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**RUMINANTS** 

## Effects of irradiation on the survival of Sarcocystis bradyzoites in beef

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#### **Abstract**

Background: Sarcocystis is a food-borne zoonotic protozoan whose final hosts are humans, dogs, cats, and other carnivores and intermediate hosts are birds and mammals, especially humans and herbivores. Humans become infected by eating raw and undercooked meat contaminated with bradyzoites or by consuming water or food contaminated with the sporocyst stage of the parasite.

Objectives: The aim of this study was to investigate the effects of gamma radiation and electron beam on the survival rate of Sarcocystis bradyzoites in infected beef and to determine the effective dose.

Methods: Three replicates of 100 g of infected meat were treated with different doses (0.5, 1, 1.5 and 2 kGy). As a control, 20 g of contaminated meat was stored separately at 4°C. The viability of the bradyzoites after digestion in pepsin solution was assessed, stained (trypan blue) and unstained, under a stereomicroscope. To assess survival of the bradyzoites, the irradiated meat samples were fed to 30 dogs. After 10 days, faecal samples were examined for sporocysts.

Results: The results showed that the highest and lowest mortality rate of Sarcocystis bradyzoites in infected organs using electron beam at a dose of 2 kGy were 92.5% and 100%, respectively, and the lowest mortality rate at a dose of 0.5 kGy were 2.5% and 7.89%, respectively.

Conclusion: The results of statistical analysis showed that the mortality rate of Sarcocystis bradyzoites was significant between different doses of gamma ray and electron beam, so that gamma rays were better compared to electron beam in destroying Sarcocystis bradyzoites.

#### **KEYWORDS**

cattle, decontamination, food-safety, meat

## 1 | INTRODUCTION

Meat plays an important role in the transmission of parasites to humans, and certain food consumption habits, such as consumption of raw or undercooked meat, increase the risk of human infection.

Toxoplasma, Sarcocystis and Trichinella are examples of parasites that humans can become infected with via consumption of undercooked meat. Meat inspection with infected meat removed from the food chain and various treatments, such as irradiation and extended exposure to freezing, can decrease the risk of parasite transmission. Irradiation of

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food (e.g., spices, poultry, and meat) is approved by the U.S. Food and Drug Administration and other regulatory bodies worldwide at does as high as 7 kGy for meat and over 10 kGy for the sterilization of food (Morehouse & Komolprasert, 2004). In the case of meat-borne parasites, irradiation has been shown to cause a loss of infectivity, loss of pathogenicity, and interruption or prevention of completion of the life cycle and/or death of the parasite (Franssen et al., 2019). Although gamma irradiation is most commonly used in food treatments, research indicates that high-energy ionization, whether produced by X-ray, electron beam or gamma, the chemical and biological effects are similar (Collins et al., 2005; Franssen et al., 2019). Similar inactivation levels of bacteria and spores have been obtained with the two methods, both of which result in DNA cell damage (Jeong & Kang, 2017; Shehata et al., 2011). Advantages of electron beam irradiation include relatively short process time, in-line process, high effectiveness, involvement of few variables, low heat, short release time, low equipment cost and controlled dose (Franssen et al., 2019; Marsh, 1997).

A disadvantage is that electron beam irradiation has limited penetration depth, requiring it to be applied to both sides of an item. In contrast, gamma irradiation has much higher penetrating power. Franssen et al. (2019) showed that gamma irradiation of 0.4-6.5 kGy can control meat-borne parasite. Alabay et al. (1992) reported that relatively high doses (3.7 kGy or more) are required to inactivate Taenia cysticercus in meat, but objectionable changes in odour, colour and texture of meat can occur at this dose. Work by Sudarmadji and Urbain (1972) indicated that the threshold dose for beef for an organoleptically detectable off-flavour is 2.5 kGy. Other studies have indicated 2 kGy should not be exceeded due to impact on meat quality (Yim et al., 2015). When considering meat-borne parasites and food treatment methods, there is considerably less information on Sarcocystis spp. compared to Toxoplasma, Trichinella and the other more commonly transmitted meat-borne parasites. However, with over 100 Sarcocystis species, many of which with unknown host ranges and a growing number of reports of clinical signs in humans, combined with the high prevalence in meat, methods of meat treatment for Sarcocystis are needed.

