Prevalence and microscopic studies of *Sarcocystis* infection in naturally infected water buffaloes (*Bubalus bubalis*) of Andhra Pradesh

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Abstract *Sarcocystis* species are the obligate tissue protozoan parasites of livestock causing clinical and subclinical disease resulting in downgrading of the meat and their products thereby leading to economic losses. The present study reveals the prevalence and distribution of sarcocystosis in water buffaloes (*Bubalus bubalis*) slaughtered at local abattoirs in A.P for a period of 1 year from June 2014 to May 2015. A total of 137 buffalo carcasses were screened grossly and microscopically organ wise viz., esophagus, tongue, heart, diaphragm and intercostal muscles. Out of 137 screened, 91 were infected with an overall prevalence of 66.42 %. Age wise analysis of 89 young male calves aged about 18–36 months old revealed 65.16 % (58/89) and 48 old she buffaloes (5–8 years) showed 68.75 % (33/48). The organ wise prevalence was highest in esophagus (51.82 %) followed by tongue (47.44 %), heart (29.92 %), diaphragmatic muscles (28.46 %) and intercostal muscles (18.24 %), respectively. Morphometric studies revealed the presence of two *Sarcocystis* species, i.e., *S.levinei* and *S.fusiformis* infection with a prevalence of 43.79 and 22.62 %, respectively, along with mixed infection rate of 43.06 %. Microscopic studies of *S.levinei* showed sarcocyst length/width/cyst wall thickness ranged between 0.31–0.69/0.09–0.12 mm/<1 µm, respectively, and bradyzoites with an average of

6.25 µm length/2.5 µm width. Similarly, *S.fusiformis* cyst ranged between 2 and 8.5 mm/1–3 mm/2–5 µm and bradyzoites with an average of 10 µm length/2.5 µm width. The histopathological studies revealed congestion and degenerative changes of myocytes along with infiltration of mononuclear cells.

Keywords Buffaloes · Sarcocystosis · Prevalence · Microscopic studies

Introduction

Sarcocystosis is caused by a tissue cyst forming coccidian protozoan parasite with a wide geographic distribution and a wide host range affecting wild and domesticated animals as well (Abu-Elwafa et al. 2015). The protozoan parasite has an obligatory prey–predator two host life cycle with carnivores (Dogs and Cats) as definitive hosts and herbivores and omnivores as an intermediate hosts (Juyal and Bhatia 1989). Each host in turn may harbor more than one *Sarcocystis* species at a time (Dubey et al. 1989). In ruminants, this infection characteristically causes subclinical and chronic infections in striated muscles by forming macrocyst or microcyst (Al-Hyali et al. 2011). Esophagus, heart, tongue, diaphragm and intercostal muscles are most common preferred organs in this infection in cattle and buffaloes which can persist throughout life in the host but may start to disappear after 3 months of infection.

Diagnosis of *Sarcocystis* infection is mostly possible during necropsy only as the disease runs either sub-clinical and chronic course characterized predominantly by reduced weight gain, poor feed efficiency, anorexia, fever, anemia, abortions, muscle weakness, reduced milk yield and even death in heavy infection in the intermediate host (Dubey et al.

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1989). Epidemiological studies of *Sarcocystosis* in different domestic animals from various parts of the India (Shah 1983) revealed that the infection is very common in adult buffaloes, attributable to some *Sarcocystis* species (Dubey et al. 1989). Four species of *Sarcocystis* have been reported in the water buffaloes *S.fusiformis*, *S.buffalonis*, *S.levineii* and *S.dubeyi* (Dissanaike and Kan 1978; Huong et al. 1997; Huong and Ugglä 1999; Huong 1999a, b; Dubey et al. 2014). *S.fusiformis* (Dubey et al. 1989) and *S.buffalonis* (Huong et al. 1997) form macrosarcocyst and has cat acts as a definitive host. *S.levineii* (Dubey et al. 1989; Claveria et al. 2000) forms microscopic sarcocyst and dog acts as definitive host (Dubey et al. 1989), whereas newly identified *S.dubeyi* forms the microscopic sarcocyst and definitive host has not yet been identified (Huong and Ugglä 1999; Oryan et al. 2010).

