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Research Article

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Investigating the Infection Rate of *Sarcocystis* in Hamburgers Sold in Shiraz City, Iran

Mohammad Javad Hooshyar[1], Ali Karimi[2], Pouria Zarei[3*], Armin Ghahremani[1]

[1]Gratuated, Department of Parasitology, Collage of Veterinary Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran
[2]Assitant Professor, Department of Parasitology, Collage of Veterinary Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran
[3]Student, Department of Parasitology, Collage of Veterinary Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran

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Abstract

Background and aim: *Sarcocystis* is one of the most common diseases between humans and animals in most parts of the world. This parasitic disease is caused by *Sarcocystis* protozoa. *Sarcocystis* cysts are mainly found in the striated muscles of cattle, sheep, and goats and vary in size from a few microns to a few millimeters. In this study, we tried to determine the infection rate of Burgers offered in Shiraz city with *Sarcocystis* parasite to investigate this popular meat product in terms of *Sarcocystis* contamination.

Materials and Methods: To perform the laboratory steps of the project, 100 samples of hamburgers (70 samples of industrial hamburgers and 30 samples of handmade hamburgers) were collected from stores and shopping centers of Shiraz and transferred to the laboratory to be studied and examined by observing the necessary hygienic points. Macroscopic observation, impression smear (DOB smear) method, and digestion method were various methods of examining the samples.

Results: In the macroscopic, DOB smear, and digestive methods contamination of the samples was zero, 28, and 21, respectively. Also, it was found that out of 70 samples of industrial hamburgers, 29 samples (41.42%) were infected with *Sarcocystis*. Of these, 18 samples (62%) were collected in autumn and 11 samples (37.93%) were collected in summer. Out of 20 samples of traditional shop hamburgers, 13 samples (65%) were infected with *Sarcocystis*. Of these, 8 samples (61.5%) were collected in autumn and 5 samples (38.46%) were collected in summer. Out of 10 samples of traditional hamburgers presented in hand-sold units, 7 samples (70%) were infected with *Sarcocystis*. Of these, 4 samples (57.14%) were collected in autumn and 3 samples (42.85%) were collected in summer. Data analysis using the chi-square test showed that there is no significant relationship between industrial and traditional burgers with a contamination rate with a 95% confidence interval. Also, there was no significant relationship between autumn and summer samples with the amount of contamination.

Conclusion: The results of this study show that the prevalence of *Sarcocystis* parasite contamination of industrial and traditional hamburger samples in Shiraz city is high. Therefore, it is suggested that preventive measures and detailed inspections be carried out at the place of production and sale of hamburgers.

Keywords: *Sarcocystis, Hamburger, Zoonotic disease, Shiraz, Iran*

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**** Corresponding Author***
Student, Department of Parasitology, Collage of Veterinary Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran.
E-mail: pouriazarei95@gmail.com, Orcid: https://orcid.org/0000-0002-9298-3512

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Introduction

Sarcocystis is one of the most common diseases between humans and animals in most parts of the world. This parasitic disease is caused by *Sarcocystis* protozoa. *Sarcocystisis* is a genus of protozoan parasites that infect most species of mammals and some reptiles and birds. The life cycle of a typical member of this genus includes two host species, a definitive host and an intermediate host. Often, the definitive host is a predator and the intermediate host is its prey (herbivorous or omnivorous). This parasite reproduces in the intestines of the definitive host, is excreted with feces, cysts are formed in the body of intermediate hosts, and the final host is infected by swallowing these cysts (Gabriele *et al.,* 2006).

When the intermediate host is eaten by the definitive host, the cycle is complete. The definitive host usually shows no signs of infection. Oocysts of this family have two sporocysts, and each sporocyst contains four sporozoites. (Claveria *et al.,* 2001). Sarcosporidia, which may also be referred to as small tubular cysts or Miescher's tubes in some sources, more than a century ago as a completely normal parasite in the striated and cardiac muscles of aquatic birds, mammal (Sibley, 2010; Dubey *et al.,* 1989).

