 45 s, and extension at 72 °C for 45 s, followed by a final extension at 72 °C for 10 min. The PCR products were analyzed by electrophoresis of 5 μ l aliquots in 1.5% agarose gels in TBE buffer with the aid of ethidium bromide staining under UV light.

For PCR-RFLP, the amplified products (16 μ l) were digested with 5 units of the restriction enzyme Bsh1236I (Fermentas, USA) in a final volume of 20 μ l for 4 h. Restriction fragments were visualized by gel electrophoresis through a 3% ethidium bromide-stained agarose gel.

To verify the results of the PCR-RFLP, five PCR products from five different worms were randomly purified and submitted for sequencing (Bioneer, South Korea) in two directions with the use of the same forward and reverse primers, which were employed in the PCR.

3. Result and discussion

During the post-mortem examinations, thirty-eight (38%) dogs were found infected with *E. granulosus* (21 males and 17 females; 3 juveniles and 35 adults) with a mean worm burden of 60 worms (range = ten to 200 worms). No significant differences ($p > 0.05$) in prevalence were observed between females (43.5%) and males (34.4%). However, the prevalence of *E. granulosus* was significantly higher in adult stray dogs compared to the juveniles ($p < 0.05$).

Thirty-eight worms isolated from 38 parasitized dogs (one worm per each dog) were used for the molecular identification by PCR-RFLP method. The PCR products of the ITS1 region showed an expected fragment size of 462 bp (Figs. 1). The PCR-RFLP results of the ITS1 showed that all worms were *E. granulosus* G1 genotype (Fig. 2). The sequences of the G1 strains showed 99% homology with a G1 sequence, which was accessible under accession number AF 13269501 in GenBank. Due to the lack of representative and well-documented data on the genotypes of *E. granulosus* originating from dogs in Northeastern Iran, the present study provides the first comprehensive strain characterization of dog isolates from this region.

The results presented here demonstrated that the *E. granulosus* G1 genotype was the dominant in dogs in Northeastern Iran. The results were in agreement with previous studies in other regions of Iran (Parsa et al., 2012; Shariatzadeh et al., 2015). Available molecular data from sheep, goats and camel in the Khorasan Razavi province (Fadakar et al., 2015; Moghaddas et al., 2015) reveal that *E. granulosus* G1 genotype

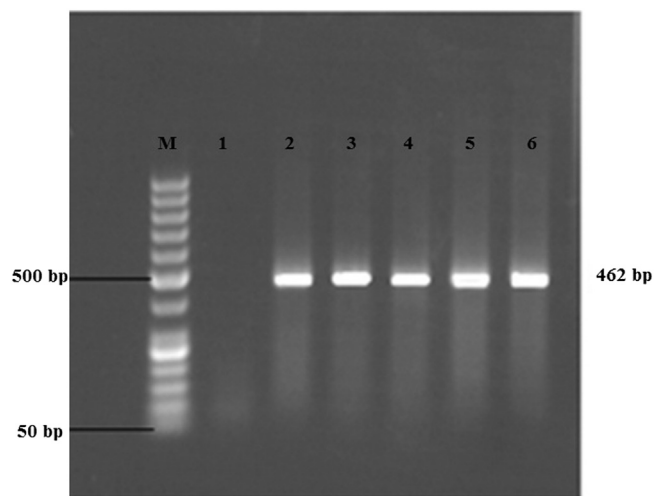


Fig. 1. Agarose gel electrophoresis of PCR amplification (462 bp) provided from dogs samples (Lane 1: positive control, Lane 2: negative control, Lanes 3–6 positive samples), M: Ladder 50 bp.

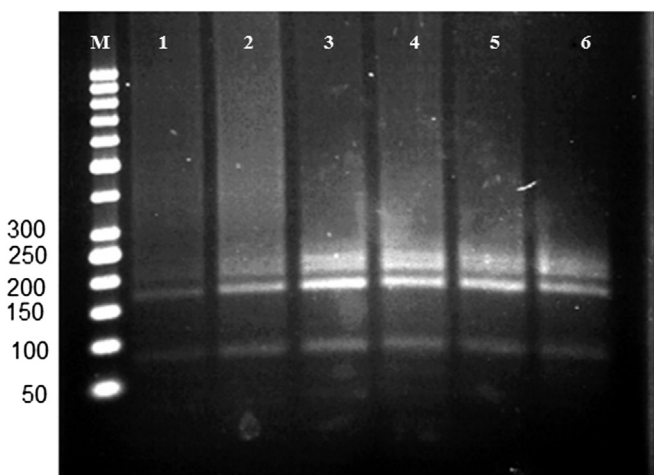


Fig. 2. Agarose gel electrophoresis of ITS1-PCR products of *E. granulosus* isolates from dogs after digestion with the restriction enzyme Bsh1236I. Lanes 1–6 cutting products that are G1 genotype, M: ladder 50 bp.

was circulating in this region confirming the existence of a sheep-dog cycle. This information will have an important impact on the preventive measures against hydatid disease in the region.

