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Identification of Sarcocystis spp. in Hamburgers Distributed in Fouman City, North of Iran Using Dab Smear and Digestion Methods

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ABSTRACT

Introduction: Human infection with *Sarcocystis* species can be attributed to the consumption of raw or inadequately cooked meat, such as hamburgers, containing encapsulated parasites.

Materials and Methods: This cross-sectional study was conducted between April and September 2022 in Fouman, Iran. A total of 182 hamburger samples, comprising two types of traditional hamburgers and industrial hamburgers, were collected.

Result: Out of 182 hamburger samples of different types, 34% of them were found to be infected with *Sarcocystis* spp. The infection rate within industrial and traditional hamburgers was 21.5% and 56%, respectively. Notably, a statistically significant difference was observed between *Sarcocystis*-infested traditional and industrial hamburgers ($p < 0.05$). Among the two distinct groups of 182 hamburgers, the microscopic digestion method revealed the presence of *Sarcocystis* bradyzoites in all 51 infected traditional beef burgers and 19 positive industrial beef burgers. However, in the dab smear method, just 33 and 12 positive samples were respectively identified in traditional and industrial beef burgers and a statistically significant difference was observed between efficiency of these two methods ($p < 0.05$). Infection with macroscopic parasite cysts was not observed in any of hamburger samples. Similarly, no statistically significant association was identified between the meat content percentage and the infection rate in industrial hamburgers ($p > 0.05$).

Conclusion: Considering the high abundance of *Sarcocysts* micro cysts in frozen raw hamburgers, it is recommended to fully cook this food product and avoid consumption when only partially cooked.

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Introduction

Sarcocystis species, a type of intracellular apicomplexan protozoan parasite, are distributed worldwide. Both carnivorous animals and humans are considered definitive hosts, while intermediate hosts include herbivores such as sheep, goats, cattle, and poultry [1,2]. These parasites follow a two-host life cycle, wherein sexual and asexual phases occur in carnivorous hosts and herbivorous hosts, respectively. Oocysts are released through the feces of definitive hosts and can infiltrate intermediate hosts, leading to the formation of cysts after undergoing multiple developmental phases within skeletal and cardiac muscles [3,4]. This

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protozoan parasite causes symptoms, such as loss of appetite, emesis, loose stools, and respiratory problems in its host [4]. *Sarcocystosis* has been prominently observed in bovine carcasses; with its occurrence is almost 100% in different regions across the world [5]. Three important types of this parasite are *S. cruzi*, *S. hirsuta*, and *S. bovifelis* that have been identified in cattle, and they could create cysts of various sizes in the muscles of the cow [5]. This issue annually results in significant losses for the livestock industry and leads to financial losses, since it causes the meat look unpleasant even after removing infected parts [6]. Human infection can be attributed to the consumption of raw or inadequately cooked meat that contains encapsulated parasites [6]. Industrial hamburgers are manufactured in meat processing facilities under the supervision of the Iranian Ministry of Health and Medical Education [1]. Traditional hamburgers are homemade products sold in street markets without rigorous safety oversight and adherence to health organization standards. Therefore, measures should be taken to check infection by different pathogens in meat products. Among various methods used for detecting *Sarcocystis* cyst in meat, digestive techniques are the most commonly utilized approaches. Preparation of slides using dab smear method is another method used to identify Sarcocysts. Considering the importance of *Sarcocystosis* in public health, the present study investigated the degree of contamination of industrial and traditional hamburgers by *Sarcocystis* spp. in Fouman city using two methods of digestion and dab smear.

