Molecular identification of *Sarcocystis hominis* in native cattle of central Iran: A case report

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**Abstract.** *Sarcocystis* spp. are two-host protozoan parasites belonging to the phylum Apicomplexa. Among different known species of *Sarcocystis* in cattle, only *Sarcocystis hominis* is important from the public health viewpoint, because of its zoonotic characteristics. This study presents the first molecular identification of *S. hominis* in native cattle in central Iran. A sample of diaphragm muscle from a 6-year-old native cow slaughtered at Yazd Slaughterhouse, Yazd, central Iran, was collected in May 2013. DNA extraction was performed, using the salting-out method. DNA purification and precipitation were performed consecutively. The amplicon and digestion results were analyzed using agarose gel electrophoresis. A PCR product with 926 bp in length was obtained after amplification, and 376 bp and 550 bp in length after digestion that identified *S. hominis*. To the best of our knowledge, this study is the first of its kind to be reported from Iran.

**INTRODUCTION**

*Sarcocystis* spp. are two-host obligate protozoan parasites belonging to the phylum Apicomplexa (Ghisleni *et al.*, 2006). Of the numerous species of the genus *Sarcocystis*, at least three are known to infect cattle as the intermediate host. These are *Sarcocystis hominis*, *Sarcocystis cruzi* and *Sarcocystis hirsuta* with final hosts being humans, canids and felids, respectively. It is estimated that more than 90% of cattle populations in most regions of the world are infected by at least one species of *Sarcocystis* (Moré *et al.*, 2011); thus, sarcocystosis is one of the most widespread parasitic diseases of cattle.

The life-cycle of *Sarcocystis* spp. has two different phases including asexual and sexual stages occurring in the intermediate and final hosts, respectively (Jehle *et al.*, 2009; Bucca *et al.*, 2011; Oryan *et al.*, 2011). Cattle are infected through consumption of food or water contaminated with oocysts expelled by the feces of the definitive hosts. The oocysts consist of two sporocysts, each containing four sporozoites. After several replications, the sporozoites develop to cysts (sarcocysts) in skeletal and cardiac muscles of the intermediate host. The final hosts may be infected after ingestion of tissues of cattle or other intermediate hosts containing infectious sarcocysts (Oryan *et al.*, 1996; 2010; Gracey *et al.*, 1999; Tappe *et al.*, 2013).

Although sarcocystosis in cattle generally has non-pathogenic features, however, in some instances it may result in clinical signs, notably eosinophilic myositis. The symptoms of illness in man occurring
after consuming of infected undercooked beef are often diarrhoea, nausea, abdominal discomfort and vomiting (Fayer, 2004; Akhondzadeh Basti & Hajimohammadi, 2011; Bucca et al., 2011).

Among different known species of Sarcocystis in cattle, only S. hominis is important from the public health viewpoint, because of its zoonotic feature. This study presents the first molecular identification of S. hominis in native cattle in Iran.

**Case report**

During a pilot study which was undertaken to determine a practical approach to molecular species identification of Sarcocystis, using PCR-RFLP, in cattle slaughtered at Yazd Slaughterhouse, Yazd, central Iran, in May 2013, about 10 gr sample of diaphragm muscle from a 6-year old native cow was collected. The sample was transferred to the laboratory and stored at -20°C. DNA extraction was performed, using the salting-out method; briefly, the samples were lysed with NET buffer (NaCl, 50 mM; EDTA pH 8, 25 mM; Tris-HCL pH 7.6, 50 mM). DNA purification and precipitation was consecutively performed by saturated salt solution and cold ethanol, respectively. The target gene (18S rRNA) was selected for identification, based on the study of Yang et al. (2002) with some modification such as primers and enzyme of BclI which substitute with BfaI. The target region was amplified with specific primer pairs of SarF (5’-CGTGGTAATTCTATGGCTAATACA-3’) and SarR (5’-TTGTGGTTAAGACTACGACGGTA-3’) for a PCR product with a size of approximately 900 bp with an initial denaturation of 94°C for 5 min, followed by 30 cycles of 94°C for 60 sec, 58°C for 60 sec and 72°C for 60 sec and finalized with extension of 72°C for 5 min. RFLP was followed by incubation of PCR product with 1X specific enzyme buffer and 10 U either Rsal or BfaI digestion for 1 hour at 37°C. The amplicon and digestion results were analyzed, using agarose gel electrophoresis. Our results showed a PCR product with 926 bp in length after amplification. Based on the databases, restriction enzyme digestion with BfaI results in 376 bp and 550 bp in length and with Rsal remains uncut for S. hominis (Yang et al., 2002) (Figure 1). For verifying this identification, the sample was sequenced. BLAST analysis showed that the sample was S. hominis.

![Figure 1. PCR-RFLP analysis](image-url)

Lane 1: 100 bp DNA ladder; Lane 2: RFLP with Rsal (no digestion for S. hominis); Lane 3: RFLP with BfaI (376 bp and 550 bp digestion results for S. hominis); Lane 4: Undigested PCR product of target gene
The three *Sarcocystis* species of cattle, *S. cruzi*, *S. hirsuta* and *S. hominis*, may be differentiated based on the morphological features of the sarcocyst walls in musculature samples by electron microscopy. However, application of this method has a considerable limitation because of being time consuming and too costly, especially when many samples are going to be ultrastructurally examined. Histopathological studies on fresh tissue samples differentiate the thick- (>3µm) and thin-walled (<1µm) cysts, but this method is not able to distinguish *S. hominis* from *S. hirsuta* (the thick-walled cysts) (Fayer, 2004; Nourollahi-Fard et al., 2009; Moré et al., 2011). Therefore, the molecular assays are the most efficient methods in epidemiological studies and species identification of *Sarcocystis* in intermediate hosts.

Identification of *S. hominis* in cattle has been successfully performed by several investigators in different parts of the world, such as in northern Vietnam (Jehle et al., 2009), Argentina (Moré et al., 2011) and Nigeria (Obijiaku et al., 2013). In addition, the few Iranian researchers who have studied the epidemiology of *Sarcocystis* spp. indicated a high prevalence of *Sarcocystis* infection in cattle in the country (Nourollahi-Fard et al., 2009, 2013; Nourani et al., 2010). Recently, molecular identification of *S. cruzi* and *S. hirsuta* were reported by Shekarforoush et al., 2013 and Kalantari et al., 2013, respectively, in Iran. However, to the best of our knowledge, there is no molecular study that has identified *S. hominis* in Iran so far. Considering the presence of *S. hominis* infection in cattle population of Iran, to achieve useful prevention programs, it is necessary to do more studies on the epidemiology, prevalence and distribution pattern of this parasite at different parts of this country in future.

As the risk of *S. hominis* is well known for humans, it is necessary to design local or national programs to investigate the prevalence of this species in cattle of Iran. In addition, those human populations who are possibly at risk should also be tested by application of molecular techniques for detection of oocysts or sporocysts in their fecal samples (Xiang et al., 2009). Beef storage at freezing temperature at least for 2 days could be a proper preventive measure in endemic regions (Fayer, 2004). In areas where semi-cooked beef may be consumed, people must be aware of the risk of sarcocystosis.

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