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Short communication

Molecular study for detecting the prevalence of *Fasciola gigantica* in field-collected snails of *Radix gedrosiana* (Pulmonata: Lymnaeidae) in northwestern Iran

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ABSTRACT

Fasciolosis is an important disease in veterinary medicine worldwide, and is a cause of great economic loss in livestock husbandry in Iran. This study was aimed to determine prevalence of *Fasciola gigantica* infection in field-collected snails of *Radix gedrosiana* in northwestern Iran. The snails were collected from 28 perennial and seasonal freshwater habitats from May to December 2010 and identified. A fragment of 618 bp of 28s rRNA gene was amplified by polymerase chain reaction (PCR). The PCR products were subjected to restriction fragment length polymorphism (RFLP) using *Dra*II and *Ava*II enzymes. PCR-RFLP patterns revealed that 3.12% of the snails were infected with *F. gigantica*. It was also found that the infected snails had a limited distribution over the water bodies located in the central part of the region. It was concluded that PCR-RFLP was a reliable approach to detect *Fasciola* infection in pond snails and may be useful to establish control measures for livestock and humans' fasciolosis in the region.

1. Introduction

The liver fluke *Fasciola gigantica* Cobbold, 1856 (Trematoda: Fasciolidae) is the etiological agent of fasciolosis that primarily infects domestic livestock in tropical areas, but can also infect humans (Ashrafi et al., 2004). Fasciolosis is estimated to be responsible for the annual loss of thousands of dollars in the Iranian agricultural industries. In Iran, fasciolosis in domestic ruminants is mainly caused by *F. hepatica* (Linnaeus, 1758); however, the occurrence of *F. gigantica* has also been reported from different parts of the country (Yakhchali and Ghobadi, 2005).

Aquatic snails of the family Lymnaeidae play a role, as the intermediate hosts, in the transmission of liver flukes (Ashrafi et al., 2004). To date, seven species of this family have been reported from different parts of Iran, most of which were found to be the intermediate hosts for *F. gigantica* or *F. hepatica* (Mansourian, 1986; Ashrafi et al., 2007). Of those, *Radix gedrosiana* (Annandale and Prashad, 1919) is the most widely distributed lymnaeid snail throughout the country (Mansourian, 1986; Imani Baran et al., 2011). This snail was reported to be a preferred intermediate host for *Trichobilharzia* spp. (Farahnak and Essalat, 2003), *F. gigantica* (Ashrafi et al., 2004), Plagiorciids and *Clinostomum* (Ghobadi and Farahnak, 2004), and *Ornitobilharzia turkestanicum* (Motamedi et al., 2008).

Microscopic examination, i.e., cercarial shedding, dissection and crushing, is the most frequently used technique to detect larval stages of *Fasciola* in the intermediate host (Kaplan et al., 1997). However, this technique has low sensitivity and/or specificity because of difficulties in detection

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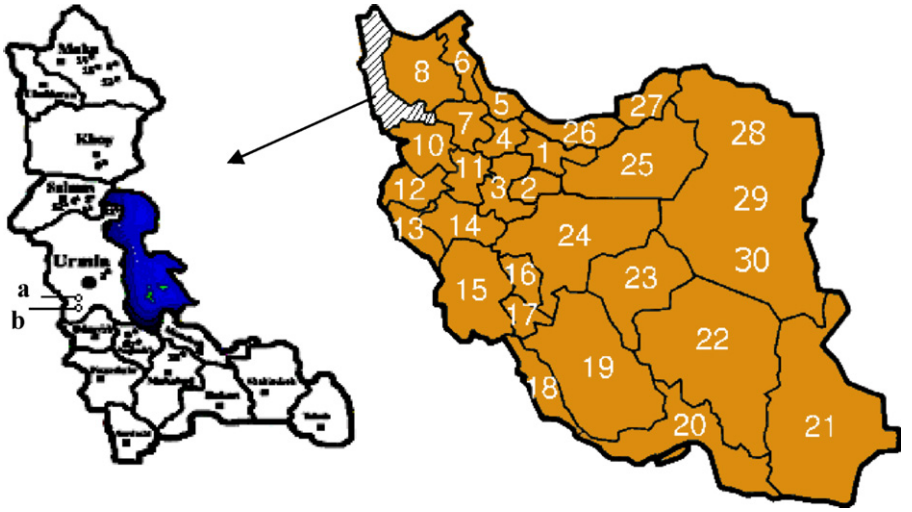

