

## Original papers

# Genetic identification of cattle hydatid cyst isolates from northeast and southwest of Iran

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**ABSTRACT.** Cystic Echinococcosis (CE) caused by the larval stages of taeniid cestodes of the genus *Echinococcus* is a neglected helminth zoonosis affecting humans and various animal species. The aim of this study was to assess the fertility of cattle from two provinces in the northeast and southwest of Iran, and molecularly characterize the hydatid cysts collected from them. From 5000 carcasses of naturally infected cattle inspected from October 2013 to December 2015, 70 hydatid cysts were collected in Mashhad in the northeast and 50 from Ahvaz in the southwest. All 120 samples were identified as *E. granulosus sensu stricto* (G1, common sheep strain) by PCR-RFLP analysis and sequencing of the ITS1 gene. All cysts recovered from these animals were sterile, highlighting the lower importance of cattle in the transmission of the “sheep strain”. The fact that the zoonotic *E. granulosus s.s.* is dominant makes CE an important public health concern in Iran.

**Key words:** cattle, *Echinococcus granulosus*, genotype, PCR-RFLP

## Introduction

Cystic echinococcosis (CE or hydatidosis) caused by the larval stages of taeniid cestodes of the genus *Echinococcus* is an important zoonotic infection that is widespread in many countries. The life cycle of this parasite is indirect, requiring two mammalian hosts. The adult worm, which lives in the small intestine of dogs and other canids (definitive hosts), lays eggs that are excreted with the faeces of the infected animal, thus contaminating the environment. Domestic or wild ungulates (intermediate hosts) acquire the infection through accidental ingestion of the eggs, which in turn develop into the larval stage (metacestode) in internal organs and ultimately cause the pathology associated with CE. The transmission cycle is completed when definitive hosts eat these infected organs.

Molecular genetic studies have confirmed that *E. granulosus* is in fact a complex of species/genotypes which show a marked genetic variability. Therefore, at least ten different genotypes (G1-G10) have been identified within the *E. granulosus*

complex [1]. These include two sheep strains (G1 and G2), two bovid strains (G3 and G5), a horse strain (G4), a camel strain (G6), two pig strains (G7 and G9) and two cervid strains (G8 and G10). Genotypes G1-G3 cluster firmly together to form the taxon *E. granulosus sensu stricto* (*E. granulosus s.s.*). Each of these genotypes can successfully develop fertile cysts in specific intermediate hosts, while the most frequent outcome of infection in non-typical intermediate hosts is the production of infertile cysts [1].

Cattle are accidental hosts for *E. granulosus* of sheep origin, and the resulting cysts in cattle grow poorly and are rarely fertile in several parts of the world [2,3]. However, a form of *Echinococcus* named *E. ortleppi* has been well characterized throughout Europe, as well as parts of Africa (Sudan, Kenya and Ethiopia), Asia (India) and South America (Brazil) [4]. Genotypes G5 (*E. ortleppi*) has been warranted species status in recent years, showing strong host specificities which affect only cattle [1].

Molecular epidemiological surveys aiming to investigate the genetic diversity of *E. granulosus* in



Fig. 1. Map of Iran, showing the geographical locations of Khorasan Razavi and Khuzestan provinces where *E. granulosus* samples were collected from cattle

cattle have been carried out in most regions of Iran, and these indicated that the G1 genotype was the most prevalent [5–9]. Moreover, genotypes G3 and G6 have been reported from cattle in some regions of Iran [6,10–12]. However, there is limited available information concerning the distributions of genetic variants of this parasite in cattle of the northeast and southwest of Iran [13]. Therefore, the aim of the present study was to extend our data in these regions of Iran to investigate the fertility and molecular epidemiology of hydatid cysts obtained from this animal.