Sarcocystis has two stages of sexual and asexual reproduction. The asexual stage includes schizogony and cyst formation in the intermediate host, and the sexual stage includes gametogony and sporogony in the final host. Gametogony and sporogeny generally occur in the intestine of the definitive host, while both schizogony (occurring in various tissues) and sarcocyte formation (containing bradyzoites and metrocytes) occur primarily in the muscles of the intermediate host. In some cases, a single species may act as a definitive and intermediate host (Abubakar *et al.,* 2013; Beaver *et al.,* 1979).

Carnivores might get infected with *Sarcocystis* cysts by eating infected tissues. The *Sarcocystis* cyst ruptures in the digestive tract of carnivores as the digestive enzymes are released. After entering the intestinal mucosa, gametes are produced. Fertilization takes place and an oocyte is formed without a spore. Two sporocysts are created in each oocyst, and four sporozoites are created in each sporocyst. The intermediate host becomes infected by eating material containing sporocysts. The sporozoites are released in the digestive system of the intermediate host and invade the intestinal wall and undergo two stages of schizogony inside the vascular endothelium cells. Then it enters blood lymphocytes and creates merozoites that attack muscle cells and turn into cysts. Sometimes a cyst occurs in the brain tissue. Damage to the walls of the vessels causes bleeding and anemia of the host (Sibley, 2010; Sahl Poulsen *et al.,* 2014).

Oocysts are excreted in the faeces of the definitively infected host. By passing through the sporogenous stage, the oocyst creates two sporocysts. Once this step is complete, the oocyst itself undergoes lysis and releases the sporocysts into the environment. Sporocysts usually contain four sporozoites and measure 15-19 by 8-10 µm (Kappe *et al.,* 2004; Markus *et al.,* 1974).

Then intermediate hosts such as cattle or pigs ingest the sporocysts. The sporozoites are then released into the body of these intermediate hosts and migrate into the vasculature, where they undergo the first two generations of asexual reproduction. These rounds lead to the growth of the meronts. This stage lasts about 15 to 16 days after consumption of sporocysts. The merozoites leave the second-generation meronts and enter the mononuclear cells, where they develop endodyogenically. Subsequent generations of merozoites develop downstream in the direction of blood flow into arteries, capillaries, veins, and veins throughout the body and subsequently develop into the final asexual generation in muscle (Markus *et al.,* 1978; Pereira & Bermejo, 1988; Claveria *et al.,* 2001; Schmidt *et al.,* 1989).

The merozoites that enter the muscle cells form metrocytes and initiate the formation of sarcocytes. Sarcocytes begin as unicellular bodies containing a single metrocyte and accumulate through asexual proliferation of multiple metrocytes, and the sarcocyte increases in size. As the sarcocyte matures, small, round, noninfectious metrocytes form crescent-shaped bodies called bradyzoites (also known as bradyzoic merozoites), which are infective to the definitive host. The time required to mature varies by species and may take 2 months or more (Wong *et al.,* 1992).

In species in which symptoms develop, these symptoms usually occur 20 to 40 days after ingestion of the sporocysts and during subsequent migration of the sporozoites through the body's vasculature. Acute lesions (edema, hemorrhage and necrosis) occur in the affected tissues. This parasite tends to skeletal

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muscle (myositis), cardiac muscle (cardiac muscle petechial hemorrhage and serous) and lymph nodes (edema, necrosis and hemorrhage). These lesions are associated with the maturation of the second generation of meronts in endothelial and subendothelial cells. Sometimes infiltration or mononuclear hyperemia has been observed in the lamina propria of the small intestine. After the acute phase, cysts may be found in various muscle tissues, generally without pathology (Abubakar *et al.,* 2013).

Infection with this parasite is known as sarcosporidiosis. The pathology of this parasite to cause disease in humans is of two types:

1) A rare aggressive form associated with vasculitis and myositis. The invasive form may involve a wide variety of tissues, including lymph nodes, muscles, and the larynx.

