Prevalence and histopathological finding of thin-walled and thick-walled *Sarcocysts* in slaughtered cattle of Karaj abattoir, Iran

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Abstract Sarcocystosis is a zoonotic disease caused by *Sarcocystis* spp. with obligatory two host life cycle generally alternating between an herbivorous intermediate host and a carnivorous definitive host. Some species of this coccidian parasite can cause considerable morbidity and mortality in cattle. The present study was set to investigate the prevalence of *Sarcocystis* spp. and type of cyst wall in slaughtered cattle of Karaj abattoir, Iran. For this purpose 125 cattle (88 males and 37 females) were investigated for the presence of macroscopic and microscopic *Sarcocystis* cysts in muscular tissues. No macroscopic *Sarcocystis* cysts were found in any of the samples. In light microscopy, 121 out of 125 cattle (96.8 %) had thin-walled cysts of *Sarcocystis cruzi*, while 43 out of them (34.4 %) had thick-walled *Sarcocystis* cyst. In this survey, the most infected tissue was esophagus and heart and the less was diaphragm. Thin-walled cysts (*S. cruzi*) mostly found in heart and skeletal muscle showed the less. However, thick-walled cyst (*S. hominis* or *S. hirsuta*) mostly were detected in diaphragm, heart muscle showed no thick-walled cyst. No significant relation was observed between age and sex and the rate of infection. The results showed that *Sarcocystis* cyst is prevalent in cattle in the North part of Iran and the evaluation of infection potential can be useful when considering control programs.

Keywords *Sarcocystis* · Thin-walled cysts · Thick-walled cyst · Cattle · Histopathological finding

Introduction

Sarcocystosis caused by different *Sarcocystis* species is a protozoal infection with worldwide distribution in man and many species of animals. *Sarcocystis* spp. are obligate two-host parasites, generally alternating between a herbivorous intermediate host and a carnivorous definitive host (Dubey et al. 1989).

The genus *Sarcocystis* is composed of approximately 130 species of heteroxenous cyst-forming coccidia which are different in life cycle and pathogenicity. The intermediate hosts for pathogenic *Sarcocystis* spp. are usually cattle, sheep, pigs, and wild cervids (Guclu et al. 2004). Cattle are most common intermediate hosts of sarcocysts. The prevalence of *Sarcocystis* in adult bovine muscle is close to 100 % in most regions of the world (Vangeel et al. 2007). In immunocompetent patients, this coccidian parasite can cause intestinal and muscular sarcocystosis (Velázquez et al. 2008). Meat that is heavily infected may be condemned as unfit for human consumption. It means, eating raw or undercooked beef and pork containing mature sarcocysts of *Sarcocystis hominis* and *S. suis*, respectively, has resulted in humans acquiring intestinal sarcocystosis. On the other hand, there is high infection rate of sarcocysts in cattle in Iran. So, this work aimed to survey the prevalence of different *Sarcocystis* species in slaughtered cattle of Karaj, Iran.

Materials and methods

During April 2010 to September 2010, out of cattle slaughtered for human consumption in Karaj abattoir, Karaj, Iran, 125 cattle were investigated for the presence of macroscopic and microscopic *Sarcocystis* cysts in muscular

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tissues. The animals included 88 males and 37 females and their ages ranged from 1 to 4 years. The investigated cattle were classified into groups according to the age (under 12 months and over 12 months), sex and breed. Ages of the investigated cattle were assessed by visual inspection of teeth. The heart, esophageal, diaphragmatic and skeletal muscles were chosen for inspection and sampling. Immediately after the animals were slaughtered, direct observation was made in order to detect macroscopic sarcocysts. For light microscopy, tissue samples were taken from the above mentioned tissues fixed in 10 % neutral buffered formalin. Then they were processed and embedded in paraffin. Sections of 5 μ m thickness were cut and stained with hematoxylin and eosin.

Statistical analysis

The results were subjected to analysis by SPSS 17.0 (SPSS Inc., Chicago, IL, USA) package. Evaluation of significant difference between the means of different groups was performed with using one way analysis of variance (One-way ANOVA) followed by the Tukey test as post hoc. Values were expressed as mean $\pm$ SEM (standard error of mean). The significance level considered was $P < 0.05$.

Results

The prevalence of microscopic sarcocysts in different age and sex of cattle are shown in Table 1. No macroscopic sarcocysts were found in any of the samples. In light microcopy, 121 out of 125 cattle (96.8 %) had thin-walled cysts of *S. cruzi*, while 43 out of them (34.4 %) had thick-walled sarcocysts. The positive rate of thin-walled cyst was 62.4 % for esophagus, 96.8 % for heart, 66.4 % for diaphragm and 60 % for skeletal muscles. The positive rate of thick walled cyst was 34.4 % for esophagus, 0 % for heart, 18.4 % for diaphragm and 26.4 % for skeletal muscles which could represent either *S. hominis* or *S. hirsuta*. In this survey, the most infected tissue was esophagus and heart and the less was diaphragm. No significant relation was observed between age and sex and the rate of infection ($P > 0.05$).