Materials and Methods

Sampling

This cross-sectional study was carried out between April and September 2022 in Fouman, Iran. A total of 182 hamburgers were collected using a simple random sampling from butcher shops, grocery stores and supermarkets, and street food vendors and after that were transported to the laboratory and kept with ice. Industrial burgers were selected randomly from different companies. A total of 182 hamburger samples, including two types of traditional hamburgers and industrial hamburgers, were collected. Traditional hamburgers originated from traditional butcheries and comprised exclusively of 100% beef (n: 91). Meanwhile, the remaining samples were manufactured by factories and contained meat ranging from 60% to 95% as specified by their respective industrial trademark (n:91). Upon reaching the laboratory, all samples underwent a process of slicing into 5 mm segments. Subsequent visual examination by the naked eye was carried out meticulously to detect the presence of macro cysts of *Sarcocystis*. The analysis was performed in two ways including digestion method and dab smear for all samples. In this process, for doing digestion method, after weighting the hamburgers to seventy grams, they were placed overnight at a temperature of 28 °C in a digestion solution containing 0.5% pepsin, 1.5% HCL in distilled water, and subsequently, it was combined with a 50 ml digestion solution, comprising 1.3 g of pepsin, 2.5 g of NaCl, and 3.5 mL of concentrated HCl, and the prepared solution was dissolved in 500 mL of sterile distilled water. The mixture was incubated at a temperature of 40°C for 2 hours, and centrifuged at 1500 rpm for 5 minutes. The deposit resulting was washed three times in phosphate-buffered saline (PBS) (centrifuged at 2000 rpm for 3 minutes). After that, two 3 × 1 inch (75 × 25-mm) clean glass slides were used for making two thin smear that were Finally stained with Giemsa. In the dab smear method, a piece of hamburger tissue was cut to the one square centimeter and after that was pressed by several times on a slide with Tissue Forceps. Ten pieces and 10 slides were prepared by this way for each sample, and finally were mixed by Giemsa stain. After that, the slides were placed under a microscope to identify the presence of Sarcocystis cyst.

Statistical analysis

Statistical analysis was performed using the SPSS software (Version: 1.0.0.1406) and the t-test statistical test.

Results

In this study, 182 hamburger samples of two

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different types were examined. A substantial 38.5% of all samples of two types were found to be infected with *Sarcocystis* spp. The infection rate in traditional hamburgers (56%) exhibited a statistically significant increase compared to industrial hamburgers (21.5%) ($p < 0.05$) (Table 1). Among the two distinct groups of hamburgers, the microscopic digestion method revealed the presence of *Sarcocystis* bradyzoites in all 51 infected traditional beef hamburgers and 19 cases in industrial beef hamburgers. However, unlike 33 and 12 infected samples of traditional and industrial beef hamburgers were respectively identified using the dab smear method. Therefore, a statistically significant difference was observed between the efficiency of these two methods ($p < 0.05$) (Table 2). Infection with macroscopic parasite cysts was not observed in any of the samples.

Table 1: Percentage and the rate of *Sarcocystis* in industrial and traditional hamburgers

Hamburger type	Number	Infected hamburgers	Percentage of infection
Industrial hamburger number	91	51	56%
Traditional hamburger number	91	19	21.5%
Total number	182	70	38.5%

Table 2: The rate of *Sarcocystis* detection in industrial and traditional hamburgers by digestion method and dab smear method and differences between them

Detected hamburgers	Number	Digestion method	Dab smear method	Differences between methods
Industrial hamburger number	91	51	33	18
Traditional hamburger number	91	19	12	7
Total number	182	70	55	25

