

The Prevalence of Infection of *Sarcocystis* Species in Cattle in Zanjan Province, Northwest Iran

Reza Hamidi, Asghar Fazaeli* and Abbas Mahmoodzadeh Poornaki

Department of Parasitology and Mycology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

*Corresponding Author: Asghar Fazaeli, Department of Parasitology and Mycology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.

Received: October 10, 2020; Published: November 07, 2020

Abstract

Background: *Sarcocystis* is a protozoan parasite, capable of infecting many species of mammalian animals and birds worldwide. Transmission of some species to humans can happen through eating raw or under-cooked meats. Cattle as a major source of food production, harbor several species of *Sarcocystis*, including *S. hominis*, *S. cruzi*, *S. hirsuta* and one or two more species yet to be further validated. Little is known about the prevalence of *Sarcocystis* in livestock, especially cattle as a major human food source, in north-west of Iran.

Aim: This study was aimed to investigate the infection prevalence of *Sarcocystis* species in slaughtered cattle in Zanjan, northwest Iran.

Methods: Two hundred seventy one cattle were randomly selected from Zanjan slaughterhouse. Macroscopic survey of three organs, including esophagus, diaphragm and heart was performed to detect possible existence of macro-sarcocysts (macrocyts). Consequently, samples were taken from the above organs of the cattle and subjected to pepsin-hydrochloric acid digestion, stained smear preparation, and microscopic examination for detection of the bradyzoites released from sarcocysts.

Results: No macro-sarcocyst was detected, by naked eye observation, in the organs of the 271 cattle, indicative of the absence or rare presence of *S. hirsuta*, as a macrocyst producing species in cattle, in the study region. Meanwhile, microscopic examination of digested samples showed that 100% of the samples were infected with *Sarcocystis* spp. that should be microcyst producing species. The infection rate was not different between male and female and among different age groups, as the samples were totally infected.

Conclusion: The results indicated that *Sarcocystis* infection is widely distributed in cattle in Zanjan province. The findings imply that the microcyst producing species, i.e. *S. cruzi* and/or *S. hominis*, are predominant in this region. However, further studies are required to clearly identify the parasite different species, particularly *S. hominis* as a zoonotic one, to provide better strategies for the food source infection control.

Keywords: *Sarcocystis*; Prevalence; Cattle; Zanjan; Iran

Introduction

There are many species of *Sarcocystis* distributed worldwide in a broad range of animal hosts. It is an obligatory heteroxenous food born parasite, each species of which circulates in two hosts including a definitive host mostly from carnivores (as predators) and an intermediate host mostly from herbivores (as prey) [1].

Cattle harbor intermediate cystic forms (sarcocysts) of three distinct species of *Sarcocystis*, i.e. *S. hominis*, *S. cruzi*, *S. hirsuta*, infective for human, canine and feline respectively [2]. Other species named as *S. rommeli* and/or *S. sinensis*-like, may also be detectable in cattle [3,4]; however, they are not yet sufficiently characterized and the latter is not validated as a true cattle species [5]. *S. hominis* is more important with regard to its public health involvement. Oocysts or sporocysts of *S. hominis* are shed in human feces as the definitive host. Ingestion of oocysts by cattle leads to their infection following the release of sporozoites and development and transformation into sarcocysts in muscles, potentially transmissible to humans through eating raw or undercooked beef [1].

Sarcocystosis may have impact on both animal and human public health. It can cause myositis, myocarditis, encephalitis, hepatitis and even abortion in animals. Intestinal infection in human can lead to mild or severe clinical consequences such as acute or chronic enteritis, diarrhea, fatigue, nausea and vomiting; though asymptomatic infections may appear in many cases [1,6]. Further, consumption of animal meats harboring cystic forms of *Sarcocystis* spp., even those with no human role in their life cycle, may cause food poisoning in humans. For example, a case was reported with description of ingestion of raw venison infected with cysts of *S. truncata* [7].

Some species in cattle, like *S. hirsute*, produce sarcocysts in muscles visible by naked eyes which are known as macro-sarcocyst/macro-cyst; while other species, *S. hominis* and *S. cruzi*, produce microscopically visible sarcocysts (microcysts) [4]. So, meat inspection by naked eye cannot be reliable for the detection of sarcocystis infection in carcasses. Digestion method, encompassing meat sample processing prior to microscope slide preparation, has been reported to facilitate better diagnosis of the infection in cattle [8].

The prevalence of sarcocystosis in cattle is high in most countries, i.e. 69.9% in Germany [9], 100% in the USA [10], 97.4% in Belgium [11], 42.5% in Zaria, Nijeria [12], 78.1% in Italy [13]. High prevalence has also been reported from some regions of Iran. It was 100% in Kerman, southeast [14], 93.3% in Sanandaj, north [15], 100% in Tabriz city, northwest [16], about 90% in Shiraz, south [17]. However, little is known about this infection in northwest region including Zanjan province.