Materials and methods

A total of 137 buffalo carcasses belonging to native and graded Murrah breeds that were slaughtered at local abattoirs in different parts of Andhra Pradesh from June 2014 to May 2015 were screened for the present study. According to age, the animals were categorized into two age groups: first group were young buffaloes (89) aged between 18 and 36 months and the second group were senile/unproductive buffaloes (48) aged about 5–8 years. At the time of slaughter, the fresh tissues were examined grossly for the presence of macrocyst, and simultaneously the suspected tissue samples from different organs viz., esophagus, tongue, heart, diaphragm and intercostal muscles were collected and tagged properly, preserved in 4 °C for further processing (Huong and Ugglä 1999). For microscopic examination, trichinoscopy method was used. In this method, freshly collected samples were subjected to –20 °C for a few hours (2–4) until they become tough enough to cut into thin slices with the help of a fine scalpel blade. Approximately 15- to 20-µm thick sections were taken in few drops of normal saline and compressed gently between the two microscopic slides until it become transparent; then, it was screened for the presence of microscopic sarcocysts. The specimens found positive by macroscopic and microscopic examination were fixed in 10 % neutral buffered formalin solution and further processed for histopathological studies (Avapal et al. 2004; Mahran 2009). Macroscopic sarcocysts obtained from esophagus

were dissected carefully without any rupture and were kept in normal saline for further investigation (Ghosal et al. 1988). The macrosarcocysts were measured and crushed between two slides for description of metrocytes and bradyzoites under phase contrast microscope. Micrometry was conducted for measurement of cyst wall thickness, length and width of the sarcocyst including bradyzoites and metrocytes in both fresh and stained preparations for species identification.

Results and discussion

In the present study, out of 137 slaughtered buffaloes, 91 were found to be positive for the presence of sarcocysts with an overall prevalence of 66.42 % with macrocysts found as 22.62 % and microscopic cysts as 43.79 %.

Similar infection rates were also reported earlier as 69 % in Egypt (Nahed et al. 2014), 65 % in Philippines (Claveria et al. 2000). However, a higher infection rate had been recorded in the previous reports as 87 % in India (Mohanty et al. 1995), China 94 % (Xiao et al. 1988), 100 % in Thailand Muangyai and Chalermchaikit (1988), 79 % in Vietnam (Huong and Ugglä 1999) and 82.9 % in Iraq (Latif et al. 1999), respectively, whereas a lower prevalence rate as 53.5 and 57 % in Iran (Haddadzadeh et al. 2004; Ghorbanpoor et al. 2007) was also recorded. Age wise prevalence in the present study revealed 65.16 % (58/89) of *Sarcocystis* in samples from young male calves aged between 18 and 36 months old and 68.75 % (33/48) in samples from senile she buffaloes (Table 1). The present investigation revealed slight variation in prevalence rates as 68.75 % in aged animals followed by 65.16 % in younger animals which were similar to the observations of Huong (1999b), Oryan et al. (2010), EL-Dakhly et al. (2011) and Mohammad (2012). There was no significant difference in age wise prevalence in this study, but (Huong and Ugglä 1999) opined that highest infection rates in older animals are most likely due to a long exposure to the infection. The organ wise prevalence was highest in esophageal muscles (51.82) followed by tongue (47.44 %), heart (29.92 %), diaphragmatic muscles (28.46 %) and intercostal muscles as 18.2 % (Table 2) in the present study and was in agreement with the earlier reports by Fayer and Dubey (1986), Huong and Ugglä (1999) and Haddadzadeh et al. (2004). In contrast to the present study,

Table 1 Prevalence of *Sarcocystis* infection in slaughtered buffaloes of different age groups in A.P

Age group	Sex	No. of animals examined	No. of animals found positive	%
18–36 months	Male	89	58	65.16
>5 years	Female	48	33	68.75

Table 2 Distribution pattern of *Sarcocystis* infection (macroscopic and microscopic) in different organs of slaughtered buffaloes

