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Muscular *Sarcocystis* infection in ruminants slaughtered at Municipality abattoir and selected Hotels in Hawassa city, southern Ethiopia: Prevalence and associated risk factors

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ABSTRACT

Sarcocystosis is a parasitic zoonosis caused by *Sarcocystis* spp. which are Apicomplexan parasites requiring intermediate and definitive hosts to complete their life cycle. Although the parasite has worldwide distribution in man and many species of animals, the prevalence in most parts of Ethiopia is not clearly known. This cross sectional study was conducted between Nov. 2016 and May 2017 to estimate the prevalence of *Sarcocystis* infection in ruminants slaughtered in the municipality abattoir and at selected hotels of Hawassa city and to assess the potential risk factors. Data were collected through gross and histopathological examination of myocardial and esophageal muscles sampled from a total of 561 ruminants (176 sheep, 181 goats and 204 cattle). The overall prevalence of *Sarcocystis* infection in ruminants was 68.98%. All of the cysts were microscopic, and found more frequently ($p < .05$) in heart (62.08%) than esophageal muscle (45.45%) although concurrent infection of both organs was observed in 33.87% of the ruminants examined. No significant association was noted between *Sarcocystis* infection and the origin, sex and species of ruminants examined ($p > .05$). The higher prevalence recorded in the study area can be explained by the abundance of stray dogs, cats and wild carnivores that are roaming in the villages, lack of proper latrine, uncontrolled disposal of condemned offals and carcass, and provision of uncooked meat for dogs and cats. Thus, farmers' awareness creation and strategies targeted at breaking the life cycle of the parasite are required to reduce the prevalence of the parasite and thereby the foreseen zoonotic and economic impact.

1. Introduction

Sarcocystis are coccidian parasites belonging to the phylum Apicomplexa and Family Sarcocystidae. This protozoal parasite has an intracellular nature and affects several species of animals including human beings. They complete their life cycle in specific intermediate and definitive hosts or within closely related host species (Fayer, 2004). The infection is characterized by cyst formation in muscular tissues (muscular sarcocyctosis) in the intermediate host or colonization of the lamina propria of the intestines (intestinal sarcocystosis) in the definitive host (Stojecki et al., 2012). Each host may be infected with more than one *Sarcocystis* spp. (Dubey and Fayer, 1983). Accordingly, ruminants can act as an intermediate host for several species of *Sarcocyst* (*Sarcocystis cruzi*, *Sarcocystis hirsuta* and *Sarcocystis hominis* in cattle; *Sarcocystis hircicanis, Sarcocystis capracanis, Sarcocystis moulei* and *Sarcocystis cuprifelis* in goats and *Sarcocystis arieticanis, Sarcocystis tenella, Sarcocystis medusiformis* and *Sarcocystis gigantea* in sheep) with canids, felids and primates as definitive hosts (Dubey and Lindsay, 2006; Stojecki et al., 2012; Bowman, 2014; Dubey et al., 2016).

Human beings can acquire intestinal sarcocystosis by consuming raw/undercooked beef infected with *Sarcocystis fusiformis* (syn. *Sarcocystis bovihominis*, *S. hominis)* (Tappe et al., 2013; Poulsen and Stensvold, 2014; Fayer et al., 2015). Clinically infected individuals with highly pathogenic species can develop dyspnea and digestive disturbances, such as nausea, vomiting, stomach ache and diarrhea (Fayer, 2004; Pal, 2007). The prevalence and/or severity of intestinal sarcocystosis can be unusually higher in stressed and immune compromised populations (e.g., with HIV or other immunodeficiency disorders), where the parasite causes an opportunistic infection (Stojecki et al., 2012; Fayer et al., 2015; Meistro et al., 2015).

The pathogenic species affecting ruminants can lead to abortion, placentitis, reduced milk yield, neurologic signs, anemia, loss of weight

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and hair and even death especially when large numbers of sporocysts are ingested (Prakas and Butkauskas, 2012; Fayer et al., 2015; Dubey et al., 2016; Portella et al., 2016). Moreover, most species of *Sarcocystis* are known to cause economic losses through condemnation of infected portions of carcasses during meat inspection in abattoirs (Vangeel et al., 2013; Fayer et al., 2015).

