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Review

Inactivation of *Sarcocystis bradyzoites* in food; a review

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ABSTRACT

The eradication of *Sarcocystis*-infected corpses costs the meat industry millions of dollars each year. Because this parasite is most commonly found in skeletal and cardiac muscles, preventative and control techniques such as inactivating or destroying the bradyzoites in infected meat are critical. The goal of this research was to look at the various methods for inactivating this parasite and to compare the results of these methods. Using internet databases from many fields and around the world, a systematic review of the literature was conducted. Heating, freezing, irradiation, and marination were all utilized to inactivate this parasite, and each had a distinct effect, according to the studies. Inactivation can be achieved by heating at 60°C for 20 min or freezing at -4ºC for 2 days. Also, 2 kGy of gamma rays and marination in 6% NaCl and 3% acetic acid for 48 h are enough.

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1. Introduction

Sarcocystis is a parasite that affects a variety of domestic animals, including cattle, goats, and sheep. Weight loss, lameness, anorexia, fever, paralysis, anemia, muscle weakness, miscarriage, decreased milk supply, and mortality is among symptoms of *sarcocystis* infection in intermediate hosts such cattles, pigs, sheeps, and goats (1-3).

According to the internal and overseas studies, the elimination of *Sarcocystis*-infected corpses costs the meat industry millions of dollars each year. Some *Sarcocystis* species cause digestive difficulties in humans, including nausea, diarrhea, and vomiting.

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In humans, sarcocystosis is caused by eating raw or undercooked beef and buffalo meat containing *Sarcocystis bovihominis* cysts, or pork containing *Sarcocystis suihominis* cysts (1,4,5). In Iran, this parasite is highly common, and it has been found in hamburgers and minced meat (6). Because this parasite is most commonly found in skeletal and cardiac muscles, it is critical to implement preventative and control measures such as inactivating or destroying the bradyzoites in contaminated meat, which is one of the phases of infection and the parasite's life cycle.The goal of this research was to look at the various methods for inactivating this parasite and to compare the results of these methods.

2. Materials and Methods

Using internet databases from many fields and around the world (In the last 50 years), a systematic review of the literature was conducted. Pub Med, CIVILICA, Elsevier, Wiley, Science Direct, SID, Sage, Irandoc, Megapaper, Elmnet, Magiran, Google Scholar, and the WHO (World Health Organization) were among the databases used. Keywords used included: Sarcocystis, inactivation, viability, heat treatment, freezing, irradiation, marination, and high pressure (Figure 1).

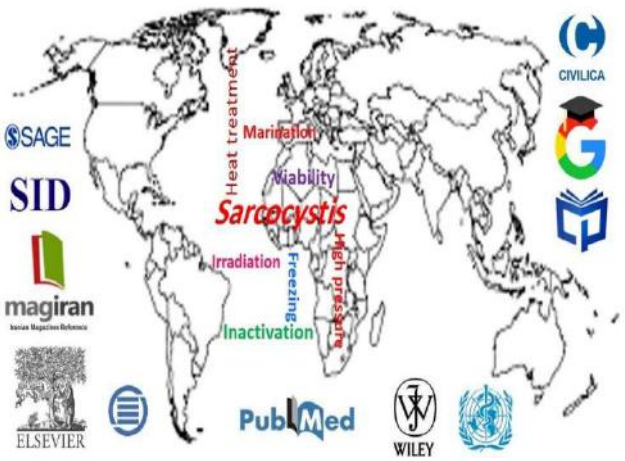

Figure 1. Internet databases and Key words

At first, more than 20 references were found, but only 6 references were completely relevant to our topic. Non-related papers were rejected based on systematic review criteria, and after screening the publications, all documents whose contexts were in agreement with the study's goal were chosen for final review. The following data were retrieved from the documents reviewed: treatment, method, effect, and matrix. All of the information was compiled into Table 1.

3.Results

Heating, freezing, irradiation, and margination in NaCl and acetic acid were all utilized to inactivate this parasite, and each had a distinct effect, according to the studies. Table1 contains a list of these methods as well as other information such as matrix, evaluation method, and effect. *Sarcocystis* survival has also been assessed using Bioassay and Digestion methods.