2) Intestinal form that appears with nausea, abdominal pain and diarrhea and is usually mild and lasts less than 48 hours, intestinal form may sometimes be severe or even life-threatening. (Clavel *et al.,* 2001)

Clinical cases have been associated with acute fever, myalgia, bronchospasm, itchy rash, lymphadenopathy, subcutaneous nodules with eosinophilia, increased erythrocyte sedimentation rate, and increased creatinine kinase levels. Symptoms may last up to five years. Segmental necrotizing enteritis was reported in one case. Infection can be prevented by cooking meat before consumption. On the other hand, freezing meat at -5°C for a few days before consumption destroys *Sarcocystis* (Fayer *et al.,* 1982).

Four known species of *Sarcocystis* infect cattle. These four species are *S. bovihominis, S. bovifelis, S. cruzi (S. bovicanis*) and S. hirsute. *Sarcocystisis cruzi* is the only species known to be pathogenic in cattle. Several clinical syndromes have been reported in association with this parasite: eosinophilic myositis. Abortion, stillbirth and death in pregnant cows; Two cases of necrotizing encephalitis in heifers have also been reported. Common clinical signs of acute bovine *Sarcocystis* include anorexia, fever (42°C or higher), anemia, cachexia, enlarged palpable lymph nodes, excessive salivation, and tail tip hair loss. Sheep may also be affected by four types of *Sarcocystis*. These four species are S. *arieticanis, S. tenella, S. gigantea and S. medusiformis*. Infection with these parasites is common in the United States, with more than 80 percent of sheep examined showing evidence of Infection (Dubey *et al.,* 1988).

The high prevalence of *Sarcocystis* in cattle, sheep and livestock in many developed and developing countries is confirmed (Sahl Poulsen C et al., 2014). Many common diseases between humans and animals are transmitted to humans through meat and meat products. Among these diseases is *Sarcocystis*. Therefore, considering the importance of the topic and considering the increasing role of fast foods, especially hamburgers, in our diet and the fact that hamburgers are made from beef, which is considered as the intermediate host of human *Sarcocystis*, and considering who it is. Bovine *Sarcocystis* are microcysts that cannot be seen with the naked eye, and there is no comprehensive study on the prevalence of *Sarcocystis* in hamburgers sold in the hamburgers offered in Shiraz city. In this article, it is tried to determine the extent of contamination of Shiraz hamburgers with *Sarcocystis* parasites so that it can be made a comparison between this popular meat product and unprocessed meat in terms of *Sarcocystis* contamination. Although researches has been conducted on sheep and cattle *Sarcocystis* in the meat of slaughtered animals in Shiraz and Kazerun slaughterhouses, but no study has been conducted on the contamination of meat products with *Sarcocystis* parasites in Fars province and Shiraz city. Based on this, this study was done.

Material and Methods

The research and laboratory stages of this project were carried out in the summer and autumn seasons of 2021 in Shiraz city, the capital of Fars province. The city of Shiraz, with a population of over 2 million people and an industrial slaughterhouse, and due to having important animal breeding centers, is one of the important hubs of red meat production in Iran, and with different hamburger production factories, it plays an important role in supplying edible hamburgers. According to previous surveys, the prevalence of *Sarcocystis* in cattle and sheep is about 50% on average. For this purpose, 100 hamburger samples were selected for examination and parasitology tests and were analyzed in terms of contamination with the parasitic protozoan *Sarcocystis*. These 100 hamburger samples were selected from 2 different categories:

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First category, industrial hamburgers: the products of hamburger factories located in Shiraz and its surrounding areas are known as industrial hamburgers. From this group of hamburgers, 70 samples from five different brands were selected to study, because this category of hamburgers are used more by consumers.

Second category: traditional or handmade hamburgers: hamburgers produced by food vendors are known as handmade hamburgers.

The handmade hamburgers used in this project are placed in two separate groups. The first group were hamburgers produced by fast food shops, and 20 samples were examined in this group. The second group was the hamburgers offered by hand-selling units that set up stalls on the side of the streets and prepare and sell hamburgers. From these units, 10 samples were taken to check the parasitism.

This product is usually made from beef. Industrial hamburgers are divided into two main groups based on the manufacturing formula:

1. Hamburgers containing 30% of meat under the name of normal hamburger, in which soy, onion, bread flour, salt and spices are used in their production in addition to beef.
2. Premium hamburger which contains at least 60% meat and its other components are similar to regular hamburgers, with the difference that soy is not used in its preparation.

