A study on rate of infestation to *Sarcocystis* cysts in supplied raw hamburgers

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Abstract This study was carried on for determination of presence of *Sarcocystis* cysts in raw hamburgers in Tabriz North West of Iran. Ninety-six samples of industrial (70 % meat content) and traditional (30 % meat content) hamburgers (80 samples industrial and 16 samples traditional) were obtained from retail fast food stores. The samples were examined by gross examination, and microscopic examination methods consist impression smear and peptic digestion. Macroscopic cysts did not observed in any of the samples in gross examination. Microscopic study showed that from 96 samples 54 (56.25 %) samples were infected by at least one bradyzoites of *Sarcocystis*. From 54 infected samples, 45 industrial hamburgers and nine traditional hamburgers samples were infected. Statistical analysis showed that there was not significant differences between industrial and traditional hamburgers in infection to *Sarcocystis*. Infestation of hamburgers to *Sarcocystis* in summer was higher than other seasons but this difference was not significant. In Iran, beef meat is used for preparation of 70 % of hamburger and infestation of cattle to sarcocystosis was reported in many investigations in Iran. With

regard to the high prevalence of *Sarcocystis* infection in meat products such as hamburgers in this study, it is strongly recommended to avoid eating raw or undercooked hamburgers or keep them at freezing temperature for at least 3–5 days.

Keywords Infestation · Raw hamburgers · *Sarcocystis*

Introduction

Sarcocystosis is a parasitic zoonosis caused by species of *Sarcocystis*; an intracellular protozoan parasite in the phylum Apicomplexa and family Sarcocystidae. The infection is characterized by cyst formation in muscular tissues (muscular sarcocystosis) in the intermediate host or colonization of the lamina propria of the intestines (intestinal sarcocystosis) in the definitive host. *Sarcocystis* has a requisite two-host life cycle based on a prey-predator (intermediate-definitive) host relationship (Fayer 2004). Cats and dogs have been recognized as competent definitive (predator) hosts and cattle, pigs, horses, sheep, goats, birds, rodents, camelids, wildlife, reptiles and humans are known as intermediate hosts that cysts of *Sarcocystis* are formed in their skeletal muscles (Dubey et al. 1989). Humans can serve as both intermediate and definitive host. In humans, eating raw or undercooked meat containing mature *Sarcocystis* has led to acquire intestinal sarcocystosis with infected individuals that they exhibit diarrhea, bloat, dyspnea, tachycardia, nausea and loss of appetite (Fayer 2004). Currently, sarcocystosis is considered as one of the opportunistic infections that is exacerbated in acquired immune deficiency syndrome (AIDS) patients (Velasquez et al. 2008). Several methods can be used to detect sarcocystosis including pathological and serological methods. Gross examination, impression smear and digestion methods

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are the usual methods in histopathological methods and indirect fluorescent antibody test (IFAT) and Enzyme-linked immunosorbent assay (ELISA) are used in serological methods (Dubey et al. 1989). The prevalence of sarcocystosis in slaughtered animals has been investigated in many studies in different parts of Iran which indicate the infection rate in range between 3.5 and 100 % in different food animals that use different methods (Hamidinejat et al. 2010; Najafian et al. 2008; Nematollahi et al. 2011; Razavi et al. 2003; Razmi 1989). In Iran beef meat is used in 70 % of hamburger preparation and recently consumption of hamburger as fast food is increasing due to easy preparation and delicious taste. Current study is carried on for determining the presence of *Sarcocystis* cysts in raw hamburgers in Tabriz North West of Iran.

Materials and methods

Study area

The study was conducted in Tabriz (northwest Iran). Tabriz is located in the East-Azarbaijan province (36°43'–39°25'N and 45°3'–48°19'E). The region is mountainous, with an altitude of 1351.4 m. The climate is temperate with relatively hot and dry summers and cold winters.

Sample size determination

The sample size was calculated by using the formula that was described by Thrusfield (1992). Because the prevalence of sarcocystosis at previous studies was reported 56 % (Nematollahi et al. 2011), the desired sample size was used with 95 % level of confidence and 5 % desired absolute precision that has been estimated 96 (Thrusfield 1992).

Sampling

This study was carried on since October 2011 to August 2012 in Tabriz, North West of Iran. Ninety-six samples of industrial and traditional hamburgers (80 samples industrial and 16 samples traditional) were obtained from retailer fast food stores. The content of meat in industrial and traditional hamburgers was 70 and 30 % respectively according to factory information. Sampling was achieved in four seasons (24 samples in each season).

