


Studies on prevalence and histomorphology of *Sarcocystis* species infecting cattle in Andhra Pradesh, India

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Abstract The present study was carried out to determine the prevalence of bovine sarcocystosis and identify the different species of *Sarcocystis* cysts in cattle in different regions of Chittoor district, Andhra Pradesh. A total of 150 slaughtered cattle over a period of 1 year were examined both macroscopically and microscopically for the presence of *Sarcocystis* infection. Out of 150 slaughtered cattle examined, macroscopic sarcocysts were observed in nine cattle and were exclusively found in oesophagus. Examination of tissue samples collected from different predilection sites from each of 150 cattle by pepsin–HCl digestion method revealed presence of live banana shaped bradyzoites under light microscopy in 134 cattle. Six cattle that were positive by macroscopic examination were also positive by microscopic examination of tissues. The overall prevalence of *Sarcocystis* infection in cattle of Chittoor district was 91.33%. The prevalence of macroscopic and microscopic sarcocysts was 6.57 and 93.43% respectively. Statistically a significant relationship between the prevalence of infection among different age groups ($P < 0.001$)

and no significant relationship ($P > 0.001$) between the prevalence of *Sarcocystis* infection in male (91.76%) and female (90.76%) cattle was observed. In both male and females the prevalence of *Sarcocystis* infection increased with age. Microscopically sections of tissues from cattle that were positive by tissue digestion technique revealed thin walled cysts ($4.5 \pm 0.5 \mu\text{m}$) in 131 samples and thick walled cysts ($0.5 \pm 0.12 \mu\text{m}$) in six cattle.

Keywords *Sarcocystis* species · Cattle · Thin walled cysts · Thick walled cysts · Prevalence

Introduction

Parasitic diseases are thought to be the primary restraint to the economy of farmers by reducing the growth and production of cattle. Sarcocystosis caused by *Sarcocystis* spp., a tissue coccidian parasite involves carnivores and humans as definitive hosts and domestic animals as intermediate hosts. Cattle become infected with the parasite by ingestion of sporocysts or sporulated oocysts of *Sarcocystis* through contaminated food or water and show symptoms of reduced weight gain, poor feeding efficiency, muscle weakness, reduced milk yield, abortion, condemnation of meat due to macroscopic sarcocysts and mortality depending on the immune status of the host and dose of ingested sporocysts (Dubey et al. 1989). Cattle are mainly infected with *Sarcocystis cruzi*, *S. hirsuta* and *S. hominis*. Out of these three species, *S. hominis* is zoonotic and infect humans by consumption of undercooked beef containing the larval stage sarcocyst resulting in digestive upset (Fayer 2004). It was reported about 50% of parasitic cysts found in muscles of cattle belong to *S. hominis* (WHO 1981). Sarcocystosis is routinely diagnosed by pathological (gross examination,

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impression smears, histopathology and digestion methods) and serological methods (IFAT and ELISA) that identify genus level of *Sarcocystis* spp. Prevalence of disease has been reported from different parts of world including India (More et al. 2008; Hamidinejat et al. 2010; Dafedar et al. 2011; Chiesa et al. 2013). However the reports on prevalence of sarcocystosis in cattle in Andhra Pradesh are scanty (Venu and Hafeez 2000). Hence owing the zoonotic significance of sarcocystosis and the importance of epidemiology of disease to follow an appropriate control strategy, the present study was carried out to know the prevalence of sarcocystosis in cattle in Chittoor district of Andhra Pradesh.