Sarcocystis is a protozoan parasite commonly found in domestic and wild animals. In Iran, prevalence in ruminants is estimated to be 74% and estimates from elsewhere in the world range from low to well over 97%, depending on the meat source and Sarcocystis species (Anvari et al., 2020; Shams et al., 2022; Zolfaghari Emameh et al., 2018). The parasite has a heteroxenous life cycle, whereby tissue cysts are formed in the striated muscles of intermediate hosts (usually herbivores) and sexual developmental stages are found in the intestinal mucosa of definitive hosts (usually carnivores).

Humans are known to serve as definitive hosts for at least three *Sarcocystis* species (*S. bovihominis* (syn. *S. hominis*) and *S. heydorni* from cattle and *S. suihominis* from pigs). In these cases, humans consume beef or pork infected with *Sarcocystis* cysts containing bradyzoites. Many human infections are asymptomatic; however, clinical signs in infected individuals have included vomiting, nausea and acute or chronic enteritis according to the species and a number of ingested cysts (Dubey

et al., 2015; Faver et al., 2015; Rosenthal, 2021), It is likely that the significance of intestinal infection and frequency in humans are underestimated (Fayer et al., 2015; Rosenthal, 2021). Humans also can be accidental intermediate hosts or aberrant hosts for some Sarcocystis species, resulting in muscular sarcocystosis. In these cases, humans have consumed sporocysts from the environment excreted in the faeces of definitive hosts. Most muscular sarcocystosis patients are asymptomatic with only 10 cases reported with acute inflammation of muscles in the world (Fayer et al., 2015; Harris et al., 2015; Rosenthal, 2021). Although only two species of Sarcocystis are known to have humans as the definitive host and there are few reports of illness when humans are intermediate hosts, there is a lack of information on many Sarcocystis species and their impact on human health (Zolfaghari Emameh et al., 2018). For example, S. fayeri resulted in illness in several people that consumed raw horse meat. It has been hypothesized that a toxin produced by the parasite resulted in the food poisoning (Kamata et al., 2014). In another case, S. nesbitti was identified as the potential cause of an outbreak in 93 people (Rosenthal, 2021). In addition, S. cruzi oocysts were recovered from the faeces of an immunodeficient women, suggesting that some species can be opportunistic in regards to the final host, at least when immunity is compromised (Agholi et al., 2021). Given the prevalence of Sarcocystis in meat, the zoonoses of some species and the lack of certainty regarding others, surveillance and control in food is an important but neglected area (Harris et al., 2015; Rosenthal, 2021; Shams et al., 2022; Zolfaghari Emameh et al., 2018).

To prevent sarcocystosis in humans, as with other protozoan with tissue cysts in meat, freezing (-4 to  $-20^{\circ}$ C for 2 or fewer days, depending on the temperature used) or heating ( $60^{\circ}$ C or greater for 10-20 min) of meat can inactivate the bradyzoites (Valizadeh, 2021). Few studies have assessed the irradiation of *Sarcocystis*-infected meat. Before the application of irradiation in food-born control programs, the irradiation dose which prevents transmission of the parasite without altering the quality of the food must be determined.

The purpose of the present study is to evaluate and compare the efficiency of gamma and electron beam irradiation (in different doses), on the survival of the tissue cyst of *Sarcocystis* spp. in beef muscles.

### 2 | MATERIALS AND METHODS

### 2.1 | Sample preparation

A total of 100 fresh samples were collected from the diaphragm (n=70), heart (n=10) and skeletal muscles (n=20) of cattle carcasses at the industrial abattoir of Tabriz, Iran. Subsequently, the tissue samples obtained from each organ were examined by the naked eye for macro-cysts. Samples were then transferred under chilled conditions to the Food and Aquatic Health Laboratory of the Faculty of Veterinary Medicine, University of Tabriz. As described previously by Saito (1984), a morphological detection assay for *Sarcocystis* spp. was used to identify cysts in the samples.