Preparation methods

The samples were examined in three ways: (1) gross examination, and microscopic examination methods, (2) impression smear and (3) peptic digestion.

Gross examination: This method was done by naked eye examination. All the hamburgers were dissected and sectioned 2–3 mm slices and were observed carefully for probable macroscopic cysts.

Impression smear: About 1 g of hamburger was cut into small pieces, approximately 3–5 mm thick and crushed firmly between two glass slides and was stained by Giemsa staining method and examined under the microscope (40×).

Peptic digestion: A modification method by Dubey et al. (1989) and modified by Hamidinejat et al. (2010) was used for digestion of tissues (Dubey et al. 1989; Hamidinejat et al. 2010). Approximately 20 g of tissues were minced and digested for 30 min at 40 °C in 50 ml of digestion medium containing 1.3 g pepsin, 3.5 ml HCl, and 2.5 g NaCl in 500 ml of distilled water. The digested was poured through a fine-meshed sieve into a beaker and the filtrate was centrifuged 1,500 rpm for 5 min. The digested samples were sieved through mesh and after discarding the supernatant fluid, the sediment were stained by Giemsa and examined microscopically for detecting *Sarcocystis* bradyzoite. Each sample was considered positive by observation at least one bradyzoite of *Sarcocystis*.

Statistical analysis

Analysis of data was performed by using SPSS version 16 package. Significant difference between seasons and kind of hamburgers was evaluated by Chi square test.

Results

Gross examination: Macroscopic cysts were not observed in any of the samples.

Impression smear and Peptic digestion: In these methods, microscopic study showed that from 96 samples 54 (56.25 %) samples were infected by at least one bradyzoites of *Sarcocystis*. From 54 infected samples, 45 industrial hamburgers (70 % meat content) and nine traditional hamburgers (30 % meat content) samples were infected. Statistical analysis showed that there were not significant differences between industrial and traditional hamburgers in infection to *Sarcocystis*. These results are showed in Table 1.

In this study, 96 samples of hamburgers in four seasons (24 samples in each season) were studied for infestation to *Sarcocystis*. Result showed that infestation of hamburgers to *Sarcocystis* in summer was higher than other seasons but this difference was not significant. The results of infestation of hamburgers are showed in Table 2.

Table 1 The prevalence of *Sarcocystis* infection in hamburger by microscopic examination methods

Hamburger (meat content)	No. of examined	No. of positive	% of positive
Industrial (70 % meat content)	80	45	56.25
Traditional (30 % meat content)	16	9	56.25
Total	96	54	56.25

Discussion

The frequency of fast food consumption has dramatically increased since the early 1970's. For example the number of fast food outlets has increased from about 30,000 in 1970 to more than 233,000 locations in the United States in 2004, generating sales in excess of \$242.5 billion annually (Paeratakul et al. 2003). In fast foods the consumption of hamburger is higher than others due to easy preparation and delicious taste (Prayson et al. 2008). In Iran, beef meat is used for preparation of 70 % of hamburger and infestation of cattle to sarcocystosis was reported in many investigations in Iran. For example Razmi (1989) reported that the prevalence rates of microscopic sarcocystosis cysts in cattle, goat, sheep and buffalo of Iran are 73.79, 70.45, 60.93 and 40.90 % especially. Also infestation to sarcocystosis in cattle in other regions of Iran such as Shahrekord (91 %), Ahwaz (53.5 %), Shiraz (99 %) and Shahryar (99.9 %) were reported by digestion and histopathologic methods. Therefore infestation to *Sarcocystis* is more probable in these hamburgers.

In this study, macroscopic cysts were not observed in any of the samples. This result was according to another investigation regarding sarcocystosis and hamburgers (Hosseini et al. 2007; Jahed-Khaniki and Kia 2006; Rahdar and Salehi 2011). Macrocystic cysts of *Sarcocystis* are not observed in any investigations. Because macroscopic cysts have very low prevalence in beef and are deleted in general inspection on beef in slaughterhouse.

The result that was obtained in this study was similar to those recorded in a study by Hosseini et al. (2007) and Rahdar and Salehi (2011) which recorded 56 % prevalence

rate of *Sarcocystis* in hamburgers by using digestion method in Tehran and Ahwaz (South of Iran) respectively.