Materials and methods

A total of 150 slaughtered cattle that were slaughtered at Tirupati, Chandragiri, Chittor, Renigunta and Pakala regions in Chittor district, Andhra Pradesh were examined macroscopically and microscopically for a period of 1 year to study the prevalence of sarcocystosis. Age and sex of cattle were taken into account. Different organs of cattle including predilection sites viz., oesophagus, diaphragm and heart were examined thoroughly for the presence of macroscopic sarcocysts. Further tissues samples of each slaughtered cattle from different predilection sites were collected and subjected to Pepsin–HCl tissue digestion technique for microscopic examination. Twenty grams of tissue samples (pooled tissues from different predilection sites) collected from each cattle carcass, irrespective of visible macroscopic cysts were incubated in 50 ml of acid pepsin solution for 20 min at room temperature (2.6 g pepsin, 5 g NaCl, 7 ml 1 M HCl, 993 ml distilled water). The suspension was then filtered using a 53 µm pore sieve, and centrifuged at 5000 rpm for 3 min. Finally, the sediment was suspended in 0.5 ml distilled water and a drop of the solution was examined for the presence of bradyzoites under a light microscope. The representative tissue pieces were also fixed in 10% neutral buffered formalin and processed routinely for histopathological evaluation. Data obtained was classified according to age and sex and was analyzed as per standard statistical techniques (Petrie and Watson 2013). Chi-square test of association was used to establish association between the infection status and variables such as age and sex of the cattle.

Results and discussion

Macroscopic sarcocysts were observed only in nine out of 150 cattle screened and were observed in oesophagus but not in other predilection sites viz., skeletal muscles, cardiac

and diaphragmatic muscles. Grossly, sarcocysts were elongated, milky-white in colour and were embedded in the muscular tissue (Fig. 1). Examination of tissue samples collected from different predilection sites from each of 150 slaughtered cattle by pepsin–HCl digestion revealed presence of live banana shaped bradyzoites (Fig. 2) in 134 cattle under light microscopy. In general, three cattle were exclusively positive for macroscopic cyst, 128 were positive for microscopic sarcocysts and six cattle were positive for both microscopic and macroscopic sarcocysts. The overall prevalence of *Sarcocystis* infection in slaughtered cattle of Chittoor district, based on macroscopic and microscopic sarcocysts was 91.33% (Table 1). The infection was highly prevalent in Tirupati (97.14%) compared to that of other regions of Chittoor district. It was observed that there was an increase in prevalence over the past 15 years in Tirupati region wherein the prevalence was 71.50% in 1996 (Venu and Hafeez 2000). The increased prevalence rate might be due to the increased source of sporocysts in pasture to cattle from definitive hosts. Similarly very high rate of prevalence between 91.0 and 100% was also reported across the globe viz., United States (Fayer and Dubey 1986), Iraq (Latif et al. 1999), South-West of Iran (More et al. 2008), Argentina (Hamidinejat et al. 2010), Southern Italy (Bucca et al. 2011), Karnataka, India (Dafedar et al. 2011), Italy (Chiesa et al. 2013) and Yazd and Hungary (Hornok et al. 2015). In contrast to the present findings, low prevalence of *Sarcocystis* spp. has been reported from Egypt (Badawy et al. 2012) and Malaysia (Latif et al. 2013). The diversity in rate of prevalence could be due to the several factors such as system of breeding, climatic factors, age of sampled animals and type and anatomical site of diagnosis (Dubey et al. 1989) in addition to different regions and availability of definitive hosts.

Macroscopic sarcocysts in meat are probably a serious meat sanitation problem and economic problem due to condemnation of infected carcass. The prevalence of macroscopic sarcocysts in the present study was low