Handmade hamburgers are often made from beef or a mixture of beef and sheep, and there is no special formula for their production, but they often contain at least 50% meat. (30) Although a research has been conducted on sheep and cattle *Sarcocystis* in the meat of slaughtered animals in Shiraz and Kazeroon slaughterhouses, but no study has been conducted on the contamination of meat products with *Sarcocystis* parasites in Fars province and Shiraz city. Based on this, this review was done. During this investigation, 100 hamburger samples were prepared from different geographic locations of Shiraz city (4 regions, north, south, west and east) during two seasons (50 samples each season) of industrial and handmade hamburgers. 70 samples of industrial hamburgers, 20 samples of shop hamburgers and 10 samples of hand-made hamburgers were transferred to the laboratory in the vicinity of ice. Then, after macroscopic examination, the samples were examined for the presence of microscopic cysts in the following ways:

1. Macroscopic examination:

Although due to the special processes that exist in the production of hamburgers, both industrially and traditionally, the probability of seeing macroscopic cysts on the hamburger was far from the mind, and almost all the *Sarcocystis* cysts in the hamburger samples were visible by eye. They are not seen unarmed, but the purchased hamburgers were examined for the presence of macroscopic cysts. In this way, the outer surface of the samples and then the depth of each sample were checked for the presence of macroscopic cysts by creating grooves and sheet cuts.

2. DOB Smear Method:

In this method, after the sample was homogenized, small pieces of it were removed and repeatedly pressed on the slide so that the extract was placed on the slide as a thin layer. Then the slides were dried in the vicinity of air and after fixing with methanol, they were stained by Giemsa method and subjected to microscopic examination.

3. Digestion Method:

In this method, based on the method presented by Dubey et al, (1989), 50 grams of each homogenized sample was placed separately in Erlenmeyer flasks. Then, 100 ml of digestive solution was poured on each of the samples (the digestive solution contains 1.3 grams of pepsin powder, 2.5 grams of sodium chloride, 3.5 ml of 40% hydrochloric acid and 500 ml of distilled water) until the surface of the samples was covered. Then, the samples were thoroughly stirred in the digestion solution to mix well. Then the samples were transferred to a 37°C hot water bath. In order to the enzyme reaction to be carried out well, the samples inside the hot water bath were stirred at different minutes. After half an hour to one hour, the samples were removed from the hot water bath. Then the solution was passed through a filter (cloth) and the filtered solution was centrifuged at 1500 rpm for 10 minutes. After that, we made a spread from the sediment and after fixing it with ethanol, we stained it with Giemsa and put it under microscopic examination.

The obtained results were statistically analyzed using SPSS software (Version 20) and Chi-square test. *P-value*<0.05 was considered significant.

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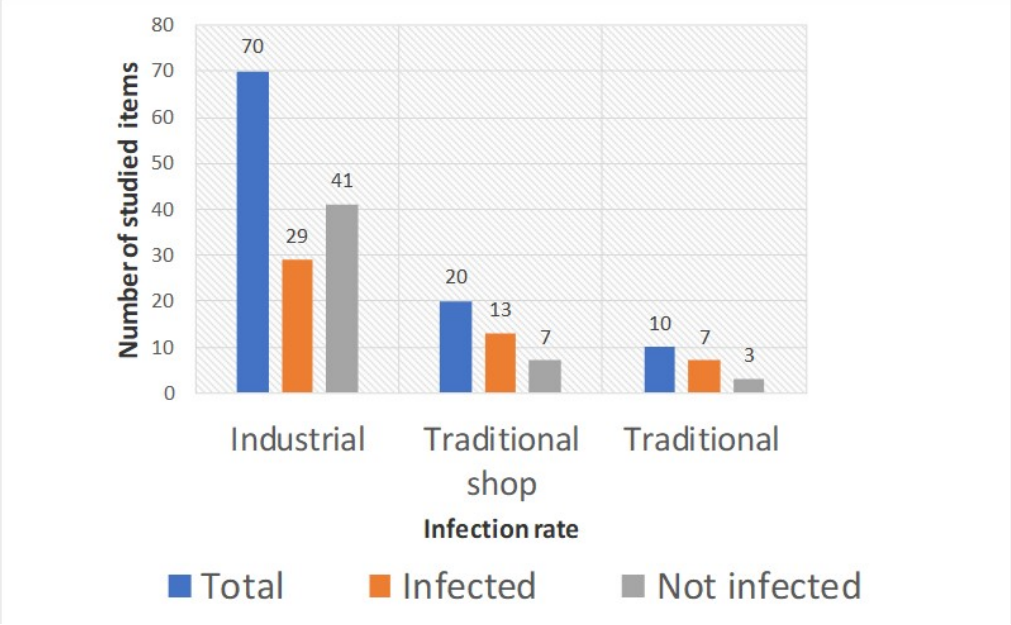
Results