Discussion

Many studies have been conducted to assess the prevalence of *Sarcocystis* in meat products. Faghiri et al. described a parasite contamination rate of 87.9% in traditional hamburgers in Zabol, which was significantly higher than the rate observed in industrial hamburgers (67.8%) [7]. Jafari, et al. reported that 87.5% of hamburgers, 83.3% of sausages, and 66% of cocktail sausages were found to be infected by *Sarcocystis* spp. in Hamadan using the digestion method[1]. Haj Mohammadi et al. found a *Sarcocystis* infection rate of 77.9% among 190 industrial and traditional hamburgers in Yazd [8]. In contrast to the findings of the present study, some investigations showed different results, which could be due to sampling time and months of sampling. However, these studies did not show any statistical difference between the infection rate of industrial and traditional hamburgers [9]. In the present study, the infection rate of hamburgers with *Sarcocystis* cysts was significantly high. For *Sarcocystis* infection prevention, it is very important to ensure that livestock are kept in sanitary conditions and away from felids. This approach is necessary to mitigate the risk of *S. bovifelis* transmission. Most of studies primarily employed techniques such as impression smear, histology, and digestion methods like this study and reported similar results with the current study. Furthermore, according to the existing scientific literature database, none of the investigations conducted to detect *Sarcocystis* spp. in hamburgers has successfully identified different species of *Sarcocystis*. This limitation is often attributed to the inefficiency of the applied methods in identifying specific species of this parasite [10-16]. Hosseini et al. reported *Sarcocystis* infection in 47.9% (56 out of 117) of hamburger samples through an impression smear assay; however, they did not specify the *Sarcocystis* species present in the samples [17]. Similarly, a study in Ahvaz, southern Iran, by Rahdar and Salehi (2011) utilized the digestion method and found a *Sarcocystis* infection rate of 56.0% in hamburgers

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[18]. Nematollahi et al. studied both the impression smear and peptic digestion methods to determine the prevalence rate of *Sarcocystis* spp. The infection rate in both traditional and industrial hamburgers was 56%[19]. The infection rate is not only determined by geographical area, age, or gender of the intermediate host, but is significantly influenced by the methodology employed for *Sarcocystis* detection. Smear and peptic digestion methods indicated infection rates ranging from 47.9% to 56.0%, respectively, but Jahed-Khaniki and Kia reported a much lower infection rate of 6.25% in Garmsar, Iran, using the histological method [20]. Hosseini et al. and Nematollahi et al. did not find a significant difference in infection rates between industrial and traditional hamburgers, but the present study revealed a significant contrast [9, 17]. The prevalence of *Sarcocystis* spp. in traditional hamburgers (56%) was notably higher than industrial hamburgers (21%) ($p < 0.05$). Industrial hamburgers are commonly prepared using the whole carcass from single cattle, resulting in a batch of hamburgers with more uniform meat content. The absence of macroscopic *Sarcocysts* in the current study is consistent with the findings of most previous studies [5,8,14,18,20]. This could be attributed to meat inspectors identifying and discarding macro cysts during official inspections at slaughterhouses. The present findings emphasize a significant concern due to the high prevalence of *Sarcocystis* spp. in hamburgers, posing a considerable risk of infection for the Iranian population consuming this meat product. Considering the zoonotic nature of some species like *S. hominis*, it is important to pay attention to the infestation rate of *S. hominis* in Iran. *S. hominis*, have microscopic cysts that are not visible in appearance, so the chance of transmission increases. It is necessary to aware individuals who consume semi-cooked hamburgers about the life cycle, public health implications, and pathogenicity of this parasite. For food safety and the inactivation of this food-borne protozoa in beef burgers, it is necessary to fully cook and reach a core temperature of at least 70°C. Alternatively, freezing the hamburgers at -20°C overnight before consumption is recommended.

Conclusion

The level of contamination with microscopic *Sarcocystis* cysts in the studied hamburgers using the digestion method was higher than dab smear method. Both techniques showed a high level of *Sarcocystis* in samples. Careful cooking of meat and meat products as well as raising awareness will be effective in preventing contamination. Also, improving the sanitary conditions of keeping and feeding livestock, as well as cutting off contact with the main hosts of the parasite, can reduce livestock contamination. Considering the high abundance of Sarcocysts micro cysts in frozen raw hamburgers, it is recommended to fully cook this food product and avoid half-cooked consumption to prevent transmission.

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Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' contributions

Study concept, design, original idea, conducting laboratory research, and the development of the protocol: Ahmadiara E. Writing the manuscript and sampling: Rahimzadeh A. Analyzing data and preparing the final edition of manuscript: Alian Samakkhah, Sh.

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