## 2.2 | Examining the collected micro-cyst samples in terms of infection

Samples, for which cysts were not seen macroscopically or seen using the method by Saito (1984), were subjected to the squash technique and acid pepsin digestion. For the squash technique, small pieces of muscle (approximately  $2.0 \times 5.0 \times 0.5$  cm³) were compressed between two glass slides and examined microscopically for *Sarcocystis* cysts as per the method described by Singh et al. (1990) and Dubey et al. (2000). For the digestion technique, 20 g of muscle were incubated for 20 min at  $40^{\circ}$ C in 50 mL of acid pepsin solution (pepsin 2.6 g [Merck], Nacl 5 g [Merck], 7 mL 1 M HCL [Scharlab S. L.] plus 993 mL of distilled water). This suspension was filtered through a 40-mesh membrane then the filtrate solution was centrifuged at  $400 \times g$  for 10 min at  $4^{\circ}$ C. The precipitate was collected and washed with phosphate-saline buffer (PBS) (pH 7.2) three times. A drop of the suspension was examined for the presence of *Sarcocystis bradyzoites* using a light microscope at  $100 \times magnification$  (Drummond Scientific).

# 2.3 | DNA extraction and identification of *Sarcocystis* spp

To confirm presence of *Sarcocystis* spp., DNA extraction was performed on 50 mg of each sample using a commercial kit (QIAamp DNA Mini Kit, Qiagen). DNA was extracted according to manufacture instructions and stored at  $-20^{\circ}$ C until further analysis.

### 2.4 | Irradiation

In preparation for irradiation, the cattle meat samples known to be positive for *Sarcocystis* were cut to a size of approximately  $2.0\times5.0\times0.5$  cm³. Each sample was separately exposed and each treatment was replicated three times. Gamma irradiation was carried out at the Northwest Research Complex (Bonab, Iran) and each sample was exposed to a  $^{60}$ Co source (GammaceH220). Electron beam irradiation was provided by the Institute of Nuclear Research of Karaj (Iran) with a 10-MeV Dynamitron electron beam accelerator (Radiation Dynamics, Inc.). Doses of 0.5, 1.0, 1.5, and 2 kGy/30 min were used. A control sample at dose of 0 kGy was also used. After exposure, the samples were inserted inside sterile 50 mL tubes and held at 4°C until the next experiments.

#### 2.5 | Assessment of irradiation treatments

After irradiation, the muscle samples were examined using the squash technique and acid pepsin digestion as already described. The viability of bradyzoites in sarcocysts was assayed using the trypan blue dye exclusion. A bradyzoite suspension on a glass slide was added to an equal volume of 0.4% trypan blue solution (0.4 g trypan blue in 100 mL of PBS, pH 7.4) (Thermo Fisher Scientific) and immediately examined

under a light microscope. All groups separately were analysed by trypan blue staining for the detection of live (colourless) and dead (blue) bradyzoites of *Sarcocystis* spp. The mortality rate of bradyzoites was calculated as follows:

The mortality rate (%)

 $= \frac{\text{number of dead bradyzoite}}{\text{number of live bradyzoite} + \text{number of dead bradyzoite}} \times 100$ 

## 2.6 | Bioassay for viability of bradyzoites of *Sarcocystis* spp

The results were assessed by bioassay using 30 specific pathogens free (SPF) dogs. The young SPF dogs, 600–1000 g in body weight, were divided into 2 groups of 15 with 1 group allocated to each irradiation method. Within each group, three dogs were fed contaminated samples not exposed to radiation and three dogs were fed each radiation dose. Faecal examination for sporocysts of *Sarcocystis* spp. by means of simple sedimentation was carried out on alternate days starting 10 days after infection. The presence of a *Sarcocystis* sporocyst in the faeces of a dog indicated that the irradiation method was ineffective (Figure 1).