In Ethiopia, published reports on the prevalence of *Sarcocystis* infection both in animals and humans are scanty. In ruminants, a single available study revealed a very high prevalence of *Sarcocystis* infection, that is, 93% in sheep, 82% in cattle and 81% in goats in northwestern Ethiopia (Woldemeskel and Gebreab, 1996). Moreover, another study conducted in camels slaughtered in Neghelle Borana and Dollo Addo slaughter houses located in pastoral areas of southern Ethiopia showed a prevalence of 45.45% (Woldemeskel and Gimi, 2001). However, no data could be retrieved on the prevalence of *Sarcocystis* infection in ruminants in other parts of the country including animals slaughtered in Hawassa city. Therefore, the current study aimed to estimate the prevalence of muscular *Sarcocystis* infection in ruminants slaughtered in the municipality abattoir and at selected hotels in Hawassa city and to assess the potential associated risk factors.

2. Materials and methods

2.1. Study population and origin

The study population included the total number of ruminants presented to the Hawassa Municipality abattoir and selected hotels for slaughter. The animals were originated primarily from Tula, Leku, Arsi Negele and Tikur Wuha areas located 5 to 46 km away from Hawassa city. The study animals were selected from the study population using a systematic random sampling technique. This was done by selecting every 10th cattle entering the lairage at the abattoir and every other sheep/goat slaughtered in the selected hotels. The selected animals were then identified based on paints (numbers) on their body.

2.2. Sample size and sample size determination

The sample size required for this study was determined according to Thrusfield (2005) assuming 50% expected prevalence, 95% confidence level and 5% absolute precision. Accordingly, a minimum of 384 ruminants were to be sampled. However, a total of 561 ruminants (176 sheep, 181 goats and 204 cattle) were sampled and examined to increase precision. Data about the species, sex, origin and body condition score of the selected animals were recorded before slaughter. The age of the slaughtered animals were more or less similar within the group (species of animal considered) as estimated by dentition (i.e. 1.5 to 2.5 years for sheep and goats and 4 to 6 years for cattle).

2.3. Study methodology

2.3.1. Post mortem examination

Soon after slaughter, the heart and esophageal muscles of selected animals were thoroughly inspected by visualization, palpation and making systemic incisions to check for the presence of macrocyst or multifocal gray to pale lesions. The pathological lesions were differentiated according to guidelines on meat inspection for developing countries (Herenda et al., 1994).

2.3.2. Histopathology and microscopic examination

For light microscopy, representative tissue samples were collected deftly and quickly from the above mentioned tissues, placed in a universal bottle containing 10% neutral buffered formalin, labeled and transported to the parasitology and pathology laboratory of the Faculty of Veterinary Medicine, Hawassa University. All of these samples were then processed for paraffin technique, sectioned (5 µm thickness), stained with Hematoxylin-Eosin (H&E) stain and then examined under a light microscope (Makhija, 2012).

Table 1
Prevalence of *Sarcocystis* infection in ruminants examined at Hawassa municipality abattoir and selected hotels.

Species	№ examined	№ positive	Prevalence (%)	95% CI
Goat	181	124	68.50	61.1–75.1
Sheep	176	119	67.61	60.1–74.4
Cattle	204	144	70.59	63.8–76.6
Total	561	387	68.98	64.9–72.8

CI: confidence interval.

2.4. Statistical analysis

All the collected data were entered into a Microsoft Excel spreadsheet, coded and then analyzed using STATA statistical software (STATA, 2013; window version 13.1). Association between various risk factors (sex, species, origin and tissue) and the prevalence of *Sarcocystis* infection was determined using a chi-square independent test. In all the analyses significance was set at $p < .05$.

3. Results

The overall prevalence of *Sarcocystis* infection in ruminants was 68.98% (387/ 561). On the basis of animal species, the prevalence was 70.6% in cattle, 68.5% in goats and 67.6% in sheep (Table 1). The difference in prevalence between the three hosts was not statistically significant ($p > .05$). Moreover, sex and origin of the animals did not have significant influence on the prevalence of *Sarcocystis* infection. Out of 559 heart and 506 esophagus samples examined histopathologically, the cysts were observed in 347 (62.08%) and 230 (45.45%) of the organs, respectively. Occurrence of *Sarcocystis* was significantly higher ($p < .001$) in the heart than esophagus (Table 2). Concurrent infection of myocardium and esophageal muscles was noted in 190 (33.87%) of the ruminants examined. All sarcocysts observed were microscopic in nature and had globular to oval shape or occasionally fusiform appearance (Fig. 1A-D).