Fig. 1 Cattle oesophagus: note macroscopic sarcocysts

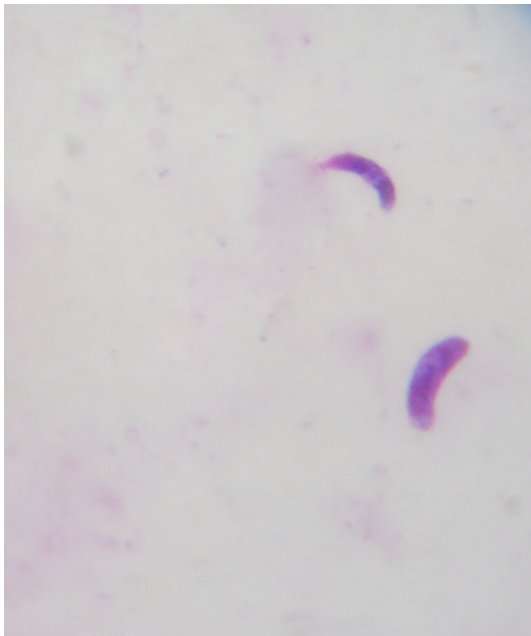


Fig. 2 Giemsa stained Bradyzoites ×1000

Table 1 Prevalence of *Sarcocystis* infection in cattle in different regions of Chittoor district

Location	No. of samples collected	No. of samples positive	Prevalence (%)
Tirupati	70	68	97.14
Chandragiri	40	37	92.5
Chittoor	20	16	80
Renigunta	10	9	90
Pakala	10	7	70
	150	137	91.33

(6.57%), compared with that of microscopic sarcocysts (93.43%) which could be due the connection between cattle and faecal sample of feline definitive hosts in pasture is less than canine definitive hosts in the area under study as macroscopic sarcocysts are of cat source. Additionally, development of macrocysts of *Sarcocystis* spp. takes for several years and cattle are slaughtered prior to the maturation of cysts as is also opined by Nourani et al. (2010). Similarly Latif et al. (1999), Hamidinejat et al. (2010) and Nahed et al. (2014) also reported the low prevalence of macroscopic sarcocysts in cattle in Iran and Egypt, respectively. Macroscopic sarcocysts were exclusively found in the oesophagus that was in accordance with the findings of Domenis et al. (2011) and Xiang et al. (2011).

The results of age-wise and sex-wise prevalence were summarized in Table 2. There was a significant relationship between the prevalence of infection among different age groups ($P < 0.001$). The prevalence was found to increase with advancement of age with high infection rate

Table 2 Age-wise and sex-wise prevalence of *Sarcocystis* infection in cattle

Sex	Age group in years	No. of cattle examined	No. of cattle infected	% of infection
Male	1–2	9	3	33.33
	2–4	46	40	86.95
	Above 4	37	35	94.59
	Total	85	78	91.76
Female	1–2	4	1	25
	2–4	29	27	93.1
	Above 4	32	31	96.87
	Total	65	59	90.76

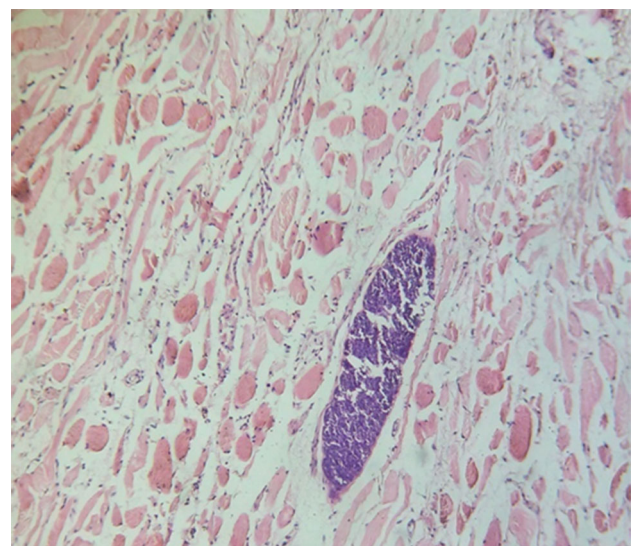


Fig. 3 Cattle diaphragm muscle: note section showing thick walled sarcocyst H&E ×100