Table 1. The effect of different treatments on the inactivation of *Sarcocystis*

Treatment method	Condition	Evaluation method	Effect	Matrix	Reference
Heating	40-45°C; 20-25 min	Bioassay	Infective	Buffalo heart	7
	50-60°C; 20-25 min	Bioassay	Infective	Buffalo heart	7
	65-75°C; 20-25 min	Bioassay	non-Infective	Buffalo heart	7
	50°C; 20 min	Bioassay	Infective	Pork	8
	60°C; 20 min	Bioassay	non-Infective	Pork	8
	70°C; 15 min	Bioassay	non-Infective	Pork	8
	100°C; 10 min	Bioassay	non-Infective	Pork	8
	100°C; 5 min	Bioassay	non-Infective	Pork	8
	40°C; 10 min	Digestion	8.8% Inactivation	mutton	9
	40°C; 20 min	Digestion	34% Inactivation	mutton	9
	50°C; 10 min	Digestion	29.2% Inactivation	mutton	9
	50°C; 20 min	Digestion	63.8% Inactivation	mutton	9
	60°C; 10 min	Digestion	49.5% Inactivation	mutton	9
	60°C; 20 min	Digestion	93.7% Inactivation	mutton	9

	70ºC; 10 min	Digestion	83.8% Inactivation	mutton	9
	70ºC; 20 min	Digestion	97.43% Inactivation	mutton	9
	80ºC; 10 min	Digestion	89.8% Inactivation	mutton	9
	80ºC; 20 min	Digestion	100% Inactivation	mutton	9
	90ºC; 10 min	Digestion	96.7% Inactivation	mutton	9
	90ºC; 20 min	Digestion	100% Inactivation	mutton	9
	100ºC; 10 min	Digestion	100% Inactivation	mutton	9
	100ºC; 20 min	Digestion	100% Inactivation	mutton	9
Freezing	-20ºC; 3 days	Bioassay	Complete loss of infectivity	Beef	10
	-2ºC; 24 h	Bioassay	Infective	Buffalo heart	7
	-4ºC; 48 h	Bioassay	non-Infective	Buffalo heart	7
	-4ºC; 3 days	Bioassay	non-Infective	Pork	8
	-4ºC; 2 days	Bioassay	non-Infective	Pork	8
	-4ºC; 1 day	Bioassay	Infective	Pork	8
	-20ºC; 2 days	Bioassay	non-Infective	Pork	8
	-20ºC; 1 day	Bioassay	non-Infective	Pork	8
	-2ºC; 24 h	Digestion	39.3% Inactivation	mutton	9
	-2ºC; 48 h	Digestion	62.4% Inactivation	mutton	9
	-4ºC; 48 h	Digestion	68.5% Inactivation	mutton	9
	-4ºC; 48 h	Digestion	89.1% Inactivation	mutton	9
	-20ºC; 24 h	Digestion	100% Inactivation	mutton	9

	-20°C; 24 h	Digestion	100% Inactivation	mutton	9
Irradiation	Electron-Beam; 0.5 KGy	Digestion and Bioassay	2.56% Inactivation	Beef and mutton	11
	Electron-Beam; 0.75 KGy	Digestion and Bioassay	10.63% Inactivation	Beef and mutton	11
	Electron-Beam; 1 KGy	Digestion and Bioassay	27.90% Inactivation	Beef and mutton	11
	Electron-Beam; 1.5 KGy	Digestion and Bioassay	78.04% Inactivation	Beef and mutton	11
	Electron-Beam; 2 KGy	Digestion and Bioassay	92.50% Inactivation	Beef and mutton	11
	Gamma; 0.5 KGy	Digestion and Bioassay	7.89% Inactivation	Beef and mutton	11
	Gamma; 0.75 KGy	Digestion and Bioassay	14.28% Inactivation	Beef and mutton	11
	Gamma; 1 KGy	Digestion and Bioassay	33.33% Inactivation	Beef and mutton	11
	Gamma; 1.5 KGy	Digestion and Bioassay	85.15% Inactivation	Beef and mutton	11
	Gamma; 2 KGy	Digestion and Bioassay	100%Inactivation	Beef and mutton	11
Marination	3% NaCl; 3 h	Digestion and Bioassay	2.8% Inactivation	mutton	12
	3% NaCl; 6 h	Digestion and Bioassay	22.7% Inactivation	mutton	12
	3% NaCl; 12 h	Digestion and Bioassay	82.31% Inactivation	mutton	12
	3% NaCl; 24 h	Digestion and Bioassay	91.26% Inactivation	mutton	12