4. Discussion

This study revealed a high prevalence of *Sarcocystis* infection in the cattle, sheep and goats examined, although it is lower than the figures reported by a previous study in the country (Woldemeskel and Gebreab, 1996). The current prevalence is also lower than the reports from Iraq (Latif et al., 1999), Mongolia (Fukuyo et al., 2002) and Iran (Saeid et al., 2013). In contrast, a lower prevalence than the present has been reported from Algeria (Nedjari, 2003), Nigeria (Obijiaku et al., 2013), Malaysia (Latif et al., 2013) and Italy (Meistro et al., 2015). The difference in the prevalence of *Sarcocystis* infection between the present and previous studies could be emanated from the difference in the diagnostic technique employed, exposure of ruminants to faces/stool of definitive hosts and the diverse management systems of livestock. Ruminants become infected with *Sarcocystis* when sporulated oocysts/ sporocysts of the parasite are ingested while grazing on pasture or drinking water contaminated with human and carnivore stools containing sporozoits or sporocysts. The high prevalence of *Sarcocystis* infection in ruminants observed in this study could be explained by the high number of stray dogs, cats and other wild carnivores in the areas where the animals originated, the practice of defecation on open fields in rural areas, and provision of uncooked offal and condemned carcasses to dogs and cats. The presence of dogs and other definitive hosts including human and non-human primates in the grazing pastures of the animals ensures shedding of the infective oocysts into the environment, which in turn infect the animals. Sporocysts or oocysts of *Sarcocystis* also remain viable for many months in the environment, and

Table 2
Analysis of the prevalence of *Sarcocystis* infection with different potential risk factors.

Variable	Category	№ examined	№ positive	Prevalence (%)	χ^2	*p* value
Species	Goat	181	124	68.50	0.20	0.656
	Sheep	176	119	67.61	0.39	0.530
	Cattle	204	144	70.59	Ref	
Sex	Female	172	125	72.67		
	Male	389	262	67.35	1.56	0.209
Origin	Tula	246	166	67.47	0.40	0.529
	Leku	148	104	70.27	0.03	0.861
	Arsi Negele	97	67	69.07	0.11	0.742
	Tikur wuha	70	50	71.43	Ref	
Organ	Heart	559	347	62.08		
	Esophagus	506	230	45.45	29.6	0.000

Ref: reference category.

they may be further spread or protected by invertebrate hosts (Dubey et al., 1989; Dubey et al., 2016).

In the present study, *Sarcocystis* infection was not significantly associated with sex, origin or species of the host. This is contrary to the finding of Savini et al. (1992), who reported that *Sarcocystis* infection in cattle in Western Australia was influenced by age, sex, environmental and management factors. The absence of association between *Sarcocystis* infection and the considered risk factors and the higher prevalence recorded in the current study could potentially reflect the cosmopolitan nature of the parasite.

Regarding the organs affected, a significantly higher *Sarcocystis* infection was found in the myocardial muscle than esophageal muscle. This finding is concordant with the observation of Fukuyo et al. (2002) from Mongolia who reported that heart was the most commonly infected in cattle (100%), yak (86.7%), and hainag (100%). In line with the present finding, Bucca et al. (2011) also recorded the highest rate of infection in heart compared to other organs of cattle slaughtered in southern Italy. According to Woldemeskel and Gebreab (1996), however, the infestation rate of diaphragm, masseter, cardiac and esophageal musculature was similar. In contrast to the current finding, a study conducted in Iran revealed a higher prevalence of infection in esophagus than in other organs (Dehaghi et al., 2011).

