

Sarcocystis and sarcocystosis in India: status and emerging perspectives

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Abstract *Sarcocystis* spp. are a group of tissue cyst-forming coccidia which infect a vast range of animals as well as human beings. Found frequently in animal carcasses at slaughter, undermining their value, they have also been found associated with clinical disease. Dogs and cats are involved in the transmission. Studies in India point to a vast reservoir of infection with high prevalence rates in various livestock species. However, there is a glaring paucity of reports on the horse and *Sarcocystis* of the camel has remained totally unexplored so far. At least two different *Sarcocystis* spp. can parasitize each livestock host species. Experimental transmission studies have provided additional parameters for distinguishing the species. The clinical symptoms are generally non-specific and diagnosis in the living animal, by the presently available means, is almost impossible. Immunodiagnosis till now is beset with problem of cross-reactivity. Treatment with anti-coccidials presently tried do not seem satisfactory. Of the two zoonotic species with cattle-man and pig-man cycles, only the latter seems of some significance in India due to backyard pig-rearing and slaughter practices. It is a paradox that despite high prevalence of *S. suihominis* in pigs, reports of human cases are limited. This and some of the existing grey areas of information in the Indian context, have been highlighted as also possible directions for future research.

Keywords *Sarcocystis* · Sarcocystosis · Coccidia · Dog · Cat · Zoonosis

Introduction

Sarcocystis, originally described from the muscle of the pig, was recognized only as a common parasite in the musculature of herbivores for nearly a century. It was not until 1972 that its obligatory two host life cycle was unravelled and helped to identify it as a group of faecally-transmitted coccidians involving herbivores as intermediate hosts and carnivores as the definitive hosts (Dubey et al. 1989a). Since then, there has been a vast upsurge of interest and accumulation of information. Broadly, it has been found that each of the livestock species has two or more types of *Sarcocystis* parasitizing them depending on the specific definitive host (carnivores like dog, cat and man) utilized for the completion of its life cycle. Their clinical and zoonotic significance has increasingly emerged in the last three decades. During this period, a large number of studies have been carried out at various centres in India, but reviews on this subject have dealt mainly with epidemiological or zoonotic aspects (Shah 1990; Juyal 1991; Shah 1995). As such, a review aimed at providing a comprehensive update of this important parasitism from an Indian perspective, seems justified.

Epidemiology

Unlike previously known coccidia, *Sarcocystis* develops gamonts and oocysts in the lamina propria of the carnivore definitive hosts. These oocysts get sporulated in situ and sporocysts are passed in the faeces of these hosts (dog, cat,

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man). Earlier workers observed the faecal sporocysts in natural infections but identified only up to the level of genus. *Sarcocystis* was detected as part of coccidia and helminths in faeces of domiciled cats (Chhabra et al. 1984) and dogs (Singh et al. 1987). The latter reported high prevalence in stray dogs which had easy access to the source viz. slaughter house and meat shops. Likewise, Shastri (1989) found an overall sporocyst presence in 67.79 % of 385 dog samples in Parbhani town (Marathwada), higher (76.50 %) from outskirts areas as against only 28.57 % in mainly domiciled dogs from the town's interior. On the other hand, Mamatha et al. (2006) could detect sporocysts in only two of 140 faecal samples from dogs and none from 100 cats in metropolitan Bangalore. Close association with dogs was also considered a factor in the transmission of *Sarcocystis* infections in ruminants (Venu and Hafeez 2000). *Sarcocystis* spp. manifest rigid host specificity for their intermediate hosts as indicated by the failure of cross-transmission even between closely related species like cattle and buffalo. In experimental studies, attempts to transfer *Sarcocystis capracanis* of goats to sheep (Pethkar and Shah 1982) failed. Likewise, large number of sporocysts of *S. cruzi* of cattle origin fed to naïve buffalo calves (Jain and Shah 1985a) and similar attempt on *S. fusiformis* and *S. levinei* of buffalo origin fed to cow calves (Ghoshal et al. 1987a) were unsuccessful. Prevalence in adult animals appeared much higher than young (<1 year) ones (Devi et al. 1998; Swarnkar et al. 1999; Srinivasa Rao and Hafeez 2002a). Highest prevalence was observed during monsoon/post-monsoon or even winter months depending on location (Jain and Shah 1985b; Mohanty et al. 1995a; Devi et al. 1998; Swarnkar et al. 1999), which is apparently related to the availability of moisture favouring the development and survival of the faecal sporocysts voided by the definitive hosts. The effect of a range of temperatures on the viability of *S. levinei* from cardiac muscle of buffalo (Srivastava et al. 1986) and *S. miescheriana* in pork (Saleque et al. 1990) indicated that infectivity was not affected except by extremely high or extremely low temperatures, which for *S. capracanis* was found to be 100 °C and –20 °C, respectively (Singh and Shah 1990a).

