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Original Article

Sarcocystis bovifelis in Raw Hamburgers Marketed in Hamadan City, Western Iran

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Abstract

Background: We aimed to evaluate *Sarcocystis* contamination in conventional and industrial raw beef burger samples from butcheries and retail stores in Hamadan, western Iran.

Methods: Overall, 80 samples including 30 conventional and 50 industrial hamburgers were randomly obtained from different butcheries and supermarkets. All specimens were studied by digestion method following microscopic examination. Samples` genomic ribosomal DNA were amplified and nucleotide sequences were analyzed by BLAST for comparison with the sequences in the gene bank of the NCBI.

Results: *Sarcocystis* bradyzoites were detected in 46 of 80 (57.6%) samples. Positive specimens were included as 46 (57.6%) and 30 (37.5%) by digestion and molecular method, respectively. Differences between two studied (digestion and molecular) methods was statistically significant (P=0.00). Twenty-six (86.5 %) of 30 conventional beef burgers and 20 (40%) of 50 industrial burgers were positive for *Sarcocystis* sp. by digestion method. There was a significant difference between *Sarcocystis* infested conventional and industrial beef burgers (P=0.01).

Conclusion: The parasitic contamination of beef burgers implied a high level of infection in cattle. Felids as the definitive hosts for *S. bovifelis* urged on the improvement of the hygienic conditions of keeping and feeding livestock in order to reduce the infection. Molecular techniques confirm species in meat products with high sensitivity and distinguish it from human species.

Introduction

Sarcocystis is an intracellular apicomplexan protozoan parasite with a worldwide distribution. Carnivorous as well as human are considered as definitive and herbivorous including cattle, sheep, goat, poultry and pig as intermediate hosts (1). Ingestion of cyst-containing tissues via raw or poorly cooked meat or meat products by the defini-

tive hosts leads to sexual reproduction of the *Sarcocystis* in the digestive tract, followed by the discharge of sporocysts in the feces (2, 3). The parasitic protozoan causes symptoms such as anorexia, mild fever, vomiting, diarrhea and respiratory problems in human (4).

Most of the *Sarcocystis* species are host-specific. *S. hominis*, *S. heydorni* and *S. suihominis* are found to use humans as definitive hosts. Sarcocystosis infection illustrated high incidence among bovine carcasses, whose prevalence can nearly reach 100% in various parts of the world (5-7). Cattle (*Bos tarus*) act as intermediate hosts for *S. cruzi*, *S. hirsuta* and *S. bovifelis* (8). *Sarcocystis*-infected meat causes economic losses due to the unpleasant appearance of meat, which annually enforces huge damages on the livestock industry (9).

Human infection can be related to eating raw or under-cooked meat containing encysted parasites (10). Processed meat products such as hamburgers, are one of the most popular meat products in different countries of the world, for example, about 5 billion burgers are consumed in the United States per year (11).

Although there is no accurate information about the per capita meat products in Iran, it is worth noting that the annual consumption of hamburgers in this country is remarkable. Unlike the conventional burgers which are made just by meat, industrial beef burgers are not mainly made from beef, their mostly ingredients are a mixture of minced meat, onions, garlic, flour, soybeans and other food additives (12). In recent years, high sensitive molecular methods have identified parasites even in small and insignificant parasitic loads (13). Digestive methods are the most used techniques in detection of *Sarcocystis* bradyzoites in meat or its products while molecular diagnostic methods used in few studies to detect *Sarcocystis* species (14).

Investigations on *Sarcocystis* in meat products reported a frequency of 56% and 80% of hamburgers in Iran (15, 16). Therefore, due to the increasing demand for fast foods, especially burgers, and the high frequency of sarcocystosis in slaughtered cattle, it can be regarded as a potential foodborne problem.

Therefore, we aimed to identify the *Sarcocystis* bradyzoites in beef burgers obtained from butcheries and supermarkets in the city of Hamadan, western Iran.

Materials and Methods

Sampling

This cross-sectional study was conducted during February 2020 to February 2021 in Hamadan, west of Iran. A total of 80 hamburgers were purchased from different butcher shops and grocery stores in simple random sampling. The samples (n: 30) which were traditionally made by the butcheries consisted of 100% beef while the meat content of the other ones (n:50) was 60 to 90% according to the industrial trademark.

