J Parasit Dis (Oct-Dec 2015) 39(4):770–772
DOI 10.1007/s12639-014-0425-7

ORIGINAL ARTICLE

First identification of *Sarcocystis hominis* in Iranian traditional hamburger

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Received: 16 October 2013 / Accepted: 13 January 2014 / Published online: 31 January 2014

Abstract Zoonotic concerns of cattle sarcocystosis are of importance, because humans are the final host for *Sarcocystis hominis*. Therefore the meat products containing beef may encompass sarcocysts which endanger food safety. In this study, we described the first report of molecular identification of *S. hominis* in Iranian traditional hamburgers using PCR-RFLP. Throughout a pilot research that was carried out to setup a molecular approach to identify the *Sarcocystis* spp., using PCR-RFLP, a sample of raw Iranian traditional hamburger was purchased from a street food seller located in Yazd, central Iran in May 2013. DNA extraction was done, by salting out method; briefly, the sample was lysed with NET buffer. The DNA purification and precipitation was then performed. Amplicon and digestion results were analyzed, using gel agarose electrophoresis. The results showed a PCR product with 926 bp in length after amplification and 376 and 550 bp in length after digestion. This product was identified as *S. hominis*. To the best of our knowledge, this is the first report of *S. hominis* infection in Iranian hamburger.

Keywords *S. hominis* · PCR-RFLP · Iranian hamburger

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Introduction

Hamburger is one of the most popular fast foods at different countries all over the world. Each year, about five billion hamburgers are consumed just in USA (Prayson et al. 2008). Although, there is no exact information regarding per capita of meat products in Iran, but it is estimated that the annual consumption of hamburger is considerable in this country. Iranian traditional hamburger is mainly made up of mixture of minced meat, onion, garlic, wheat flour, vegetable protein (such as soybean), edible oils, salt, pepper, and occasionally traditional spices. Meat achieved from cattle, sheep, goat, camel or buffalo composes about 50–90 % of this meat product. In spite of most countries, pork is a forbidden ingredient in composition of Iranian traditional hamburger because of religious aspect of Muslims (Doosti et al. 2011). There is no national standard for formulation of this traditional product in Iran. On the other words, the Iranian traditional hamburger is a home-made meat product which is prepared and sold in street market without any considerable surveillance. Therefore, there is a gap for safety and control of this product.

Sarcocystis spp. are intracellular protozoan parasites pertaining to the phylum Apicomplexa and family Sarcocystidae with an obligate two-host life cycle between the predators as the final hosts and prey animals as the intermediate hosts (Nematollahia et al. 2013; Oryan et al. 2011). Oocysts expelled within feces of the infected final

hosts are the source of infection for the intermediate hosts. After several developmental stages, sarcocysts are formed in musculature organs such as heart, tongue, diaphragm, intercostal muscle, esophagus and locomotors muscles of the intermediate hosts (Bucca et al. 2011; Moré et al. 2011; Rahdar and Salehi 2011; Oryan et al. 1996; Oryan et al. 2010).

Among the numerous species of this genus, only *S. hominis* and *S. suihominis* which cattle and pig are their intermediate hosts, respectively, have been recognized as the zoonotic parasites. Intestinal infection in human beings may occur after consumption of undercooked beef or pork containing sarcocysts. The clinical signs are digestive system disorders including nausea, vomiting, stomachache and diarrhea especially in immunocompromised patients (Fayer 2004; Rahdar and Salehi 2011).

The zoonotic concerns of cattle sarcocystosis are of importance, because humans are the final host for *S. hominis* (Bucca et al. 2011). Therefore the meat products containing beef may encompass sarcocysts which endanger food safety. In this study, we described the first report of molecular identification of *S. hominis* in Iranian traditional hamburgers using PCR-RFLP.

Materials and methods

Throughout a pilot research that was carried out to setup a molecular approach to identify the *Sarcocystis* spp., using PCR-RFLP, a sample of raw Iranian traditional hamburger was purchased from a street food seller located in Yazd, central Iran in May 2013. The sample was immediately transferred to laboratory and stored at −20 °C. DNA extraction was done, by salting out method; briefly, the sample was lysed with NET buffer (NaCl, 50 mM; EDTA pH 8, 25 mM; Tris-HCl pH 7.6, 50 mM). The DNA purification and precipitation was then performed. The target gene (18S rRNA) was amplified with specific primer pair of SarF (5′-CGTGGTAATTCTATGGCTAATACA-3′) and SarR (5′-TTTATGGTTAAGACTACGACGGTA-3′) for a PCR product with around 900 bp and followed by *Rsa*I and *Bfa*I digestion. The amplicon and digestion results were analyzed, using gel agarose electrophoresis.