Cattle

The prevalence reported from various states was highly variable viz. 75.3 % of 1,030 animals in Bihar (Sahai et al. 1982), 80.3 % in Madhya Pradesh (Jain and Shah 1985b), 80.3 % of 86 in Orissa (Mohanty et al. 1995a) and 58.74 % of 143 from Tirupati in Andhra Pradesh (Venu and Hafeez 2000). These workers surveyed cattle as part of the overall prevalence of *Sarcocystis* in ruminants and as such, either did not identify the species or considered it solely as the dog-transmitted *S. cruzi* (Venu and Hafeez 2000).

Buffalo

According to Gill et al. (1978), *Sarcocystis* is the most common protozoan infection in the buffalo (*Bubalus bubalis*) and in general, the prevalence rate is higher than in cattle (Ramanujachari and Alwar 1951). Most subsequent workers confirmed this trend in their observations: 86.6 % of 60 examined in Orissa (Mohanty et al. 1995a), 79.9 % of 169 examined in A.P. (Venu and Hafeez 2000), with Sahai et al. (1982) who found 59 % positive of 283 buffaloes in Bihar, being an exception. In M.P., natural occurrence of *Sarcocystis* was recorded in 80 % buffalo carcasses (Ghoshal et al. 1986) with *S. levinei* as the predominant species in Marathwada (Deshpande et al. 1983a) as also in Bihar (Saha et al. 1986).

Goat

Chhabra and Mahajan (1978) observed *Sarcocystis* in the oesophagus and diaphragm tissues of slaughter goats by trypsin digestion in the course of attempted isolation of *Toxoplasma* at Chandigarh. At Hisar, Haryana, Gupta and Gautam (1982) found 55 % positivity by pepsin digestion of muscles from 152 goats. In M.P. Pethkar and Shah (1982) recorded 66.46 % positive in 650 goat carcasses, of which the dog-transmitted *S. capracanis* was predominant (97.20 %). A later report from Hisar (Juyal et al. 1989a) found microcysts identical to *S. capracanis* in 62.6 % of 235 slaughter goats. Other reports documented an alarming 100 % *S. capracanis* in 35 goats in Marathwada (Shastri 1990), 67.10 % of 76 at Jabalpur, M.P. (Singh et al. 1990), 33.33 % of 80 goats in Tripura (Saha and Ghosh 1992), 68.3 % of 703 from Bikaner, Rajasthan (Singh et al. 1991), 73.33 % in Uttar Pradesh (Daya Shankar and Bhatia 1993), 56.18 % in Maharashtra (Wadajkar et al. 1994), 76.6 % of 120 goats in Orissa (Mohanty et al. 1995a), 55.26 % (Aulakh et al. 1997) from Punjab, 34.62 % of 800 goats at Nagpur (Jumde et al. 2000), and 72 % of 111 goats in A.P. (Venu and Hafeez 2000).

Pig

Sarcocystis infection in the musculature of slaughter pigs was reported from various centres. The prevalence of the dog-transmitted microcysts (likely *S. miescheriana*) was 53.5 % of 170 pigs in Bihar (Sahai et al. 1982), 68.8 % at Hisar (Gupta and Gautam 1984), 68.98 % in slaughter pigs in western U.P. (Agnihotri et al. 1987; Saleque and Bhatia 1991) 84 % in M.P. (Solanki et al. 1991a), 76.1 % of 372 in Guwahati, Assam (Devi et al. 1998), 61.48 % of 270 in A.P. (Srinivasa Rao and Hafeez 2002a), 73.36 % of 229 pigs at Ludhiana (Avapal et al. 2003a, 2004), Prasanth (1995) reported prevalence of two species viz.