Organ	No. of animals found positive	% of infection
Esophagus	71	51.82
Tongue	65	47.44
Heart	41	29.92
Diaphragmatic muscle	39	28.46
Intercostal muscle	25	18.24

Table 3 Distribution pattern of macrocyst and microcyst in slaughtered buffaloes

No. of samples	Buffalo calves aged b/n 18–36 months	%	Buffaloes aged >5 years	%
Total positives	58	65.16	33	68.75
Macrocyst	12	13.4	19	39.51
Microcyst	38	42.69	22	45.83
Mixed infection	24	26.96	25	52.08

Fig. 1 Photograph showing *S.fusiformis* (macrocysts) underneath the serosal surface of esophageal muscles

Daryani et al. (2006) found the highest prevalence of sarcocystosis in the abdominal muscles of infected buffaloes rather than esophageal muscles. Gross and histopathological studies revealed the presence of macroscopic and microscopic cysts either in a single form of infection or mixed form and the most common affected organs were identified as esophagus and tongue (Table 3). Microscopic studies of histological sections showed mixed infections of *S.levinei* and *S.fusiformis* supporting that the buffaloes were frequently exposed to the infection due to close contact with dog, cat and other wild animals which acts as definitive hosts for this protozoa. The present investigation reports highest prevalence of microscopic sarcocysts (43.79 %) than the macroscopic sarcocyst (22.62 %) which were in line with the reports of Huong and Uggla (1999), Oryan et al. (2010) and El-Dakhly et al. (2011). The highest prevalence of microscopic sarcocyst strongly suggests that dogs are in close contact with buffaloes, constantly disseminate the large number of sporocysts that may contaminate the feed and drinking water of intermediate hosts.

The prevalence rates were mainly dependent on the number of sporocysts disseminated by the definitive host and their survivability in the environment, basically regulated by the climatic conditions like temperature, rain fall and humidity (Huong and Uggla 1999; Oryan et al. 2010; El-Dakhly et al. 2011). Grossly the macroscopic cysts were spindle or oval shaped and appear as milky white threads parallel to muscle fibers present underneath the serosal surface of the esophagus and in between the muscle tissue in case of the tongue. The macroscopic cysts were grossly opaque, measuring about 2–8.5 mm length and 1–3 mm width, which can be easily separated by the fine needles from the serosal surface of the esophagus and also from the tissues of the tongue (Figs. 1, 2). Contents of freshly collected cysts examined under the microscope revealed, gliding movement of bradyzoites along with round metrocytes (Fig. 3). Histological studies of hematoxylin and eosin stained tissue sections showed round-/ovoid-/spindle-shaped tissue cysts. The cysts were identified as *S.levinei* and *S.fusiformis* with prevalence rates of 43.79 and 22.62 %, respectively, and mixed infection

Fig. 2 Photograph showing fusiform-shaped sarcocysts (*S. fusiformis*) measuring about 2–8.5 mm length and 1–3 mm width extracted from esophagus and tongue muscles

Fig. 3 Fresh smear preparation of cyst fluid from macrocyst (*S. fusiformis*) showing crescent-shaped bradyzoites (arrow) and round-shaped metrocytes (arrow head) $\times 100$

(macroscopic and microscopic) was identified as 43.06 %. Micrometric studies of macrosarcocysts revealed 2–5 μm thickness of the cyst wall, and underneath this, the thick ground substance spread with oval- or irregular-shaped metrocytes with an average of 7.5/4 μm (length/width) were observed. The cysts were overcrowded with a number of bradyzoites centrifugally which were separated by septa, and toward the center, there was a sparse distribution of bradyzoites. The bradyzoites were categorized as mature and immature, based on their morphology. Mature bradyzoites were slender and crescent shaped with one sharply pointed end and measured about 11.5/2.5 μm ($N = 30$). Immature bradyzoites were banana shaped with blunt ends with an average of 8/3 μm ($N = 30$). Based on morphometry and micrometric analysis, the macroscopic cysts were confirmed as of *S. fusiformis* (Figs. 4, 5).