Aim of the Study

The present study was designed to investigate the prevalence of muscular sarcocystosis in cattle caused by different species of *Sarcocystis* in Zanjan province.

Materials and Methods

Study design

This is a cross-sectional study. Zanjan province is located in northwest Iran. The sample size was estimated ($n = 271$) using formula $n = Z_{1-\alpha/2}^2 [P (1- P)]/d^2$ and based on the findings of the similar studies previously performed in Iran. The samples were randomly selected from Zanjan slaughterhouse, where cattle are brought from all areas of the province for slaughtering and distributing.

Macroscopic survey and sampling

Macroscopic survey of three organs, including esophagus, diaphragm, and heart muscles was done to detect possible existence of macrocysts.

Samples were collected from the above organs of the carcasses, 50g each in a sealed plastic bag, and transferred to the research laboratory at the Department of Parasitology, School of Medicine, for further investigations.

Digestion of samples

Sample digestion was performed based on a standard protocol (8) as follows: A portion of 20g of each sample was cleaned, chopped, minced by a meat grinder and digested in 50 ml of digestion solution consisted of 1.3g pepsin, 2.5g NaCl, 3.5 ml HCl in 500 ml distilled

water and incubated at 40°C for one hour. To avoid cross contamination, after every time use of grinder, it was washed and cleared sufficiently by tap water and consequently decontaminated by a detergent, followed by a final wash with distilled water. Following incubation, the samples were filtered and spun by centrifugation with 2500 rpm for 5 minutes and the pellets were used for smear preparation.

Microscopic examination

The pellets were examined using both wet smear and permanent staining methods. The wet smears were prepared and examined under microscope with X400 magnifications. The Giemsa staining was utilized for preparation of permanent stained smears that enabled more precise microscopic examination and detection of bradyzoites released from ruptured sarcocysts with X1000 enlargement.

Results and Discussion

All three organs (esophagus, diaphragm, heart) of all 271 selected cattle were surveyed macroscopically, with no macrocyst detection. Meanwhile, microscopic examination of digested samples showed that 100% of the samples were infected with *Sarcocysts* spp. Enormous numbers of cystizotes were seen in both wet smear and permanent stained smears (Figure 1). All three organs were highly infected in all examined cattle.

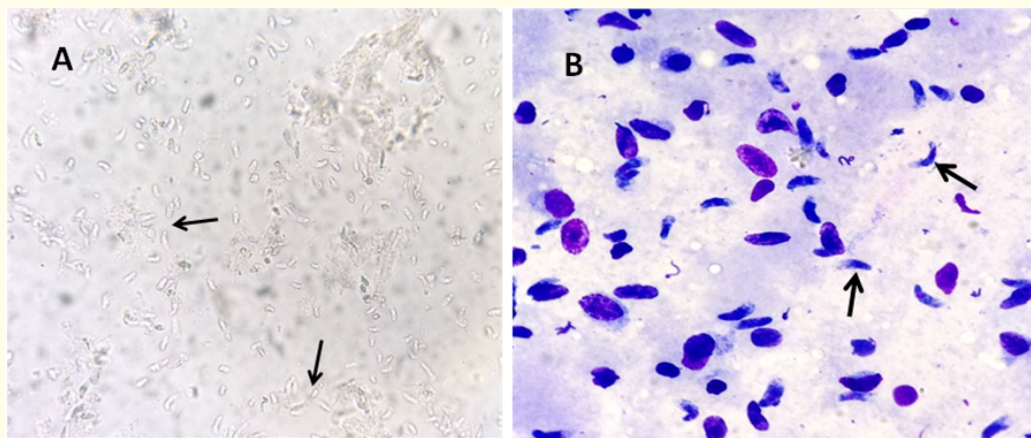


Figure 1: Representative microscope slides prepared from digested muscle samples. Panel A is a wet smear (X400) and panel B is a Giemsa stained smear (X1000). Arrows indicate bradyzoites released from tissue cysts of *Sarcocystis* spp.

The information of the cattle, including sex, age and breeding type is mentioned in table 1.

Sex No (%)	Male 217 (80)	Female 54 (20)	Total 271 (100)
Age No (%)	≤ 18 months 236 (87)	> 18 months 35 (13)	Total 271 (100)
Breeding type* No (%)	Industrialized 226 (83.4)	Local 45 (16.6)	Total 271 (100)

Table 1: Information about the cattle investigated for the infection with *Sarcocystis* spp. in Zanjan province, Iran.
*Non-improved *B. indicus* breeds.