Ethical approval

The research was performed in terms of the principles and ethical considerations of working with laboratory animals as confirmed by the ethics committee of Hamadan University of Medical Sciences (Ethics committee code: IR.UMSHA.REC.1397.304).

Microscopic examination and peptic digestion

All specimens were firstly analyzed via digestion method. Briefly, 20 grams of each hamburger was grinded thoroughly and then treated by a 50 ml digestion solution including 1.3 g of pepsin, 2.5 g of NaCl and 3.5 mL of concentrated HCl in 500 mL of sterile distilled water. The mixture was incubated at 40 ºC for 2 h and then centrifuged at 1500 rpm for 15 min. The supernatant was discarded and the precipitate washed three times and examined under microscopy for *Sarcocystis* bradyzoites (17).

DNA extraction and PCR amplification

Genomic DNA of the parasite was extracted using the tissue extraction kit (DNG-plus, SINACOLON, Iran) according to the manufacturer`s instruction. The extracted DNA was stored at -20 ^{O}C until PCR examination. The ratio of absorbance was assessed at 260-280 nm for evaluation of the purity of DNA.

PCR was performed to detect *Sarcocystis* DNA fragments. Fragments of 900 bp from 18s ribosomal DNA genes were amplified using forward (SarF: GGATAACCTGG-TAATTCTATG) and reverse (SarR: GGCAAATGCTTTCGCAGTAG) primer (18). The amplification began with an *initial denaturation* at *94 °C* for 3 *min* followed by 30s annealing at 57 °C and extension at 72 °C for 3 min. *Sarcocystis*-positive samples isolated from cattle striated muscle were used as positive controls while blank reagents (dd H_2O) were manipulated as negative controls.

PCR products were resolved through 1.5% agarose gel and imaged in a blue light transilluminator. Amplicons were purified and analyzed by the Bioner Company of South Korea. The nucleotide sequence homology of randomly selected four samples was compared with the sequences in the gene bank of the NCBI database by BLAST (Basic Local Alignment Search Tool).

Statistical analysis

All statistical analysis was performed using SPSS V.16 (Chicago, IL, USA). Variables were analyzed by Mann-Whitney and Kruskal-Wallis tests. All data were expressed as mean ± standard deviation (SD). The statistical level of significance was set at $P < 0.05$.

Results

Sarcocystis bradyzoites were detected in 46 of 80 (57.6%) samples. Among a total of 80 hamburgers, including two different groups, 46 (57.6%) and 30 (37.5%) were identified positive by digestion and molecular method, respectively (Table 1).

The PCR products were identified by size using a 100 bp ladder. The expected PCR product had a length of 900 bp (Fig. 1). The homology of randomly selected four purified amplicons were determined in Blast software and the *S. bovifelis* was identified with homology > 95%.

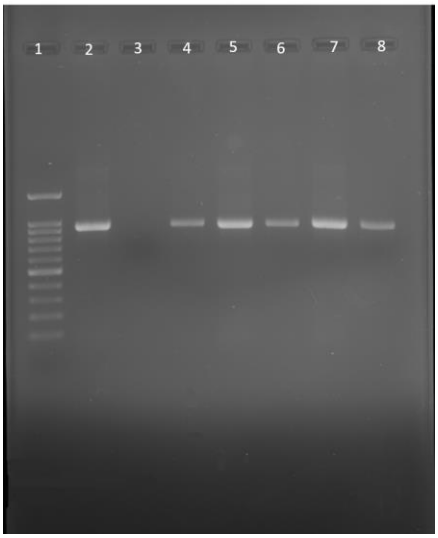

Fig. 1: PCR amplification of eucoccidial DNA fragments in samples.
Lane 1: 100bp marker, 2: Positive control (positive samples isolated from cattle striated muscle), 3: Negative control (dd H2O), 4: industrial burger sample, 5: traditional burger sample, 6: industrial burger sample, 7: traditional burger sample, 8: industrial burger sample

Table 1: Prevalence of *Sarcocystis* in two types of beef burgers. Using microscopic and molecular methods