### 2.7 | Statistical analysis

SPSS software version 21.0 (IBM Corp.) was used for statistical analysis. Parasite mortality was analysed by one-way analysis of variance (ANOVA). When significant effects (p < 0.05) were found to be present, comparison of viability of *Sarcocystis* spp. between the treated groups in different doses was performed using a post hoc test (Duncan). Moreover, differences between electron beam and gamma irradiation in different doses were performed by a General Linear Model & Univariate ANOVA test.

## 3 | RESULTS

## 3.1 | Molecular identification of *Sarcocystis* spp. in cattle by PCR

All samples collected were infected by *Sarcocystis*. Based on the PRC results, the *Sarcocystis* species was *S. cruzi*.

## 3.2 | Irradiation effects on viability of *Sarcocystis* spp. in meat

The mortality rate of *Sarcocystis* bradyzoites in infected meat increased with increasing radiation dose from 0.5 to 2 kGy with both methods (Table 1). The results showed that the highest and lowest mortality rates of *Sarcocystis* bradyzoites by electron beam irradiation were at doses of 2 kGy (92.5%) and 0.5 kGy (2.56%) and by gamma irradiation

**FIGURE 1** Morphology of *Sarcocystis* bradyzoites after staining trypan blue (1000×): (a) live bradyzoites (due to the impermeable membrane, they are not completely stained), (b) dead bradyzoites (coloured blue due to membrane permeability).

**TABLE 1** The mortality rate of *Sarcocystis cruzi* bradyzoites in infected meat following treatment with electron beam or gamma irradiation.

Irradiation dose (kGy)	Mean <u>±</u> SD <sup>a</sup>	Mortality rate (%)	p-Value
Electron beam			
0	0.00	0.00	0.000
0.5	$2.56 \pm 0.25$	2.56	
1.0	$27.90 \pm 0.25$	27.90	
1.5	$78.04 \pm 0.95$	78.04	
2	92.50 ± 2.29	92.50	
Gamma			
0	0.00	0.00	0.000
0.5	$7.89 \pm 0.04$	7.89	
1.0	$33.33 \pm 0.59$	33.33	
1.5	85.15 ± 0.98	85.15	
2	$100 \pm 0.00$	100	

 $<sup>^{</sup>a}$ Means  $\pm$  standard deviations from three replications.

were at doses of 2 kGy with 100% and 0.5 kGy with 7.89%, respectively. Significant differences were observed in the viability of *S. cruzi* between the control group (0.00 kGy) and the electron beam irradiated groups and the control group and the gamma irradiation groups (p < 0.0001). The results showed the mortality rate of *Sarcocystis* bradyzoites in different doses between electron beam and gamma irradiation is significant (p < 0.05) with gamma rays being able to eliminate *Sarcocystis* bradyzoites in infected meat better than electron beam irradiation (See Table 2).

## 3.3 | Bioassay for viability of *Sarcocystis* spp. bradyzoites

All dogs exposed to samples irradiated using electron beam and gamma at doses of 0.5–1.5 kGy were positive for sporocysts. They had no apparent difference from dogs fed non-irradiated infected meat. Similar results were obtained in the 1.0 kGy irradiation groups, but sporocysts recovery rate was lower. No sporocysts were recovered

**TABLE 2** Sporocysts recovery in dogs 10 days after infection with meat samples electron beam and gamma irradiated at various doses.

Irradiation dose (kGy)	Dogs positive for Sarcocystis spp. sporocysts number (%) (N = 3)	Sporocysts recovery rate (mean $\pm$ SD)
Electron beam		
0	3 (100)	566.6 ± 170.0
0.5	3 (100)	$416.7 \pm 143.4$
1.0	2 (66.6)	$175.0 \pm 25.0$
1.5	1 (33.3)	$90.0 \pm 0.0$
2	0	0
Gamma		
0	3 (100)	466.6 ± 169.9
0.5	3 (100)	183.3 ± 62.3
1.0	1 (33.3)	50.0 ± 0.0
1.5	1 (33.3)	20.0 ± 0.0
2	0	0

from dogs infected with meat samples irradiated at 2 kGy. These findings revealed that the minimal effective dose to control infectivity of *Sarcocystis* spp. of electron beam and gamma irradiation was close to 1.5 kGy.