None of the inspected cattle organs (esophagus, diaphragm and heart), nor the samples provided from these organs showed macrocysts. However, microscopic examination confirmed infection with *Sarcocystis* spp. in all 271 investigated cattle, indicating the presence of microcysts in all samples:

Two *Sarcocystis* species, including *S. hominis* and *S. sui hominis* are infective to human as definitive host, in common with cattle and swine as intermediate hosts, respectively; however, it is not known whether human can be involved in the life cycle of any other species detectable in hosts other than cattle, particularly the species that infect non-human primates [6].

The high prevalence of the infection presented in our study is similar to the results of several studies reported from other countries. In a study of 1080 cattle in Iraq, using pepsin digestion method and microscopic examination, the rate of infection with *Sarcocystis* spp was 97.8% [18]. It was 100% in New Zealand [19], 100% in cattle and sheep in the United States [10] and 91.33 in Andhra Pradesh, India [20]. The infection rate was lower in some other regions. For example, it was 51% in Australia [21], 40.8% in cattle and buffalo in Malaysia [22], 42.5% in Nigeria [12], 36% of cattle in Egypt [23], 15.7% to 51.1% in Hokkaido, Japan [24] and 17.9% in Romania [25].

The prevalence of muscle *Sarcocystis* infection of cattle in our study is also similar to the findings in other parts of Iran, as the most of studies presented high prevalence of the infection ($\geq 90\%$). It was 90% In Shiraz (south of Iran) [17]. Other studies showed muscle microcysts in 100% of 480 bovine sample in Kerman, southeast [14] and 93.3% of 120 cattle in Sanandaj, west [15]. Macroscopical observation did not resulted in detection of macrocysts in the above studies in Iran. However, in a study by Shekarforoush., *et al.* [26], a sample of macrocyst was detected and identified as *S. hirsuta*; in another study a rate of 8.2% of macrocyst observation (without morphological presentation) was reported in slaughtered cattle of Tabriz city [16]. The absence or rare observation of macrocysts in cattle in different studies implies that the prevalence of macrocyst producing species, i.e. *S. hirsuta*, is quite low in Iran.

Cattle as a major source of food production, harbor several species of *Sarcocystis*, including *S. hominis*, *S. cruzi*, *S. hirsuta*, and one or two more species yet to be further characterized or validated [2-5]. *S. hominis* is mildly pathogenic for cattle and may cause either symptomless intestinal infection or symptomatic gastroenteritis in humans [27]. *S. cruzi* (*S. bovicanis*) is the most pathogenic *Sarcocystis* species in cattle, causing severe disease with symptoms including exophthalmia, hepatitis, myocardial bleeding, anemia, weight loss, abortion, and death [27]. In the present study, we did not perform species identification, but evidence from other studies is in favor of predominant infection of *S. cruzi* species in Iranian cattle that can affect meat production and food supplies. However, *S. hominis* is among the species existing in cattle that is important to human health.

Conclusion

The results indicated that *Sarcocystis* infection is widely distributed in cattle in Zanjan province. The absence of macrocyst in samples studied here indicates that probably there is no *S. hirsuta* endemic in the study area, as this species is known to produce macrocysts in the intermediate hosts. The foods which are produced from cattle meats can be potentially infective for consumers, though we do not know the proportion of *S. hominis* throughout the whole species in this region. Further investigation, including molecular techniques are required for the precise identification of the parasite species and their contributions to the cattle infection to provide better strategies for infection control in livestock.

Acknowledgement

This project was a MSc thesis of Reza Hamidi and financially supported by the Deputy for Research at Zanjan University of Medical Sciences and Health Services with grant number A-12-245-6 and approved by the institutional Research Ethics Committee with code number ZUMS.rec.1394.325. We would like to greatly appreciate supervision of the veterinarians and kind co-operation of the staff of Zanjan slaughter house. The authority of Provincial Veterinary Organization also kindly facilitated the field work of this project.

Conflict of Interest

The authors declared that they have no conflicts of interest in this research and publication.