Results

The results showed a PCR product with 926 bp in length after amplification and 376 and 550 bp in length after digestion. This product was identified as *S. hominis* (Fig. 1).

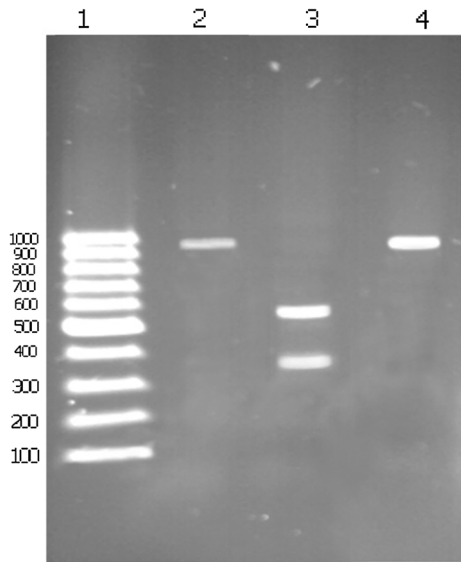

Fig. 1 PCR–RFLP analysis. *Lane 1* 100 bp DNA ladder, *Lane 2* RFLP with RsaI (no digestion for *S. hominis*), *Lane 3* RFLP with BfaI (376 and 550 bp for *S. hominis*); *Lane 4* PCR product of target gene

Discussion

Sarcocystis hominis is the only species of *Sarcocystis* in cattle that posses importance in food safety and public health. Different types of food such as hamburger composed of beef may be a source of human infection. Therefore, the hamburger consumers are at high risk in endemic areas. Identification of *S. hominis* in cattle has been tried by some researchers at different parts of the world, such as Northern Vietnam (Jehle et al. 2009), Argentina (Moré et al. 2011) and Nigeria (Obijiaku et al. 2013). While no cases of *S. hominis* have been detected in various areas of Iran, the prevalence of sarcocystosis infection with other species of *Sarcocystis* in cattle of Iran have been reported to be high (Nourani et al. 2010; Nourollahi-Fard et al. 2009; Nourollahi-Fard et al. 2013).

Several investigators have studied the infection rate of the *Sarcocystis* spp. in hamburgers. Prayson et al. (2008) reported that 25 % of the examined hamburgers in USA were infected with *Sarcocystis* spp. According to Hosseini et al. (2007), *Sarcocystis* spp. were found in 56 of 117 (47.9 %) hamburger distributed in Tehran, Iran. Similar survey carried out in Ahvaz, southwestern Iran, revealed that 56 % of the hamburgers had *Sarcocystis* spp. (Rahdar and Salehi 2011). However, this rate of infection in hamburgers sold in Garmsar, Iran was 6.25 % (Jahed Khaniki and Kia 2006). Recently, the infection rate of *Sarcocystis* spp. in hamburger of northwest of Iran was determined as 56.25 % by Nematollahia et al. (2013).

In all the previous surveys, impression smear, digestion or histological methods were used in detection of *Sarcocystis* spp. infection in hamburgers. Since none of the mentioned methods are able to identify *S. hominis*, the incidence of this species in hamburger had not been recognized yet. In this study, we applied molecular assay (PCR-RFLP) for detection of *S. hominis* as the only zoonotic species of *Sarcocystis* spp. infecting beef and its derivative meat products such as hamburger. Therefore, to the best of our knowledge, this is the first report of *S. hominis* infection in Iranian hamburger.

Since the significance of *S. hominis* in public health and human illnesses is obvious, the preventive actions should be highlighted for consumers of traditional hamburger. Keeping hamburgers at −20 °C for 1 day or −4 °C for 2 days and heating them to a core temperature of 70 °C inactivates *Sarcocystis* (Fayer 2004; Ghisleni et al. 2006). In regions where consumption of semi-cooked hamburger is common, the carcass inspectors and people should be warned to the risk of this parasite.

Acknowledgments This study was conducted at the Faculty of Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. We thank the authorities of Shahid Sadoughi University of Medical Sciences for their financial support and assistance.

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