### 4 DISCUSSION

This study confirmed that electron beam and gamma irradiation had an appropriate efficiency in inactivation of *Sarcocystis* spp. in meat. In the study presented herein, 2 kGy appeared to be an effective dose for eliminating bradyzoite development and demonstrated via staining and using an in vivo model. Gamma rays were 100% effective, whereas electron beam was 92.50% effective at the 2 kGy dose. Our results agree with those summarized by Valizadeh (2021). With gamma rays being more efficacious than electron beam, our results also agree with the findings of Collins et al. (2005) who assessed the effect of electron beam on *Cryptosporidium parvum* in oysters. Based on their study, doses of 1 and 1.5 kGy were effective in destruction of *C. parvum*, whereas a dose of 2 kGy destroyed all inoculated parasites.

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Our results indicate that a higher dose of gamma irradiation is needed to inactivate Sarcocystis than that needed for Toxoplasma, another protozoa tissue cyst in beef and other meat (Franssen et al., 2019; Mirzaei Dehaghi et al., 2013). Our results for electron beam irradiation differ from those of Eslami et al. (2020) who determined that a dose of 3 kGy was needed to deactivate Sarcocystis. However, in the work by Eslami et al. (2020), viability was assessed solely with PCR. The assay for monitoring Sarcocystis bradyzoite viability showed that Sarcocystis spp. bradyzoites in meat irradiated at doses 0.5 kGy in both electron beam and gamma irradiation were still infective to the dog hosts. These results are similar to those of Wikerhauser et al. (1988) who demonstrated that irradiation up to 0.5 kGy might not be sufficient to control the infectivity of Toxoplasma gondii cysts. Findings in our study clearly showed that the irradiation dose of 1 kGy (electron beam and gamma ray) reduced the survival rate of bradyzoites. The numbers of sporocysts of Sarcocystis spp. recovered at dose of 0.5, 1 and 1.5 kGy were lower than those of the control group. In comparison, our 100% effective doses were 2 kGy with no sporocysts recovered from the inoculated dogs. Kasprzak et al. (1993) showed that the low irradiation dose of 0.1 kGy reduced the number of adult Trichinella spiralis to single individuals and higher doses of irradiation, over 0.4 kGy, were required for a total destruction of encysted muscle larvae in hosts. It seems, the effectiveness of ionizing irradiation is highly dependent on the stage of development of a parasite (Franssen et al., 2019; Hallman, 2013). Munir and Federighi (2020) studied the relationship between the stage of development of *T. spiralis* and dose of irradiation. They indicated that a dose of 0.01 kGy is necessary for the sterilization of T. spiralis, whereas for maturation inhibition of T. spiralis, doses of 0.02-0.03 kGy are efficient, and for its destruction, doses of 1.4-6.3 kGy are needed. Therefore, the present study demonstrates that at doses 1.5 or 2 kGy but not 0.1 kGy electron, beam and gamma irradiation should be regarded strong enough to control the infectivity of Sarcocystis bradyzoites.

#### **AUTHOR CONTRIBUTIONS**

Nasser Hajipour: Conceptualization; supervision; methodology; project administration; investigation; writing – original draft preparation; visualization. Saeid Azizi: Methodology; investigation; writing – original draft preparation. Parviz Hassanzadeh: Conceptualization; methodology. Jennifer Ketzis: Formal analysis; writing – review and editing.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

#### DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study

#### **ETHICS STATEMENT**

This study was approved by the University of Tabriz Ethical Committee (Number Ethical: 43/40781/2).

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#### PEER REVIEW

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