## Materials and Methods

This study was carried out in two provinces in the northeast and southwest of Iran, including Khorasan Razavi and Khuzestan provinces, with approximately 458,470 and 190,841 cattle, respectively (<http://dla.agri-jahad.ir>). Khorasan Razavi province in the northeast of country rests on the border with both Afghanistan and Turkmenistan; it has an area of 173,115 km<sup>2</sup>, receives 100–500 mm of rain/year, and its air temperatures range from –8 to +38°C. Khuzestan province in contrast, is in the

southwest of the country; it borders Iraq and the Persian Gulf and covers an area of 63,238 km<sup>2</sup>. This province can be divided into two regions, the rolling hills and mountainous regions north of the Ahvaz Ridge, and the plains and marsh lands to its south. The climate of Khuzestan is generally very hot and occasionally humid, particularly in the south, while winters are much colder and dry. The average annual rainfall in this area is around 230 mm and air temperature ranges between 4°C in the cold season and 50°C in the warm season (Fig. 1).

In total, 5000 carcasses were inspected from October 2013 to December 2015. From these, 70 hydatid cysts were collected from naturally infected cattle in Mashhad in the northeast and 50 from carcasses in Ahvaz in the southwest. Hydatid fluid was aspirated from each cyst, centrifuged and the pellet examined for the presence of protoscoleces in order to determine cyst fertility. Sterile, calcified or caseated cysts were recorded as infertile (Fig. 2).

All 120 samples isolated from cattle had uniform characteristics and were sterile. For molecular analysis, the germinal layer of the cysts was collected, rinsed five times with sterile phosphate buffer (PBS, pH 7.4) and stored in 70% ethanol until



Fig. 2. A huge hydatid cyst from liver of one infected cattle. The weight of this cyst was over 20 kg.

DNA isolation. Genomic DNA was extracted from the germinal layer using a commercial DNA extraction kit (MBST, Iran) according to the manufacturer's protocol. A 462 bp fragment of the ribosomal DNA internal transcribed spacer 1 (ITS1) gene was amplified from each isolate and PCR-RFLP was used to genotype the isolates as described in our previous study [14].

The PCR procedure took place in a total volume of 25  $\mu$ l containing 2.5 mM  $MgCl_2$ , 250  $\mu$ M dNTP mix, 20 pmol of each primer, 5  $\mu$ l of 10 $\times$  PCR buffer, 1.25 U Taq DNA polymerase (MBI, Fermentas) and 2  $\mu$ l of template DNA (50 ng DNA) in an automated Thermocycler (Eppendorf, Germany). The following program was used: an initial denaturation step at 95°C for five minutes, 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 minutes. The PCR products were analysed by electrophoresis of 5  $\mu$ l aliquots in 1.5% agarose gels in TBE buffer with the aid of ethidium bromide staining under UV condition.

For PCR-RFLP, the amplified products (16  $\mu$ l)

were digested with five units of the restriction endonuclease Bsh1236I (5U, Fermentas) in a final volume of 20  $\mu$ l for four hours. Restriction fragments were visualised by gel electrophoreses through a 3% ethidium bromide- stained agarose gel.

Five PCR products from each of the different cutting patterns of ITS1 were purified and submitted to be sequenced (Bioneer, South Korea). Sequencing took place in two directions with the use of the same forward and reverse primers employed in the PCR.

## Results

The PCR product of ITS1 revealed an expected fragment of 462 bp in length in cattle (Fig. 3).

No differences were observed in the electrophoresis patterns of all isolates. The analysis of *E. granulosus* cysts by PCR-RFLP of ITS1, following digestion with Bsh1236I, showed that all 120 *E. granulosus* cysts in cattle (100%) were G1 strain (Fig. 4).

The sequence of the G1 strain demonstrated 99% homology with the G1 reference sequences stored



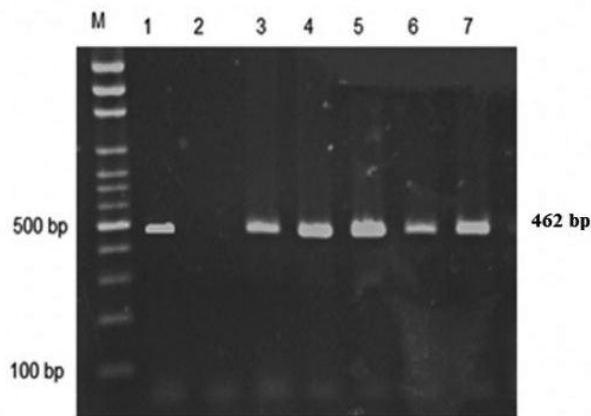


Fig. 3. Electrophoresis of PCR amplification (462 bp) provided from liver of cattle sample:

Lane 1: positive control; Lane 2: negative control; Lanes 3-7: positive samples; M: ladder 100 bp.

under accession numbers AF 13269501 and AB 68516201 in Gen Bank.