In this study, a total of 100 samples of hamburgers offered in the city of Shiraz was tested to detect the contamination rate to *sarcocystis* spp. Sampling were performed during two seasons and samples were analyzed using macroscopic and microscopic tests (DOB Smear Method and Digestion Method) after staining by Gimsa (Mirmedia brand) in terms of the presence of *Sarcocystis* parasites. The way it was done was that even with a parasite zoite, it was considered a positive sample, and if the zoite was not visible at all microscopic sections of a sample, it would be considered a negative sample. For more detailed study, 50 hamburger samples were collected in summer and 50 in the fall, of which 35 are provided in the industrial hamburgers, 10 traditional shop hamburgers and 5 traditional hamburgers were in the hands of the shops. It is noteworthy that none of the raw hamburger samples were found to be observed. Out of 100 total samples, 0 sample was detected positive in macroscopic examination and 28 samples were detected positive in DOB smear method, 21 samples were detected positive in digestion method. Contaminated samples were detected by the following examination tests:

In the microscopic study, out of 70 industrial hamburgers samples, 29 samples (41.42%) were contaminated with the *Sarcocystis*. Of these, 18 (62%) were collected in the fall and 11 (37.93%) were collected in the summer. Of the 20 traditional shop hamburgers, 13 (65%) were contaminated with the *Sarcocystis*. Of these, 8 (61.5%) were collected in the fall and 5 (38.46%) were collected in the summer. Of the 10 traditional hamburger samples presented in the sales units, 7 were contaminated with the *Sarcocystis*. Of these, 4 of the samples (57.14%) were collected in the fall and 3 (42.85%) were collected in the summer (Table 1 and Graph 1). In terms of seasonal infection, out of the total 50 samples collected in the fall, 30 samples (60%) and out of the 50 samples collected in the summer 19 samples (38%) were contaminated with *Sarcocystis*.

Infection / Type of Hamburgers	Number of items study	Positive items		Negative items	
		Number	percentage	Number	percentage
Industrial	70	29	41%	41	59%
Traditional Shop	20	13	65%	7	35%
Traditional	10	7	70%	3	30%

Table 1. Distribution table of the relative and absolute frequency of infection of the studied hamburgers with the parasitic protozoan *Sarcocystis* according to the types of collected cases.



Graph 1. Amounts of hamburgers infected by *Sarcocystisis* parasitic protozoa according to the types of cases.

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Discussion

This study was conducted in order to answer two basic hypotheses. These two hypotheses were as follows:

1) Hamburgers sold in Shiraz city are infected with *Sarcocystis* parasite.

2) Almost all *Sarcocystis* cysts in hamburger samples cannot be seen with the naked eye.

For this purpose, in this study, the contamination with macrocysts and *Sarcocystis* microcysts in raw industrial (factory) and traditional hamburgers (sold in shops and hand shops) in Shiraz during the fall and summer seasons of 2020 was investigated.

During this investigation, no parasite macrocysts were observed in any of the raw hamburger samples. Since beef is used in the production of raw hamburgers, the absence of parasite macrocysts is consistent with the studies conducted in Iran on beef.