3% NaCl; 48 h	Digestion and Bioassay	95.46% Inactivation	mutton	12
6% NaCl; 3 h	Digestion and Bioassay	7.09% Inactivation	mutton	12
6% NaCl; 6 h	Digestion and Bioassay	28.84% Inactivation	mutton	12
6% NaCl; 12 h	Digestion and Bioassay	87.06% Inactivation	mutton	12
6% NaCl; 24 h	Digestion and Bioassay	91/90% Inactivation	mutton	12
6,9 and 12% NaCl; 48 h	Digestion and Bioassay	100% Inactivation	mutton	12
9% NaCl; 3 h	Digestion and Bioassay	29.28% Inactivation	mutton	12
9% NaCl; 6 h	Digestion and Bioassay	39.32% Inactivation	mutton	12
9% NaCl; 12 h	Digestion and Bioassay	95.14% Inactivation	mutton	12
9% NaCl; 24 h	Digestion and Bioassay	96.89% Inactivation	mutton	12
12% NaCl; 3 h	Digestion and Bioassay	31.03% Inactivation	mutton	12
12% NaCl; 6 h	Digestion and Bioassay	80.32% Inactivation	mutton	12
12% NaCl; 12, 24 and 48 h	Digestion and Bioassay	100% Inactivation	mutton	12
3% acetic acid; 3 h	Digestion and Bioassay	38.21% Inactivation	mutton	12
3% acetic acid; 6 h	Digestion and Bioassay	88.57% Inactivation	mutton	12

	3% acetic acid; 12 h	Digestion and Bioassay	92.90% Inactivation	mutton	12
	3% acetic acid; 24 h	Digestion and Bioassay	93.68% Inactivation	mutton	12
	3,6,9 and 12% acetic acid; 48 h	Digestion and Bioassay	100% Inactivation	mutton	12
	6% acetic acid; 3 h	Digestion and Bioassay	73.44% Inactivation	mutton	12
	6% acetic acid; 6 h	Digestion and Bioassay	99.43% Inactivation	mutton	12
	6% acetic acid; 12 h	Digestion and Bioassay	99.70% Inactivation	mutton	12
	6,9 and 12% acetic acid; 24 h	Digestion and Bioassay	100% Inactivation	mutton	12
	9% acetic acid; 3 h	Digestion and Bioassay	97.52% Inactivation	mutton	12
	9 and 12% acetic acid; 6 h	Digestion and Bioassay	100% Inactivation	mutton	12
	12% acetic acid; 3 h	Digestion and Bioassay	99.37% Inactivation	mutton	12

Digestion method

This procedure involves crushing some of the target tissue with a meat grinder and placing it in 100 mL of digesting solution. The solution is filtered after some time at 37°C. After that, the solution is transferred to test tubes and centrifuged for 5 min at 2500 rpm . The sediment at the bottom of the test tube is then used to make a slide, which is then fixed with methanol and stained by Trypan blue dye after drying. Stained slides with immersion oil will be viewed under a microscope (12).

To make the pepsin solution (digestion solution), combine 2.5 g pepsin powder with 100 mL PBS and 10 mL Choloridric acid. Dead bradyzoites are stained under a microscope in this procedure, while live bradyzoites are not (12).

Bioassay method

The target tissue is feed to a dog or cat for this purpose, and then the animal's feces are floated after 12-15 days. The presence of a Sarcocystis sporocyst in the feces of these animals indicates that the tissue in question was infected, but otherwise, the result is negative (12).