## Discussion

Due to the lack of representative and well documented data on the genotypes of *E. granulosus* originating from cattle in the northeast of Iran, the present study provides the first comprehensive strain characterization of cattle isolates in this region.

Our findings demonstrate that G1 was the dominant genotype of cystic echinococcosis in cattle in the northeast and southwest of Iran. The results of the current study are in agreement with previous studies in other regions of Iran [5–13,15,16]. Although G6 and G3 were previously reported from cattle in some regions of Iran [6,10–12], these genotypes were not found in the present study. It is important to note that the camel strain was found to be present in the cattle: This has noteworthy implications for the achievement of hydatid control programmes based on regular drug treatment of dogs, as this strain has shorter maturation time in dogs compared with the common sheep strain [4].

Based on our previous retrospective study in two mentioned provinces [17,18], *Echinococcus* infections have been shown to be more prevalent in cattle than in sheep and goats, with typical prevalence rates 6.7% and 3.82%, respectively. Although the reasons for these differences in the prevalence of hydatidosis are not very obvious, they could be attributed to the positions of the two

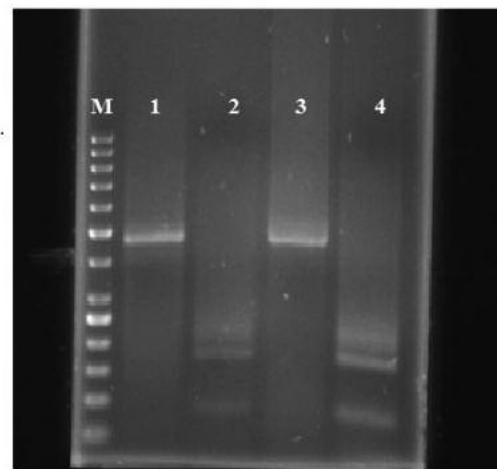


Fig. 4. Agarose gel electrophoresis of ITS1-PCR

products of *E. granulosus* isolates from cattle before and after digestion with the restriction enzyme Bsh1236I:

Lanes 1, 3 uncut products and Lane 2 and 4 cutting products that are G1 genotype, M: ladder 50bp.

provinces on the border regions of the country: the slaughtered animals originate from different locations both within and outside the region, while animals slaughtered in other reported areas tended to be from within the locality itself. Other factors, such as differences in environmental circumstances that are favourable for the maintenance of the parasite, the quantity of infected definitive hosts, the type of cattle husbandry, stocking rates and grazing patterns of animals may contribute to this variation.

In areas where there are numerous intermediate host species of *Echinococcus*, it is essential to identify whether each harbours a diverse species/strain and whether there is the chance of interaction between cycles in order to satisfactory control strategies. The epidemiological significance of Iranian cattle in two mentioned area infected by the metacestode stage of the G1 genotype of *E. granulosus* can be seen in the present results, as a large number of cysts were examined. Moreover, the analyses of cyst fertility showed that cattle infected with the G1 strain harboured non-fertile cysts. The cyst fertility of the sheep strain/G1 genotype infecting cattle has been commonly reported to be low [19], and so this intermediate host could present a low risk for dog contamination and consequently for human infection. The results of our molecular study, combined with previous molecular data from sheep, goats and camels in Khorasan province [14,20], suggest that sheep strain transmission occurs actively in this region. This information will have an important impact on the preventive

measures employed against hydatid disease.

In conclusion, Iran is a main endemic region for echinococcosis, with various intermediate hosts, cycles of transmission and new species being described [20]. It is a region that will be of strong interest for molecular epidemiological studies to elucidate cycles of transmission and the risk factors for human infection.

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