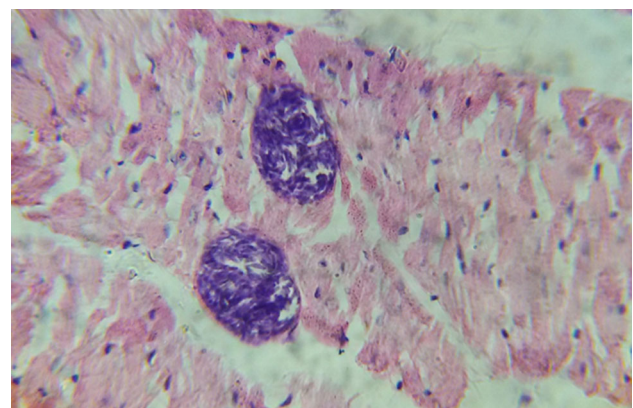


Fig. 4 Cattle cardiac muscle: note section showing thin walled sarcocyst H&E ×100

of 95.65% among the old animals which was consistent with data of *Sarcocystis* infection in cattle from other countries (Hornok et al. 2015) and in other live stock species (Abu-Elwafa et al. 2015). This could be due to prolonged exposure of aged animals to the sporocysts infection. In contrast, Bucca et al. (2011) and Hajimohammadi et al. (2014) observed no significant association between *Sarcocystis* infection and age of cattle. The present study revealed that the prevalence of *Sarcocystis* infection of both male and females increased with advance in age and there was no significant relationship ($P > 0.001$) between the rates of infection in female and male cattle within each age group, in accordance with the previous studies (Jehle et al. 2009; Hajimohammadi et al. 2014). In contrast significant association between rate of *Sarcocystis* infection and sex of cattle was reported by More et al. (2008) and Nourani et al. (2010). Microscopically sections of tissues from each cattle that were positive by tissue digestion technique revealed thick walled cysts (Fig. 3) in six samples ($4.5 \pm 0.5 \mu\text{m}$) and thin walled cysts (Fig. 4) in 131 cattle ($0.5 \pm 0.12 \mu\text{m}$). Further studies on molecular differentiation of *Sarcocystis* species is necessary as conventional techniques revealed diagnostic limitation in distinguishing the *S. hominis* from *S. hirsuta* thick walled cysts (Nourani et al. 2010).

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Author contributions KM: involved in sample collection, executed the lab work as per design. CS: monitored lab work and involved in manuscript preparation. RV and TSR: involved in sample processing and shared the lab facilities. SK: involved in experimental design of the research work and analysed the results.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest to declare which controls the content of this research work.