4.Discussion

Sarcocystis is a parasite that affects many animals' skeletal and cardiac muscles. This parasite can be found throughout the world, with a high prevalence in Iran. Humans can contract the disease by eating the contaminated raw or undercooked meat. Both economically and in terms of human health, this disease is significant. As a result, it is vital to apply proper preventative methods to the inactivation of *Sarcocystis* bradyzoites. Heating, freezing, irradiation, and marination in NaCl and acetic acid were all utilized to inactivate this parasite, and each had a distinct effect, according to the studies (Figure 2).

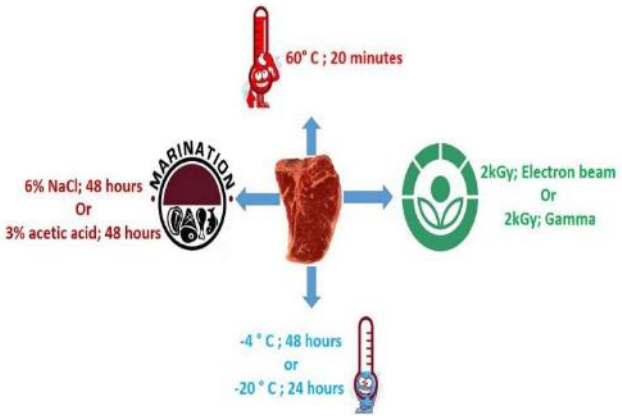

Figure 2. The preventative methods to inactivation of *Sarcocystis* bradyzoites

Heat treatment

In FAO, heat treatment is still one of the most effective parasite control strategies (13). Several studies show that heating duration is just as important as temperature and that it should be chosen so that desired temperatures are obtained, maintained, and dispersed evenly throughout the meat (14). The effect of temperatures ranging from 40°C to 100°C for 5 to 25 min on the survival rate of sarcocystis bradyzoites has been examined in earlier investigations. According to the findings, cooking (heating) the meat at 60°C for 20 min is an effective technique to disinfect it (8).

Freezing

Freezing was another approach that used temperatures ranging from -2 to -20 for one to seven days. The optimal solution for inactivating sarcocystis bradyzoites in meat, according to the results in Table 1, is to freeze it for 48 h at -4°C or for 24 h at -20°C (7,8).

Irradiation

With the persistence of some parasites under classic inactivation methods like freezing and heating, researchers are looking for new ways to kill them. However, the available data is minimal, and more testing is required. The electron beam is a microbial inactivation technique that uses high-energy electrons that have been accelerated to near-light speed. The high energies that arise can uniformly penetrate food items. For high throughput, foodstuffs are often arranged on pallets, and the dose received is adjusted by adjusting the beam current and scanning length, as well as the under-beam conveyor speed (15,16). Electron beams have been employed at dosages ranging from 0.5 to 2 kGy, and 2 kGy being considered an effective dose (11). The inactivation impact of gamma irradiation varies greatly, as seen by the wide range of recorded lowest effective doses, which is directly connected to the parasite type, parasite stage, and food product tested. Doses of 0.5 to 2 KGy gamma rays were utilized in the current research, and dose 2 KGy was also found to be effective (11).

Marination in NaCl and acetic acid

At the manufacturing factory, ready-to-eat meals of animal origin must be free of parasites. Several commonly used food-processing procedures, such as marination, fermentation, smoking, and others, have parasite-inactivating potential, frequently as a result of a combination of mechanisms, which can sometimes function synergistically. Both drying and adding salt lower the amount of accessible water and raise osmotic pressure, both of which are harmful to all living cells. Marination is the process of treating meat or fish with brines, organic acids, and, in some cases, essential oils (17). In the experiments, marination in 3 % NaCl and acetic acid for 3 to 48 h was reported. Marination in 6% NaCl and 3 % acetic acid for 48 h was found to be effective in removing all bradyzoites (12).

5. Conclusion

It is critical to implement preventative and control measures such as inactivating or destroying the bradyzoites in contaminated meat, which is one of the phases of infection and the parasite's life cycle. These methods include Heating, freezing, irradiation, and marination in NaCl and acetic acid. Inactivation can be achieved by heating at 60°C for 20 min or freezing at -4°C for 2 days. Also, 2 kGy irradiation and marination in 6% NaCl and 3% acetic acid for 48 h are enough.

Acknowledgment

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