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First molecular identification of *Sarcocystis hirsuta* in Iranian beef: A case report

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ABSTRACT

Introduction: The main agents of sarcocystosis in cattle as an intermediate host include *S. cruzi*, *S. hominis* and *S. hirsuta*. A sensitive and specific tool such as molecular-based techniques would be necessary to identify the species.

Case report: After collection of beef sample from Yazd slaughterhouse, DNA extraction was done with salting out method. The *18SrRNA* gene as a specific target gene was used for molecular detection of *Sarcocystis* spp, then Restriction Fragment Length Polymorphysm (RFLP) analysis identified the speciesusing *Rsa* and *Bfa*. Results showed that our designed molecular method could identify *S. hirsuta* in beef sample.

Conclusion: Based on our knowledge, this study indicates the first report of molecular identification of *S. hirsuta* in Iran.

Introduction

There are about 130 species of cyst-forming coccidian with differences in life cycle and pathogenicity in the genus *Sarcocystis*. These parasites have high prevalence in the livestock**.** The intermediate hosts are mainly prey herbivore animals and the definitive hosts are predator carnivore animals (Fayer, 2004; Oryan et al., 2011).

Sarcocystosis in cattle is a zoonotic disease with worldwide distribution. All species of *Sarcocystis* are cyst-forming coccidian intracellular parasites having a two-host life cycle (Nourollahi-Fard et al., 2013; Oryan et al., 1996). The main species of *Sarcocystis* infected cattle as intermediate host include *S. cruzi*, *S. hominis* and *S. hirsuta* whose definitive hosts are canids, humans and felids, respectively (Bucca et al., 2011; Nourani et al., 2010; Nourollahi Fard et al., 2009)

The intermediate hosts are infected by ingesting food contaminated by feces of final host containing one of the above three *Sarcocystis* species. The sporocyst will subsequently develop to sarcocyst in the muscles of cattle, and then the final hosts are infected by eating tissues containing sarcocyst (Oryan et al., 2010; Oryan et al., 2011).

In this study, PCR-RFLP technique was used for the first time to detect *S. hirsuta* in Iranian beef.

Case report

Sample from the diaphragm and intercostals muscles of a 5-year-old native cattle was obtained from industrial slaughterhouse of Yazd, Iran. About 30 gram of the beef sample was transferred into a sterile tube containing 70% ethanol and stored at -20 °C for the next examination.

DNA extraction was done using salting out method. Briefly, 30 mg of the sample was crushed and suspended in 900 µl NET buffer (NaCl, 50 mM; EDTA pH 8, 25 mM; Tris-HCl pH 7.6, 50 mM) supplied by 10 µl proteinase K (Fermentas, EO049, 20 mg/ml) and SDS with end concentration of 1% and incubated at 56°C for an overnight. DNA purification was done by adding 6M NaCl in 1/3 of total volume. After centrifugation, the supernatant was transferred into a new sterile 1.5 ml microtube for the next precipitation step using cold absolute ethanol. After washing with ethanol 70%, the pellet was diluted in 100 µl ddH_2O and stored at -20 °C.

Amplification was done with the target of *18SrRNA* specified for *Sarcocystis* (Yang et al., 2002). The reaction was performed

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with end concentrations of 1X PCR buffer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 10 pmol of each primer and 100 ng of template DNA in a total volume of 30µl. The amplification program was comprised of an initial denaturation of 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 sec, 58 °C for 30 sec and 72 °C for 30 sec and finalized with extension of 72 °C for 5 min. Analysis of the amplification results was done using agarose gel electrophoresis alongside with 100 bp DNA ladder. Then, the sample was analyzed using RFLP by *BfaI* and *RsaI*.

The digestion was analyzed using agarose gel electrophoresis alongside with 100 bp DNA ladder. The fragments of 397 bp and 557 bp and also the fragments of 376 bp and 577 bp detected *S. hirsuta*, by digestion with *RsaI* and *Bfa*, respectively (Fig. 1). For verification of the results, the sample was sequenced and BLAST.

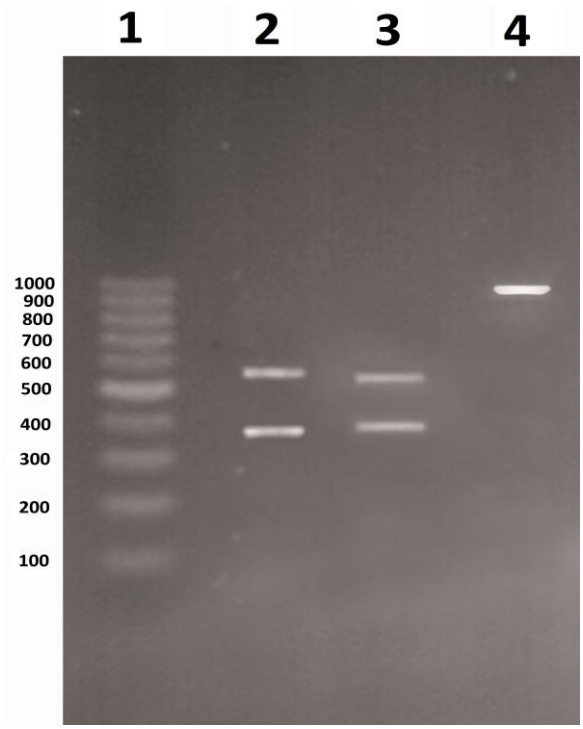

Fig.1: PCR-RFLP analysis. Lane1: 100 bp DNA ladder; Lane2: RFLP with *RsaI* (376 bp and 577 bp for *S. hirsuta*); Lane3: RFLP with *BfaI* (397 bp and 557 bp for *S. hirsuta*); Lane 4: PCR product of target gene.

Discussion

Genus of *Sarcocystis* has worldwide contribution (Fayer, 2004). Differentiation of various species of *Sarcocystis* in cattle is based on thickness of the cyst's wall. Thin-walled cysts are *S. cruzi* and thick-walled are *S. hirsuta* or *S. homoinis*. Jehle et al. (2009) reported a considerable prevalence of *S. hirsuta* (27.7%) in beef samples obtained from cattle slaughtered in Vietnam. In another study, in Argentina, 23.1% of loin samples had thick walled *Sarcocystis* including either *S. hirsuta* or *S. hominis* (More et al., 2011). Also, Domenis et al. (2011) revealed that infection rate of *S. hirsuta* in cattle of Italy was 1.8%.

Several methods such as digestion, trichinoscope, staining with methylene blue, light and electron microscopy, histological techniques and molecular methods have been found useful in detecting *Sarcocystis* spp. (Bucca et al., 2011; Moré et al., 2011; Nourani et al., 2010). The traditional methods could distinguish between the species with thick and thin cyst wall. On the other hand, electron microscopy can distinguish between *S. hominis* and *S. hirstua*, but molecular methods are more efficient and sensitive in detecting these two *Sarcocystis* species. (Jehle et al., 2009; Nourollahi-Fard et al., 2009). Shekarforoush et al. (2013) detected *S. hirsuta* in cattle in Shiraz, Iran using microscopic method. On the other hands, the first molecular identification of *S. cruzi* in Iranian beef was reported by Kalantari et al. (2013).

Conclusion

So far, there is no report of molecular identification of *S. hirsuta* in the documents in Iran. Therefore, to the authors' knowledge, this study indicates the first report of molecular identification of *S. hirsuta* in this country.

Conflicts of interest

The authors declare no conflicts of interest.

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