Sample	*Microscopic method (%)*		*Molecular method (%)*		*Total*
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	
Conventional made beef burgers	26 (86.6)	4 (13.3)	19 (63.3)	11 (36.6)	30
Industrial made beef burgers	20 (40)	30 (60)	11 (22)	39 (78)	50
Total (%)	46 (57.5)	34 (42.5)	30 (37.5)	50 (62.5)	80

Discussion

The results confirm that the overall 56.25% of conventional beef burgers and 38.75% industrial beef burgers were infected by *Sarcocystis*. Microscopic digestion method indicated 57.5% of *Sarcocystis* bradyzoites while molecular technique showed parasites` DNA in 30% of burger specimens. There was a significant difference between *Sarcocystis* infested conventional and industrial beef burgers (P=0.01).

Macroscopic investigation of *Sarcocystis* was not possible due to the process of hamburger preparation as a meat product; nevertheless, studies reported the *Sarcocystis* contamination of beef between zero to 0.4% in beef and meat products (14, 18, 19). Macrocysts may be ignored in visual inspection so, other accurate methods such as microscopic or molecular techniques are much more reliable in confirmation of infestation (19). On the other hand, according to the previous reports (5, 6), nearly 100% of beef was contaminated by *Sarcocystis* spp. so, it is highly expected to recognize the bradyzoites in the meat products including hamburgers.

Several studies have evaluated the *Sarcocystis* frequency in the meat products in Iran which have provided disputable points. For example, in accordance with the present job, the total infection of meat products with *Sarcocystis* was 80% and the highest contamination was determined for the hamburger samples (15). Besides, 87.5% of hamburgers, 83.3% of sausages and 66% of cocktail sausages in Hamadan were infected by *Sarcocystis* sp. by digestion method (15). Digestion method was the only used method in Dehkordi et al. project, so, more positive results were obtained compared to the present study. Nematollahia et al, also reported the prevalence of *Sarcocystis* 56.25%, using digestion method in hamburgers prepared in traditional and industrial procedures in Tabriz. There was no statistically significant difference between the two groups, in this regard the molecular method was not employed on detection of parasites (20).

In this study, the difference between the two types of beef burgers was statistically significant and the frequency of this parasite was higher in conventional beef burgers (87%) in comparison to industrial ones (56.6%). This difference may be due to the fact that the traditional burgers were made with a mixture of 100% fresh meat without any additives, while industrial burgers contain other vegetal ingredients beside meat. Furthermore, industrial meat products proceed a cooking process for producing safer output.

Haj Mohammadi et al. indicated the *Sarcocystis* infection rate 77.9 %, in the study of 190 industrial and traditional hamburgers in Yazd. Accordingly, PCR-RFLP technique detected the small traces of *Sarcocystis* DNA, so the rate of positive results compared to studies using peptide digestion or impression smears was higher (21). Faghiri et al described the parasite contamination of traditional hamburgers 87.9%, which is significantly higher than industrial hamburgers 67.8% (18).

In contrast to the current study, others found no statistical difference between the infection rate of the industrial and traditional hamburgers (20, 22). It may have contributed

to the method because in two studies peptic digestion was the only used method. *Sarcocystis* infection of meat and meat products were not associated with geographical area nevertheless, the applied method was the crucial element for investigation of *Sarcocystis* (21).

According to the sequencing results, *S. bovifelis* was identified in the hamburger samples. Cattle (*Bos taurus*) are as intermediate host for six *Sarcocystis* spp. which felids, canids and humans are respectively definitive hosts *for S. cruzi, S. hirsuta and S. hominis* and the three latter ones *S. bovihominis* and *S. bovini* with felids and *S.heydorni* with primates as definitive hosts (8, 23).

Sarcocystosis is very important in cattle because it can cause irreparable economic damage to the meat and meat products industry (7, 24). *Sarcocystis* species are distinguished based on the characteristics of the cyst wall by electron microscopy as well as molecular methods (25, 26). The histological examination of cattle tissue can easily differentiate thick-walled *S. hominis* (27, 28). In the case of processed meat products, including hamburgers, the histological detection of thick-walled cysts is not possible. *S. hirsuta* (*S. bovifelis*) could not be confirmed even by electron microscopy nor by RAPD-PCR (29). Due to the high prevalence of *Sarcocystis* in cattle, DNA-based procedures play a crucial role in identification of species and their contribution in meat industry. On the other hand, species identification can determine the sources of livestock infection by leading to the final host (30).