It should be noted that echinococcosis is a major public health concern in many regions of Iran (Rokni, 2009; Borji and Parandeh, 2010; Borji et al., 2012a, 2012b). In most cases, dogs are the main definitive host species involved in the transmission of *Echinococcus* infection to humans. The current molecular epidemiological situation of canine echinococcosis, however, is unknown in many regions of Iran. More research is also necessary to assess the relevance of sylvatic populations as reservoir of disease for human and domestic species. This includes not only defining the prevalence of *Echinococcus* spp. in wild host species, but also defining the isolates obtained from definitive host species by molecular studies. Finally, it is worth mentioning that canine echinococcosis is a preventable infection. In order to develop efficient preventive and control strategies for echinococcosis, better knowledge of transmission of the *E. granulosus* complex is necessary.

Conflict of interest

All authors declare no conflict of interest.

Acknowledgements

This study was supported financially by Ferdowsi university of Mashhad with Grant number 25894. We thank Mr. H. Eshrati for his technical assistance during data collection.

References

- Barnes, T.S., Deplazes, P., Gottstein, B., Jenkins, D.J., Mathis, A., Siles-Lucas, M., Torgerson, P.R., Ziadinov, I., Heath, D.D., 2012. Challenges for diagnosis and control of cystic hydatid disease. *Acta Trop.* 123, 1–7.
- Borji, H., Parandeh, S., 2010. The abattoir condemnation of meat because of parasitic infection, and its economic importance: results of a retrospective study in north-eastern Iran. *Ann. Trop. Med. Parasitol.* 104, 2010–2011.
- Borji, H., Azizzadeh, M., Kamelli, M., 2012a. A retrospective study of abattoir condemnation due to parasitic infections and its economic importance in Ahwaz, southwestern of Iran. *J. Parasitol.* 98 (5), 954–957.
- Borji, H., Azizzadeh, M., Afsai, A., 2012b. An abattoir-based study of hydatidosis in livestock in Mashhad, Iran. *J. Helminthol.* 86 (2), 233–236.
- Borji, H., Naghibi, A., Jahangiri, F., 2013. Copro-DNA test for diagnosis of canine echinococcosis. *Comp. Clin. Pathol.* 22, 909–912.
- Boufana, B., Lett, W., Lahmar, S., Griffiths, A., Jenkins, D.J., Buishi, I., Engliez, S.A., Alrefadi, M.A., Eljaki, A.A., Elmestiri, F.M., Reyes, M.M., Pointing, S., Al-Hindi, A., Torgerson, P.R., Okamoto, M., Craig, P.S., 2015. Canine echinococcosis: genetic diversity of *Echinococcus granulosus sensu stricto* (s.s.) from definitive hosts. *J. Helminthol.* 89 (6), 689–698.
- Craig, P.S., Larrieu, E., 2006. Control of cystic echinococcosis/hydatidosis: 1863–2002. *Adv. Parasitol.* 61, 443–508.
- Emampour, R., Borji, H., Naghibi, A., 2015. An epidemiological survey on intestinal helminths of stray dogs in Mashhad, North-east of Iran. *J. Parasit. Dis.* 39 (2), 266–271.
- Fadakar, B., Tabatabaei, N., Borji, H., Naghibi, A., 2015. Genotyping of *Echinococcus granulosus* from goats and sheep indicating G7 genotype in goats in the northeast of Iran. *Vet. Parasitol.* 214, 204–207.
- Hüttner, M., Nakao, M., Wassermann, T., Siefert, L., Boomker, J.D.F., Dinkel, A., Sako, Y., Mackenstedt, U., Romig, T., Ito, A., 2008. Genetic characterization and phylogenetic position of *Echinococcus felidis* (Cestoda: Taeniidae) from the African lion. *Int. J. Parasitol.* 38, 861–868.
- Moghaddas, E., Borji, H., Naghibi, A., Shayan, P., Razmi, G.R., 2015. Molecular genotyping of *Echinococcus granulosus* from dromedaries (*Camelus dromedarius*) in eastern Iran. *J. Helminthol.* 89, 100–104.
- Parsa, F., Fasihi Harandi, M., Rostami, S., Sharbatkhorji, M., 2012. Genotyping *Echinococcus granulosus* from dogs from Western Iran. *Exp. Parasitol.* 132, 308–312.
- Rokni, M.B., 2009. Echinococcosis/hydatidosis in Iran. *Iran. J. Parasitol.* 4, 1–16.
- Shariatzadeh, S.A., Spotin, A., Gholami, S., Fallah, E., Hazratian, T., Mahami-Oskouei, M., Montazeri, F., Moslemzadeh, H.R., Shahbazi, A., 2015. The first morphometric and phylogenetic perspective on molecular epidemiology of *Echinococcus granulosus sensu lato* in stray dogs in a hyperendemic Middle East focus, northwestern Iran. *Parasit. Vectors* 6 (8), 409.
- 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. *Parasitol. Res.* 92, 347–351.
- Thompson, R.C.A., 2008. The taxonomy, phylogeny and transmission of *Echinococcus*. *Exp. Parasitol.* 119, 439–446.
- Thompson, R.C.A., McManus, D.P., 2002. Towards a taxonomic revision of the genus *Echinococcus*. *Trends Parasitol.* 18, 452–457.
- Zhang, Y., Bart, J.M., Giraudoux, P., Craig, P., Vuitton, D., Wen, H., 2006. Morphological and molecular characteristics of *Echinococcus multilocularis* and *Echinococcus granulosus* mixed infection in a dog from Xinjiang, China. *Vet. Parasitol.* 139 (1–3), 244–248.