Histologically, thin-walled cysts (*S. cruzi*) mostly found in heart (Fig. 1) and skeletal muscles showed the less. However, thick-walled cyst (*S. hominis* or *S. hirsuta*) mostly were detected in diaphragm (Fig. 2) and heart muscle showed no thick-walled cyst. Histopathological examination of the esophageal muscles revealed thick-walled sarcocysts surrounded by degenerate eosinophils and necrotic muscle fiber (Fig. 3). Sections of the cattle diaphragm showing different types of sarcocysts wall (Fig. 4).

Table 1 The prevalence of microscopic *Sarcocystis* spp. in different age and sex of cattle

(%)	Oes	Heart	Diaph	SM
Thin-wall cyst	62.4	96.8	66.4	60
Thick-wall cyst	8	0	1.6	8
Mix cyst	34.4	0	18.4	26.4
Age (year)				
<1	59.8	65	52.7	67.3
1–2	63.63	62.02	61.29	60.30
>2	61.99	65	68.42	68.76
Sex				
Male	62.64	62.16	60.81	61.28
Female	61.49	65	68.22	67.08

Oeo oesophagus, *Diaph* diaphragm, *SM* skeletal muscle

Discussion

Sarcocystis spp. are common parasites with worldwide distribution in man and many species of animals, and infect the skeletal and cardiac muscles (Fayer 2004). The histological distinction between bovine thin-walled sarcocysts (*S. cruzi*) from thick-walled sarcocysts (*S. hirsuta* and *S. hominis*) is simple; whereas the distinction between *S. hirsuta* from *S. hominis* is difficult (Ghisleni et al. 2006). They occur as elongated cylindrical bodies, sometimes large enough to be visible to the naked eye, in striated muscle and sometimes in unstrained muscle. In the present study, the oesophagus, heart, cervical and abdominal muscles were used, as previous investigations have shown these organs to be the most common sites for *Sarcocystis* infection. Results of this study showed a high frequency of microscopic *Sarcocystis* infection in cattle slaughtered in the Karaj, Iran. The high prevalence rate of microscopic sarcocysts (96.8 %) in cattle shows the importance of the infection for the other intermediate host (Figs. 5, 6).

In this study, the prevalence of thin-walled sarcocysts, *S. cruzi* cyst, and thick walled sarcocysts was 96.8 and 34 %, respectively. In some countries, over 90 % of adult cattle have been found infected by *S. cruzi*, and occasionally, this *Sarcocystis* species causes abortion, acute systemic illness, and poor growth (Moré et al. 2008). *S. cruzi* was found in 100 % of examined bovine myocardium in Brazil (Santos Da Silva et al. 2002). Previous studies have shown that *S. cruzi* is most commonly found in the heart (Gajadhar et al. 1992; Ghisleni et al. 2006). Singh and Sharma investigated prevalence of *Sarcocystis* spp. in cattle in India and differentiated them on the basis of morphological characters and structure of cyst wall. The prevalence of various *Sarcocystis* spp. such as *S. cruzi*, *S. hirsuta*, and *S. hominis* was 41.70, 9.47, and 3.79 %, respectively (Singh and Sharma 2004).

Fig. 1 Thin walled sarcocysts in the myocardial fibers, H&E $\times 400$

Fig. 4 Thick walled sarcocysts in the diaphragm, H&E $\times 400$

Fig. 2 Thin walled sarcocysts in the Purkinje fibers cell in the heart, H&E $\times 400$

Fig. 5 Two thick walled sarcocysts in the skeletal muscle, H&E $\times 400$

Fig. 3 Inflammatory reaction around the degenerated sarcocysts, H&E $\times 400$

Previous studies have shown that prevalence of microscopic *Sarcocystis* was 100 % in cattle (Hamidinejat et al. 2010; Nourollahi Fard et al. 2009).

The most important factor to influence the prevalence variation of *Sarcocystis* spp. infection is likely the difficulty to complete the heteroxenous life cycle *Sarcocystis* spp. (Ghisleni et al. 2006). In our study, high infection rate of *S. cruzi* cyst (96.8 %) compared with thick-walled cysts (34.4 %) may be due to the fact that in cattle husbandry fields, there are a lot of sentinel dogs which can contaminate cattle pasture, feed, drinking water, and bedding with *S. cruzi* sporocysts. So to prevent infection of food animals; they must be prevented from ingesting the sporocyst stage from dogs feces in contaminated water, feed, and bedding. These measures will prevent the development of intestinal stages where humans might serve as definitive hosts.

Fig. 6 Thick walled sarcocysts (a) and (b), thin walled sarcocysts (c), H&E $\times 400$

No macroscopic sarcocysts were observed in the present study, and this is in agreement with the previous studies (Okur et al. 1995; Nourollahi Fard et al. 2009). The lack of macroscopic sarcocysts may be due to the fact that such cysts are of feline origin and contact between cattle and cats in this area is not common. The age of the cattle may have been the reason too because it can take years to develop (Nourani et al. 2010).

Future control measures should emphasize awareness in farmers and all the beef production chain to prevent these infections, especially by *S. hominis*. This requires the help of veterinary practitioners and a consensus message from scientists. International regulations for beef commercialization and diagnostic methods applied for *Sarcocystis* infections need to be further discussed.

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