Microscopic sarcocysts were distributed in all types of organs viz, esophagus, tongue, heart, intercostal muscles and diaphragmatic muscles, and among these, esophagus and tongue were most frequent organs affected. These cysts

Fig. 4 Section of macrosarcocyst (*S. fusiformis*) showing cyst wall thickness of 2–5 μm width (arrow) and internal compartmentalization spread with bradyzoites (arrow head) H&E $\times 40$

Fig. 5 Photomicrograph of section of *S. fusiformis* cyst. Arrow mark showing the cyst wall and arrow head showing the metrocytes H&E $\times 1000$

were spindle shaped with tightly packed bradyzoites with slight compartmentalization ranged between 0.31 and 0.69 mm length and 0.09–0.12 mm width and have a very thin cyst wall less than 1 μm thickness. The bradyzoites measuring with an average of 6.25/2.5 μm (length/width). Based on the size of cyst, wall thickness and bradyzoites, the microscopic cyst was identified as *S. levinei*. Morphometry and micrometric analysis was compared with the descriptions of previous reports and strongly supports that they are *S. fusiformis* and *S. levinei*. The cyst characters of *S. fusiformis* and metrocytes in the present study are corroborate to the findings of Kan and Dissanaik (1978), Ghosal et al. (1988), Arafa et al. (2003), Metwalley et al. (2013) and Dubey et al. (2014). However, the measurements of crescent-shaped and banana-shaped bradyzoites were slightly smaller when compared to above reports. Similarly, cyst characters and bradyzoites of *S. levinei* were in accordance with Dissanaik and Kan (1978) and Metwalley et al. (2013) and were in contrast to Claveria et al. (2000) and Arafa et al. (2003) who reported the sarcocysts with thick cyst walls of

2.3–2.75 μm in Philippine water buffaloes and 4–7 μm in Assiut Governorate buffaloes. The present study revealed all cysts with <1 μm thickness only (Figs. 6, 7).

Fig. 6 Unstained microscopic sarcocyst (*S.levinei*) in trichnoscopic method distributed in muscle tissues $\times 100$

Histopathologically, both forms of sarcocysts revealed severe congestion of blood vessels consistently in all the positive samples. Degenerative changes like cloudy swelling of myocytes and hyalinization of muscle fibers along with infiltration of mononuclear cells, predominantly eosinophils, were observed. Presence of elongated sarcocysts and numerous bradyzoites in between the muscle bundles was noticed. Atrophy of few myocytes and focal areas of necrosis surrounding the cysts were also seen in the present study (Figs. 8, 9, 10). The findings were in accordance with earlier reports of Foggin (1980), Mohanty et al. (1995) and Singh et al. (2004) who observed inflammatory cellular infiltration in *S.fusiformis* and *S.cruzi* along with loss of striations and mild hyalinization of myofibrils in buffaloes and cattle, respectively. However, ruptured macrocysts releasing merozoites and necrotic centers infiltrated by inflammatory cells predominantly eosinophils in *S.gigantea* in sheep were reported by Al-Hyali et al. (2011). The inflammatory reactions and hyalinization of muscles can deteriorate the quality of the

Fig. 7 Muscle sections of **a** tongue and **b** heart revealing microcyst (*S.levinei*) with thin cyst wall (<1 μm) and with tightly packed bradyzoites $\times 100$

Fig. 8 Photomicrograph showing **(a)**. *S.fusiformis* (macrocyst) in esophagus with degenerative changes in the surrounding muscle fibers $\times 1000$ **(b)**. t-shaped bradyzoites released between muscle fibers and

hyaline degeneration of muscle fibers in heart with microcyst (*S.levinei*) H&E $\times 1000$

Fig. 9 Photomicrographs showing **a** cysts of *S.levinei* in heart muscle H&E $\times 100$. **b** Elongated cyst of *S.levinei* in tongue showing degenerative changes in the surrounding muscle fibers H&E $\times 1000$

Fig. 10 Photomicrograph showing necrosis, infiltration of eosinophils and mononuclear cells in sarcocystosis infected muscles H&E $\times 1000$

meat and that can be attributable to the toxins released from the cysts (Singh et al. 2004).

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