Hooshyar et al showed a 29% contamination among 200 studied hamburgers in Kashan, by PCR-RFLP technique. They confirmed that 74% of the samples were infected by *S. cruzi*, which canids act as definitive host, 20% by both *S. cruzi* and *S. hirsuta* (felids as final hosts) and 3.5% revealed both infection of *S. cruzi* and *S. homminis* (human as final hosts) (14). In Italy, *S. cruzi* and *S. bovifelis* were the most isolated species of cattle in the slaughterhouses (23). *S. hominis*, unlike previous studies (14, 21), was isolated from only 1.7% of the samples. Prior studies had not considered the prevalence of *S. bovifelis* and this may indicate that the higher prevalence of *S. hominis* has been due to the inability of techniques to separate the two species (23). In the present study, although molecular methods were determined as a powerful tool for confirmation of meat infection due to the high sensitivity rather than the digestive ones, they may not be merited for food safety detection because of showing the dead particles of infectious agents. On the other hand DNA extraction was challenging, due to disruption of the parasite structure in processed food.

Conclusion

More than half (57 %) of beef burgers were infected by *Sarcocystis*. *S. bovifelis* was identified as the main isolated species. Although the predominant species of the parasite in this study was *S. bovifelis*, isolation and species identification are suggested to confirm the real contamination with the zoonotic *S. hominis*. Livestock should be maintained at hygienic condition, away from felids, in order to avoid *S. bovifelis* infection.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Dubey JP, Speer C, Fayer R. Sarcocystosis of animals and man: CRC Press, Inc.; 1989.
2. Dubey J, Calero-Bernal R, Rosenthal B, et al . Sarcocystosis of animals and humans: CRC Press; 2015.
3. Fayer R, Esposito DH, Dubey JP. Human infections with *Sarcocystis* species. Clin Microbiol Rev. 2015;28(2):295-311.
4. Fayer R. *Sarcocystis* spp. in human infections. Clin Microbiol Rev.2004;17(4):894-902.
5. Oryan A, Moghaddar N, Gaur S. The distribution pattern of *Sarcocystis* species, their transmission and pathogenesis in sheep in Fars Province of Iran. Vet Res Commun. 1996;20(3):243-53.
6. Shekarforoush S, Razavi S, Dehghan S, Sarihi K. Prevalence of *Sarcocystis* species in slaughtered goats in Shiraz, Iran. Vet Rec. 2005;156(13):418-20.
7. Vangeel L, Houf K, Chiers K, et al . Molecular-based identification of *Sarcocystis hominis* in Belgian minced beef. J Food Prot. 2007;70(6):1523-6.
8. Gjerde B. Molecular characterisation of *Sarcocystis bovifelis, Sarcocystis bovini* n. sp., *Sarcocystis hirsuta* and *Sarcocystis cruzi* from cattle (Bos taurus) and *Sarcocystis sinensis* from water buffaloes (*Bubalus bubalis*). Parasitol Res. 2016;115(4):1473-92.
9. Saeed MA, Rashid MH, Vaughan J, Jabbar A. Sarcocystosis in South American camelids: The state of play revisited. Parasit Vectots. 2018;11(1):1-11.
10. Rosenthal B, Yang Z, Yuan L. *Sarcocystis* spp. Lucy J, Robertson, Huw, Smith V (eds) Foodborne Protozoan Parasites, USDA: NOVA Science Publishers 2012:151-66.
11. Prayson B, McMahon JT, Prayson RA. Fast food hamburgers: what are we really eating? Ann Diagn Pathol. 2008;12(6):406-9.
12. Doosti A, Dehkordi PG, Rahimi E. Molecular assay to fraud identification of meat products. J Food Sci Technol. 2014;51(1):148-52.
13. Yang ZQ, Li QQ, Zuo YX, et al. Characterization of *Sarcocystis* species in domestic animals using a PCR-RFLP analysis of variation in the 18S rRNA gene: a cost-effective and simple technique for routine species identification. Exp Parasitol. 2002;102(3-4):212-7.
14. Hooshyar H, Abbaszadeh Z, Sharafati-Chaleshtori R, et al . Molecular identification of *Sarcocystis* species in raw hamburgers using PCR–RFLP method in Kashan, central Iran. J Parasit Dis. 2017;41(4):1001-1005.
15. Dehkordi ZS, Yalameha B, Sari AA. Prevalence of *Sarcocystis* infection in processed meat products by using digestion and impression smear methods in Hamedan, Iran. Comp Clin Path. 2017;26(5):1023-1026.
16. Rahdar M, Salehi M. The prevalence of *Sarcocystis* infection in meat-production by using digestion method in Ahvaz, Iran. Jundishapur J Microbiol. 2011;4(4):295-9.
17. Ayazian Mavi S, Teimouri A, Mohebali M, et al. *Sarcocystis* infection in beef and industrial raw beef burgers from butcheries and retail stores: A molecular microscopic study. Heliyon. 2020;6(6):e04171.
18. Faghiri E, Davari A, Nabavi R. Histopathological Survey on *Sarcocystis* Species Infection in Slaughtered Cattle of Zabol-Iran. Turkiye Parazitol Derg. 2019;43(4):182-7.
19. Hoeve-Bakker B, van der Giessen J, Franssen F. Molecular identification targeting cox1 and 18S genes confirms the high prevalence of *Sarcocystis* spp. in cattle in the Netherlands. Int J Parasitol. 2019;49(11):859-866.
20. Nematollahia A, Khoshkerdar A, Helan JA, et al. A study on rate of infestation to *Sarcocystis* cysts in supplied raw hamburgers. J Parasit Dis 2015;39(2):276-9.
21. Hajimohammadi B, Dehghani A, Ahmadi MM, et al . Prevalence and species identification of *Sarcocystis* in raw hamburgers distributed in Yazd, Iran using PCR-RFLP. J Food Qual Hazards Control. 2014;1(1):15-20.
22. Hosseini H, Khaksar R, Sheshadi B. Sudy of *Sarcocyst* in raw, ready to sell hamburgers in Tehran. Iranian Journal of Food Science and Technology. 2008;4(4):65-71.
23. Rubiola S, Civera T, Panebianco F, et al . Molecular detection of cattle *Sarcocystis* spp. in North-West Italy highlights their association with bovine eosinophilic myositis. Parasites & Vectors. 2021;14(1):1-8.
24. Chiesa F, Muratore E, Dalmasso A, et al . A new molecular approach to assess the