5. Conclusion

In a nutshell, the observation of high prevalence of *Sarcocystis*

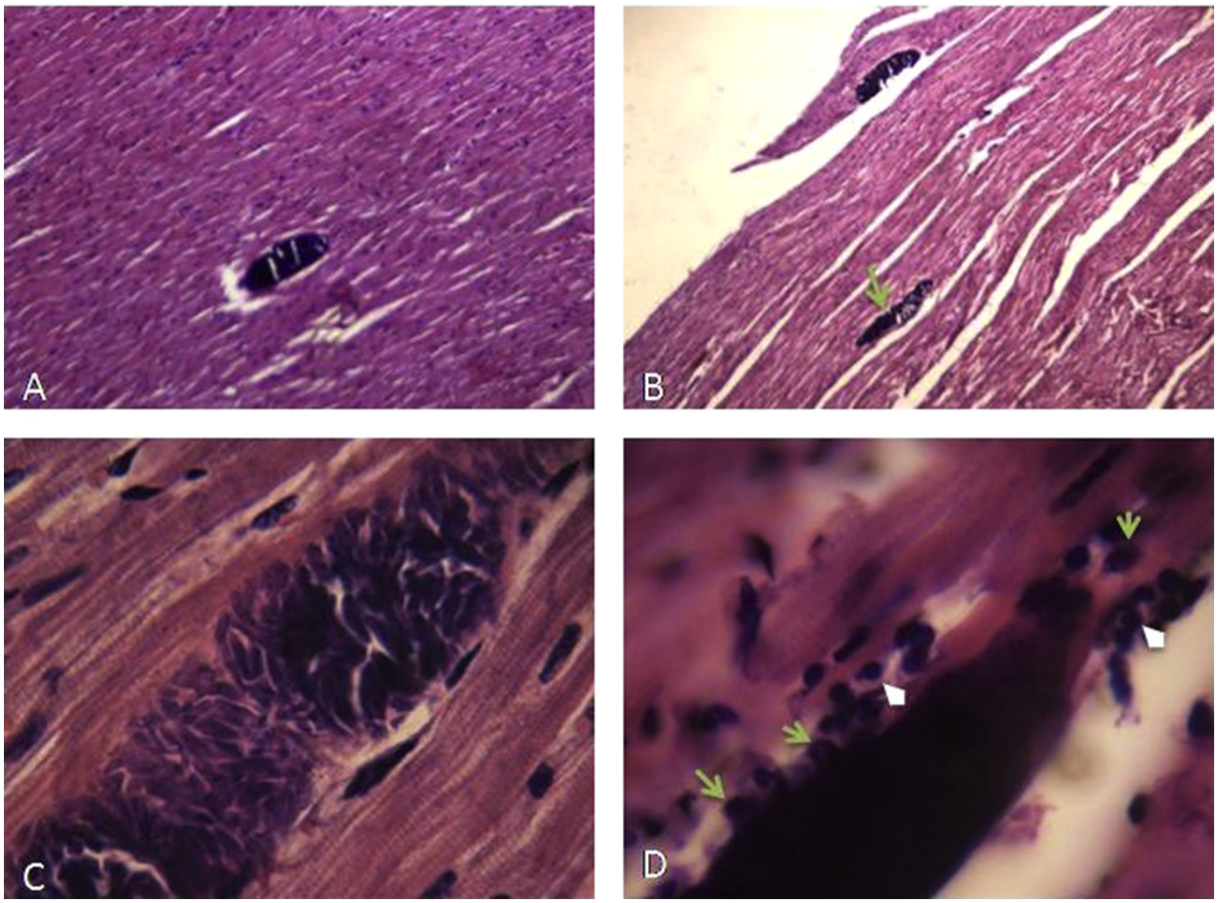

Fig. 1. Histopathological images of Sarcocyst: A. oval to caudate shaped sarcocyst, cardiac tissue. B. Oval to caudate and fusiform shaped (Arrow) sarcocyst, esophageal muscle. C. Bradyzoites and metrocytes inside the thin cyst wall. D. Mature sarcocyst surrounded by mononuclear cell (arrow head) and eosinophil (arrow) infiltrate. All are H&E stained; A&B in 10×; C&D in 100×.

infection in the current study without statistically significant difference between sex, origin, and species of ruminants strongly suggests the widespread nature of the parasite. Although species identification was not attempted in the current and previous works, *Sarcocystis* infection observed in cattle could be caused by zoonotic species. Moreover, the custom of eating raw or undercooked meat and poor latrine usage in rural and peri-urban areas can facilitate the transmission of the parasite to a level difficult to curb. For a better understanding of the problem and to make necessary measures, further study preferably based on molecular techniques should be conducted to characterize the cysts at species level and to estimate the problem on edible skeletal muscles that are consumed raw or uncooked. Moreover, to reduce the prevalence of this parasite and hence its zoonotic and economic impact, effort should be made to break the life cycle through awareness.

Ethical approval

Ethical clearance was obtained from Hawassa University Faculty of Veterinary Medicine Research Ethical Review Committee. This article does not contain any studies with human participants or experimental animals performed by any of the authors.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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