Hosseini et al. (2007) reported that infestation of raw hamburgers to *Sarcocystis* by Dab smear was 47.9 % (Najafian et al. 2008). However, by using histopathologic method, Jahed-Khaniki and Kia (2006) reported only 6.25 % infestation and Prayson et al. (2008) confirmed that two out of eight (25 %) of hamburgers were infected with the parasite. It seems this difference is related to methods of studies between the last study and others. Tissue digestion was found to be more efficient in detecting *Sarcocystis* bradyzoites in tissues than histology. Digestion of host tissues is the most sensitive method to detect light *Sarcocystis* infection because several hundreds or thousands of bradyzoites are released from *Sarcocystis* as the host tissue and *Sarcocystis* are digested for making detection of the parasites easier. In histology, only thin sections (5 µm) are taken simultaneously, which may not contain any cyst (Dubey et al. 1989). This finding agrees with the findings of Kudi (1989) and Hamidinejat et al. (2010).

The finding of the present study is in accordance with the findings were done by Hosseini et al. (2007) and Rahdar and Salehi (2011) who reported that there was not difference between industrial hamburgers (70 % meat content) and traditional hamburgers (30 % meat content) in infestation to *Sarcocystis*.

The finding of the present study is similar to the findings of Nematollahi et al. (2011) reported that there was not difference between the infection rate to sarcocystosis and season. With regard to the high prevalence *Sarcocystis* infection in meat products such as hamburgers in this study, it is strongly recommended to avoid eating raw or under-cooked hamburgers or keep them at freezing temperature for at least 3–5 days.

References

- Dubey JP, Speer CA, Fayer R (1989) *Sarcocystis* of animals and man. CRC Press, Boca raton, p 215
- Fayer R (2004) *Sarcocystis* spp. in human infections. Clin Microbiol Rev 17:894–902
- Hamidinejat H, Jalali MHR, Nabavi L (2010) Survey on *Sarcocystis* infection in slaughtered cattle in south-west of Iran, emphasized on evaluation of muscle squash in comparison with digestion method. J Anim Vet Adv 12:1724–1726
- Hosseini H, Khaksar R, Shemshadi B (2007) Study on infestation of raw hamburgers to *Sarcocystis* cyst in Tehran. Iranian Nut Sci 4:65–70
- Jahed-Khaniki GR, Kia EB (2006) Detection of the *Sarcocystis* cysts from meat supplied for hamburger in Iran by histological method. J Med Sci 6:18–21
- Kudi AC (1989) Prevalence of *Sarcocystis* species in Kaduna, Plateau, and Bauchi states of Nigeria. Unpublished MSc thesis, Ahmadu BelloUniversity, Zaria, Nigeria, p 117

Table 2 The prevalence of infestation to *Sarcocystis* in hamburgers in four seasons

Season	No. of examined	No. of positive	% of positive
Spring	24	10	41.6
Summer	24	19	79.16
Autumn	24	16	66.66
Winter	24	9	37.5
Total	96	54	56.25

- Najafian HR, Mohebali M, Keshavarz H (2008) Study on frequency of *Sarcocystis* spp. by macroscopic and microscopic methods in slaughtered cattle in Shariar district and their public health importance. *Paj Saz* 77:15–19
- Nematollahi A et al (2011) Determination of prevalence of *Sarcocystis* by using gross examination, impression smear and digestion methods in muscles of slaughtered sheep of Tabriz, Iran. In: Proceedings of 19th international congress of Fe. ME. S.P. Rum (Belgrade, Serbia), pp 131–133
- Paeratakul S, Ferdinand DP, Champagne CM, Ryan DH, Bray GA (2003) Fast food consumption among US adults and children: dietary and nutrient intake profile. *J Am Diet Assoc* 103: 1332–1338
- Prayson B, McMahon JT, Prayson RA (2008) Fast food hamburgers: what are we really eating? *Annu Diag Pathol* 12:406–409
- Rahdar M, Salehi M (2011) The prevalence of *Sarcocystis* infection in meat-production by using digestion method in Ahvaz. *Iran Jundi J Microbiol* 4:36–45
- Razavi SM, Shekarforoush SS, Farahani M, Sarihi K (2003) Prevalence of *Sarcocystis* in slaughter sheep in Shiraz. *Iran J Vet Parasitol* 17:139–141
- Razmi GhR (1989) Prevalence of *Sarcocystis* spp. in domestic ruminants of Iran. D.V.M. thesis, Veterinary Medicine University of Tehran
- Thrusfield M (1992) *Veterinary epidemiology*. Blackwell, London, p 182
- Velasquez JN, Risio C, Etchart CB, Chertcoff AV, Mendez N, Cabrera MG, Labbe JH, Carnevele S (2008) Systemic *Sarcocystosis* in a patient with acquired immune deficiency syndrome. *Hum Pathol* 39:1263–1267