occurrence of *Sarcocystis* spp. in cattle and products thereof: preliminary data. Ital J Food Saf. 2013;2(e41):148-51.
25. Jehle C, Dinkel A, Sander A, et al. Diagnosis of *Sarcocystis* spp. in cattle (*Bos taurus*) and water buffalo (*Bubalus bubalis*) in Northern Vietnam. Vet Parasitol. 2009;166(3-4):314-20.
26. Tenter AM. Current research on *Sarcocystis* species of domestic animals. Int J Parasitol. 1995;25(11):1311-30.
27. Moré G, Abrahamovich P, Jurado S, et al. Prevalence of *Sarcocystis* spp. in Argentinean cattle. Vet Parasitol. 2011;177(1-2):162-5.
28. Saito M, Shibata Y, Kubo M, et al. First isolation of *Sarcocystis hominis* from cattle in Japan. J Vet Med Sci. 1999;61(3):307-9.
29. Guclu F, Aldem-R O, Guler L. Differential identification of cattle *Sarcocystis* Spp. by random amplified Polymorphic DNA Polymerase chain reaction (RAPD-PCR). Rev Med Interne. 2004;155:440-4.
30. Rubiola S, Civera T, Ferroglio E, et al. Molecular differentiation of cattle *Sarcocystis* spp. by multiplex PCR targeting 18S and COI genes following identification of *Sarcocystis hominis* in human stool samples. Food Waterborne Parasitol. 2020;18:e00074.