Fig. 1. Map of field-collected *Radix gedrosiana* snails with infection by *Fasciola gigantica* larvae in West Azarbaijan province, Iran (a: Dolatabad, b: Golestane).

and differentiation of larval stages of *Fasciola* in snails (Caron et al., 2007). For this reason, recent studies on discerning the experimental infection of lymnaeid snails with *Fasciola* larvae (Magalhaes et al., 2004) and natural infections of field-collected snails (Ashrafi et al., 2007; Caron et al., 2007; Relf et al., 2009) have employed molecular tools. Nevertheless, large-scale molecular study resulting in the diagnosis of the infection with *F. gigantica* larvae in field-collected *R. gedrosiana* snails from northwestern Iran has not been yet performed. Collection of accurate data on snail infection rates is crucial to estimate the potential infection risk of the ruminants. Thus, objective of this study was to examine the utility of PCR-RFLP for detecting the prevalence of the infection of field-collected *R. gedrosiana* with *F. gigantica* larvae in northwestern Iran.

2. Materials and methods

2.1. Study area

The province of West Azarbaijan is located in northwestern Iran (39°47′–45°58′N, 44°30′–47°23′E) with an average altitude of 1,332 m above sea level (Fig. 1). Climate of the province is largely influenced by the rainy winds of the Atlantic Ocean and Mediterranean Sea. Annual precipitation ranges from 300 to 800 mm with large yearly and monthly fluctuations, so that dry seasons lasting for 3–5 months may also occur. Average annual temperature varies between 9.2 °C and 14.2 °C and temperatures below 0 °C may persist for more than 100 days. Numerous water bodies and reservoirs with suitable environmental conditions are present in the area to host several species of pond snails (Imani Baran et al., 2011).

2.2. Snail collection and identification

Field collection of pond snails was undertaken by searching 28 perennial and seasonal freshwater habitats in West Azarbaijan from May to December 2010. In each site, sampling was performed over a 30-min period using a standard flat wire mesh scoop (mesh size 2 mm) (Relf et al., 2009). Data pertaining to the sampling sites, i.e., type of water bodies, water temperature, salinity and pH, were recorded. The specimens were placed individually into plastic screw-capped bottles containing the water of their habitats and carried alive to the Laboratory of Malacology of Faculty of Veterinary Medicine, Urmia University. *R. gedrosiana* snails were taxonomically identified according to the keys of Mansourian (1986) and Pfleger (1999). The soft tissues of *R. gedrosiana* snails were dissected, washed several times in PBS, and stored at −20 °C until DNA extraction.

2.3. DNA extraction and polymerase chain reaction (PCR)

Snail genomic DNA was extracted by modified phenol–chloroform method (Sambrook and Russell, 2002) using cetyltrimethylammonium bromide (CTAB) at 60 °C for 1 h. A fragment of 618 bp of the 28s rRNA gene was amplified using two primers (forward: 5′-ACGTGATTACCCGCTGAACT-3′ and reverse: 5′-CTGAGAAAGTGCACTGACAAG-3′) introduced by Marcilla et al. (2002). PCR was carried out in 25 μl reaction mixture containing 2 μl of genomic DNA (diluted 1:30), 1.5 U of *Taq* DNA polymerase (Fermentas, Germany), 50 mM of each dNTPs (CinnaGen, Iran), 2 mM of $MgCl_2$, 2.5 μl of PCR buffer (10×) and 0.2 μM of each primer. The reaction was performed in a Bioer XP thermal cycler. The samples were subjected to an initial denaturation step at 94 °C for 3 min, followed by 30 cycles of 30 s at 94 °C, 30 s at 60 °C and 60 s at 72 °C, and a final extension step at 72 °C for 5 min. A volume of 10 μl of each PCR product was analyzed by electrophoresis on 1.5% (w/v) agarose gel for approximately 1.5 h at 90 V. The gels were visualized by staining with 1% ethidium bromide.

2.4. Restriction fragment length polymorphism (RFLP)

A PCR-RFLP procedure was developed to specify *F. gigantica*. Specific restriction enzymes were selected by BioEdit