Bibliography

1. JP Dubey, *et al.* "Sarcocystosis of animals and man". CRC Press, Boca Raton, FL (1989): 1-215.
2. JP Dubey, *et al.* "*Sarcocystis rommeli*, n. sp. (Apicomplexa: Sarcocystidae) from cattle (*Bos taurus*) and its differentiation from *Sarcocystis hominis*". *Journal of Eukaryotic Microbiology* 63.1 (2015): 62-68.
3. ZQ Yang, *et al.* "Analysis of the 18S rRNA genes of *Sarcocystis* species suggests that the morphologically similar organisms from cattle and water buffalo should be considered the same species". *Molecular and Biochemical Parasitology* 115.2 (2001): 283-288.
4. B Gjerde. "The resurrection of a species: *Sarcocystis bovifelis* Heydorn *et al.*, 1975 is distinct from the current *Sarcocystis hirsuta* in cattle and morphologically indistinguishable from *Sarcocystis sinensis* in water buffaloes". *Parasitology Research* 115.1 (2016): 1-21.
5. JP Dubey, *et al.* "Identity of *Sarcocystis* species of the water buffalo (*Bubalus bubalis*) and cattle (*Bos taurus*) and the suppression of *Sarcocystis sinensis* as a nomen nudum". *Veterinary Parasitology* 205 (2014): 1-6.
6. R Fayer, *et al.* "Human infections with *Sarcocystis* species". *Clinical Microbiology Review* 28.2 (2015): 295-311.
7. T Ota, *et al.* "First Case Report of Possible *Sarcocystis truncata*-induced Food Poisoning in Venison". *Internal Medicine* 58 (2019): 2727-2730.
8. M Nevole and D Lukesová. "Methods of direct detection of Sarcocysts and their diagnostic reliability". *Veterinary Medicine* 26.10 (1981): 581-584.
9. G Moré, *et al.* "*Sarcocystis sinensis* is the most prevalent thick-walled *Sarcocystis* species in beef on sale for consumers in Germany". *Parasitology Research* 113.6 (2014): 2223-2230.
10. R Fayer. "*Sarcocystis* spp. in human infections". *Clinical Microbiology Review* 17.4 (2004): 894-902.
11. L Vangeel, *et al.* "Molecular-based identification of *Sarcocystis hominis* in Belgian minced beef". *Journal of Food Protection* 70 (2007): 1523-1526.
12. FN Obijiaku, *et al.* "*Sarcocystis* infection in slaughtered cattle in Zango abattoir, Zaria, Nigeria". *Veterinary World* (2013): 346-349.
13. L Domenis, *et al.* "Detection of a morphogenetically novel *Sarcocystis hominis*-like in the context of a prevalence study in semi-intensively bred cattle in Italy". *Parasitology Research* 109.6 (2011): 1677-1687.
14. SR Nouroollahi Fard, *et al.* "Survey of *Sarcocystis* infection in slaughtered cattle in Kerman, Iran". *Tropical Animal Health and Production* 41.8 (2009): 1633-1636.
15. S Rasouli. "A Survey On Prevalence Rate Of Meat Infection With *Sarcocystis* Spp In Sanandaj Using Digestion Method". *Journal of Veterinary Pathobiology* (2010): 27-32.
16. M Mirzaei and H Rezaei. "A survey on *Sarcocystis* spp. infection in cattle of Tabriz city, Iran". *Journal of Parasitic Diseases* 40.3 (2016): 648-651.
17. M Akhlaghi, *et al.* "Molecular differentiation of bovine sarcocysts". *Parasitology Research* 115 (2016): 2721-2728.
18. B Latif, *et al.* "Prevalence of *Sarcocystis* spp. in meat-producing animals in Iraq". *Veterinary Parasitology* 84.1 (1999): 85-90.

19. A Böttner, *et al.* "The prevalence and identity of *Sarcocystis* in beef cattle in New Zealand". *Veterinary Parasitology* 24.3-4 (1987): 157-168.
20. K Mounika K, *et al.* "Studies on prevalence and histomorphology of *Sarcocystis* species infecting cattle in Andhra Pradesh, India". *Journal of Parasitic Diseases* 42.1 (2018): 77-80.
21. G Savini, *et al.* "The epidemiology of *Sarcocystis* spp. in cattle of Western Australia". *Epidemiology and Infection* 108.1 (1992): 107-113.
22. B Latif, *et al.* "Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh, High prevalence of muscular sarcocystosis in cattle and water buffaloes from Selangor, Malaysia". *Tropical Biomedicine* 30.4 (2013): 699-705.
23. AM Ahmed, *et al.* "Survey on *Sarcocystis* in bovine carcasses slaughtered at the municipal abattoir of El-Kharga, Egypt". *Veterinary World* (2016): 2231-0916.
24. Y Omata, *et al.* "Survey of *Sarcocystis* Infection in Cattle in East Hokkaido, Japan". *Journal of Veterinary Medical Science* 56.3 (1994): 557-558.
25. K Imre, *et al.* "*Sarcocystis* spp. in Romanian Slaughtered Cattle: Molecular Characterization and Epidemiological Significance of the Findings". *BioMed Research International* (2019): 1-6.
26. S Shekarforoush, *et al.* "First detection of *Sarcocystis hirsuta* from cattle in Iran". *Iranian Journal of Veterinary Research* 14.2 (2013): 155-157.
27. D Herenda, *et al.* "Specific diseases of cattle". In: Manual on Meat Inspection for Developing Countries. (FAO Animal Production and Health Paper 119) (2000): 180-186.

Volume 5 Issue 12 December 2020

© All rights reserved by Asghar Fazaeli, *et al.*