Because in none of the studies conducted in Iran on beef, parasite macrocysts have not been observed. Also, before starting the research, this proposition was mentioned as one of the hypotheses. Therefore, it can be concluded that the non-observation of macrocysts is consistent with the predetermined hypothesis and in this sense, it confirms the truth of the statement.

In this study, the total amount of contamination among 100 samples taken from industrial and traditional hamburgers was 49 cases (49%). The comparison of this rate is consistent with the rate of contamination in similar studies in Tehran and Ahvaz, where the rate of *Sarcocystis* cyst contamination is 47.9% and 56%, respectively (Hosseini *et al.,* 2006). Data analysis using the Chi-square test showed that there is no significant relationship between industrial and traditional hamburgers with the degree of contamination with a confidence interval of 95% (*P-value* = 0.0665). Also, there was no significant relationship between autumn and summer samples with the level of contamination (*P-value* = 0.0713).

Hajimohammadi et al, in 2014 showed that 77.9% of all tested hamburger samples were infected with *Sarcocystis* spp. The infection rate in the traditional hamburger (87%) was significantly (*P-value* = 0.05). (Hajimohammadi *et al.,* 2014). Nematollahia et al, in 2015 showed that 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*. Infection of hamburgers to *Sarcocystis* in summer was higher than other seasons but this difference was not significant (Nematollahia *et al.,* 2015).

Hooshyar et al, in 2017 showed that 58 (29%) of 200 tested hamburger samples were infected to *Sarcocystis* spp. The prevalence rate was 31.25 and 26.9% in the hamburgers with 90 and 60–75% meat, respectively (Hooshyar *et al.,* 2017). Dehkordi et al, in 2017 showed that 16 (80%) of samples were infected with *Sarcocystis* spp. Based on digestion method, the infection rate in hamburger, sausage, and cocktail were 87.5, 83.33, and 66.66%, respectively (Dehkordi *et al.,* 2017).

Jafari et al, in 2022 showed that *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-value* =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* infection rate in conventional and industrial beef burgers (*P-value* =0.01) (Jafari *et al.,* 2022).

In this study, the rate of contamination of industrial raw hamburgers was 41.42% and the rate of contamination of traditional hamburgers of both types was 66%, so no statistically significant difference was observed between the average rate of contamination. The results of this study are consistent with the studies of Hosseini et al, (2006) who did not find any significant difference between the level of contamination of industrial and traditional raw hamburgers offered in Tehran (Hosseini *et al.,* 2006). Irrespective of the microbial contamination of food with this parasite, the way the food is prepared, whether in an industrial process or by hand power, along with the involvement of human factors, has an effect on the degree of contamination of the muscle used in the preparation of hamburgers. In other words, the type of muscle used from infected or non-infected organs of the animal overshadows the *Sarcocystis* infection more than the influence of human factors in the preparation of hamburgers.

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Conclusion

It can be concluded that the amount of *Sarcocystis* infection in hamburgers has no relationship with whether they are traditional or industrial, and there is no significant relationship between the amount of *Sarcocystis* infection between the two seasons of autumn and summer. The most important cause of *Sarcocystis* contamination is the type of muscle used from infected or non- infected animal organs in the preparation of hamburgers.

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Conflict of Interest

The authors declare no conflict of interest.

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Kazerun Branch
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بررسی میزان آلودگی به *سارکوسیستیس* در همبرگرهای فروخته شده در شهرستان شیراز، ایران

محمد جواد هوشیار[۱]، علی کریمی[۲]، پوریا زارعی[۳*]، آرمین قهرمانی[۱]

[۱]فارغ التحصیل، گروه انگل شناسی، دانشکده دامپزشکی، واحد کازرون، دانشگاه آزاد اسلامی، کازرون، ایران
[۲]استادیار، گروه انگل شناسی، دانشکده دامپزشکی، واحد کازرون، دانشگاه آزاد اسلامی، کازرون، ایران
[۳]دانشجو، گروه انگل شناسی، کالج دامپزشکی، واحد کازرون، دانشگاه آزاد اسلامی، کازرون، ایران

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چکیده

زمینه و هدف: *سارکوسیستوزیس* یکی از بیماری های بسیار شایع و مشترک بین انسان و حیوان در اکثر نقاط دنیا می باشد. این بیماری انگلی در اثر تک یاخته *سارکوسیست* ایجاد می شود. کیست های *سارکوسیست* عمدتا در عضلات مخطط گاو، گوسفند و بز وجود دارند و اندازه آنها از چند میکرون تا چند میلیمتر متغیر می باشد. در این تحقیق سعی شد میزان آلودگی همبرگرهای شیراز به انگل *سارکوسیست* تعیین شود.