References

- Abu-Elwafa SA, Al-Araby MA, Abbas IEA (2015) *Sarcocystis fusiformis* infecting water buffaloes (*Bubalus bubalis*) in Dakahlia Province, Egypt. *Int J Adv Res* 3:116–120
- Badawy AII, Abouzaid NZ, Ahmed HA (2012) *Sarcocystis hominis* and other *Sarcocystis* species infecting cattle in Sharkia Province, Egypt. *J Am Sci* 8:271–275
- Bucca M, Brianti E, Giuffrida A, Ziino G, Cicciari S, Panebianco A (2011) Prevalence and distribution of *Sarcocystis* spp. cysts in several muscles of cattle slaughtered in Sicily, Southern Italy. *Food Control* 22:105–108
- Chiesa F, Muratore E, Dalmaso A, Civera T (2013) A new molecular approach to assess the occurrence of *Sarcocystis* spp. in cattle and products there of preliminary data. *Ital J Food Saf* 2(41):148–151
- Dafedar A, D'Souza Placid Eugene, Mamatha GS (2011) Prevalence and morphological studies on *Sarcocystis* species infecting cattle in Bangalore. *J Vet Parasitol* 25:183–184
- Domenis L, Peletto S, Sacchi L, Clementi E, Genchi M, Felisari L, Felisari C, Mo P, Modesto P, Zuccon F, Campanella C, Maurella C, Guidetti C, Acutis PL (2011) Detection of a morphogenetically novel *Sarcocystis hominis*-like in the context of a prevalence study in semi-intensively bred cattle in Italy. *Parasitol Res* 109:1677–1687
- Dubey JP, Speer CA, Fayer R (1989) *Sarcocystosis* of animals and man. CRC Press, Boca Raton, p 215
- Fayer R (2004) *Sarcocystis* spp. in human infections. *Clin Microbiol Rev* 17:894–902
- Fayer R, Dubey JP (1986) Bovine sarcocystosis. *Compend Contin Educ Pract Vet* 8:130–142
- Hajimohammadi B, Dehghani A, Ahmadi MM, Eslami G, Oryan A, Khamsepour A (2014) Prevalence and species identification of *Sarcocystis* in raw hamburgers distributed in Yazd, Iran using PCR-RFLP. *J Food Qual Hazards Control* 1:15–20
- Hamidinejat H, Jalali MHR, Nabavi L (2010) Survey on *Sarcocystis* infection in slaughtered cattle in South-West of Iran, emphasized on evaluation of muscle squash in comparison with digestion method. *J Anim Vet Adv* 9(1724):1726
- Hornok S, Mester A, Takacs N, Baska F, Majoros G, Fok E, Biksi I, Nemet Z, Hornyak A, Janosi S, Farkas R (2015) *Sarcocystis*-infection of cattle in Hungary. *Parasites Vectors* 8:69. <https://doi.org/10.1186/s13071-015-0685-9>
- Jehle C, Dinkel A, Sander A, Morent M, Romig T, Luc PV, De TV, Thai VV, Mackenstedt U (2009) Diagnosis of *Sarcocystis* spp. in cattle (*Bos taurus*) and water buffalo (*Bubalus bubalis*) in Northern Vietnam. *Vet Parasitol* 166:314–320
- Latif BMA, Al-Delemi JK, Mohammed BS, Al-Bayati SM, Amiry AM (1999) Prevalence of *Sarcocystis* spp. in meat-producing animals in Iraq. *Vet Parasitol* 84:85–90
- Latif B, Vellayan S, Heo C, Kannan Kutty M, Omar E, Abdullah S, Tappe D (2013) High prevalence of muscular sarcocystosis in cattle and water buffaloes from Selangor, Malaysia. *Trop Biomed* 30:699–705
- More G, Basso W, Bacigalupe D, Venturini MC, Venturini L (2008) Diagnosis of *Sarcocystis cruzi*, *Neospora caninum* and *Toxoplasma gondii* infections in cattle. *Parasitol Res* 102:671–675. <https://doi.org/10.1007/s00436-007-0818-6>
- Nahed H, Ghoneim WM, Nader MS (2014) Occurrence of zoonotic sarcosporidiosis in slaughtered cattle and buffaloes in different abattoirs in Egypt. *Global Vet* 13:809–813
- Nourani H, Matin S, Nouri A, Azizi H (2010) Prevalence of thin-walled *Sarcocystis cruzi* and thick-walled *Sarcocystis hirsuta* or *Sarcocystis hominis* from cattle in Iran. *Trop Anim Health Prod* 42:1225–1227
- Petrie A, Watson P (2013) *Statistics for veterinary and animal science*, 1st edn. Blackwell Publishing, Oxford, pp 101–109
- Venu R, Hafeez Md (2000) Prevalence of *Sarcocystis* infections in slaughtered domestic ruminants in Tirupati, Andhra Pradesh. *Indian Vet J* 77:165–166
- World Health Organization (1981) *Intestinal protozoan and helminthic infections*, vol 666. World Health Organization, Geneva
- Xiang Z, He Y, Zhao H, Rosenthal BM, Dunams DB, Li X, Zuo Y, Feng G, Cui L, Yang Z (2011) *Sarcocystis cruzi*: comparative studies confirm natural infections of buffaloes. *Exp Parasitol* 127:460–466