مواد و روش‌ها: به منظور انجام مراحل آزمایشگاهی، تعداد ۱۰۰ نمونه همبرگر (۷۰ نمونه همبرگر صنعتی و ۳۰ نمونه همبرگر دست ساز) از سطح شیراز جمع آوری و با رعایت نکات بهداشتی لازم به آزمایشگاه منتقل شد تا مورد مطالعه و بررسی قرار گیرد. مشاهدات ماکروسکوپیک، روش گسترش مهری و هضمی انواع روش های بررسی نمونه ها بودند.

یافته‌ها: در روش های ماکروسکوپی، گسترش مهری و هضمی، آلودگی نمونه ها به ترتیب صفر، ۲۸ و ۲۱ بود. پس از بررسی مشخص شد که از مجموع ۷۰ نمونه همبرگر صنعتی تعداد ۲۹ نمونه (۴۲/۴۱٪) به *سارکوسیست* آلوده بودند. از این تعداد، ۱۸ نمونه (۶۲٪) از موارد جمع آوری شده در فصل پاییز و ۱۱ نمونه (۹۳/۳۷٪) از موارد جمع آوری شده در فصل تابستان بودند. از مجموع ۲۰ نمونه همبرگر سنتی مغازه ای تعداد ۱۳ نمونه (۶۵٪) به *سارکوسیست* آلوده بودند. از این تعداد، ۸ نمونه (۵/۶۱٪) از موارد جمع آوری شده در فصل پاییز و ۵ نمونه (۴۶/۳۸٪) از موارد جمع آوری شده در فصل تابستان بودند. از مجموع ۱۰ نمونه همبرگر سنتی ارایه شده در واحدهای دست فروشی نیز ۷ نمونه (۷۰٪) به *سارکوسیست* آلوده بودند. از این تعداد، ۴ نمونه (۱۴/۵۷٪) از موارد جمع آوری شده در فصل پاییز و ۳ نمونه (۸۵/۴۲٪) از موارد جمع آوری شده در فصل تابستان بودند. تجزیه و تحلیل داده ها با استفاده از آزمون مربع کای نشان داد که با ضریب اطمینان ۹۵ درصد بین همبرگرهای صنعتی و سنتی با میزان آلودگی ارتباط معنی داری وجود ندارد. همچنین بین نمونه های پاییز و تابستان با میزان آلودگی ارتباط معنادار وجود نداشت.

نتیجه‌گیری: نتایج این مطالعه نشان می دهد که فراوانی آلودگی نمونه های همبرگرهای صنعتی و سنتی به انگل *سارکوسیست* در شهر شیراز بالاست. بنابراین پیشنهاد می گردد اقدامات پیشگیرانه و بازرسی های دقیق از محل تولید و فروش همبرگرها بعمل آید.

واژه های کلیدی: *سارکوسیست، همبرگر، بیماری های مشترک بین انسان و دام، شیراز، ایران*

محمد جواد هوشیار، علی کریمی، پوریا زارعی، آرمین قهرمانی. بررسی میزان آلودگی به *سارکوسیستیس* در همبرگرهای فروخته شده در شهرستان شیراز، ایران. مجله طب دامپزشکی جایگزین. ۱۴۰۲؛ ۶ (۱۶): ۹۵۳-۹۶۱.

* *نویسنده مسئول: دانشجو، گروه انگل شناسی، کالج دامپزشکی، واحد کازرون، دانشگاه آزاد اسلامی، کازرون، ایران.*
Orcid: https://orcid.org/0000-0002-9298-3512 ,Email: pouriazarei95@gmail.com

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