S. sui hominis (48.9 %) and *S. miescheriana* (40.2 %) from Western U.P. Occasionally spontaneous *Sarcocystis* were also detected in the brain of pigs (Gupta and Iyer 1984; Prakash et al. 2007).

Sheep

Prevalence of *Sarcocystis* infection in sheep has been reported from various places viz. up to 81.15 % in Haryana (Gupta et al. 1979; Hussain et al. 1986) and elsewhere ranging between 19.73 and 73.40 % in A.P. (Rao and Rama Rao 1987), 71.87 % in U. P. (Saleque et al. 1992), 81.37 % in Orissa (Mohanty et al. 1995a), 74.3 % in Western U.P. and Delhi (Banerjee 1998), 37.3 % in Rajasthan (Swarnkar et al. 1999) and 88.32 % in Tirupati, A.P. (Venu and Hafeez 2000). Majority of these investigators regarded *S. tenella* as the only or the predominant species identified, whereas Saleque et al. (1992) assigned them to two species viz. *S. tenella* and *S. arieticanis*, both dog-transmitted, on morphological grounds.

Horse

The first report in India on the occurrence of *Sarcocystis* as sparsely distributed white bodies in the oesophageal and heart muscles of a pony, came from Madras (Achuthan and Raja 1990), but the species was not identified. The first confirmed case of *S. equicanis* was recorded in Punjab (Juyal et al. 1991). Chronic sarcocystosis in a naturally infected mare was reported later (Juyal et al. 1994).

Man

Sporadic human infection has been recorded incidentally during autopsy (Vasudevan 1927; Naidu 1928). The first documented case of *Sarcocystis* cysts diagnosed by histological examination of biopsy pieces from calf and deltoid muscles during life, came from Lucknow (Gupta et al. 1973). Ten years later, again from Lucknow, two more cases from living humans, were reported (Agarwal and Srivastava 1983). Both were presented with discharging sinuses, one on the lower extremity and the other, gluteal, but the presence of cysts was unrelated to the symptoms. The source of infection was not clear but appeared to be faecal or soil contamination of food. In these two instances, the species is likely to be *S. lindemanni* for which man is the intermediate host (Dubey et al. 1989a) and definitive host is not known.

Biology and speciation

Generally, microsarcocysts (<1 mm) belong to such species of *Sarcocystis* which have the definitive hosts as

canids or humans (and possibly other primates), while the species having macrosarcocysts (>1 mm) involve felids as definitive hosts (Bhatia et al. 2010). Early investigators either did not identify beyond the genus level or relied on morphological criteria of cysts to differentiate between the species.

Cattle

Deshpande et al. (1983a) regarded the thin walled cysts as *S. cruzi* and the thick walled as *S. hirsuta/S. sui hominis*. Of these three, *S. cruzi* is dog-transmitted and has been found to be the predominant species, 66.81 % out of the overall 83.2 % positivity in M.P. (Jain and Shah 1985b, 1987). The other two species, *S. hirsuta* and *S. hominis*, both have thick radially striated walls but have different definitive hosts viz. cat and man, respectively. The comparative morphology of *Sarcocystis* of the three species has been described (Pandit et al. 1994). In experimental infections of *S. cruzi*, the pattern of discharge of sporocysts in dog faeces was reported (Jain and Shah 1986b). Heart muscles from cattle fed to pups and kittens (Sahai et al. 1983) resulted in shedding of sporocysts by pups only in 12.0–15.3 days (prepatent period) but the authors did not name the species. For *S. cruzi*, Jain and Shah (1986b) recorded the prepatent period of 9–10 days post infection (DPI) and patent period as 18–85 DPI, while Venu and Hafeez (1999) found the prepatent period as 9–11 DPI.

Buffalo

The gametogenous development of bubaline *S. fusiformis* in pups in an experimental study (Chauhan et al. 1977) was reported. The first sporocyst appeared in faeces after 18–21 days and shedding continued for >140 days. On histological examination, the parasites were located mainly in the sub-epithelial tissues of the villi of the jejunum and upper ileum. On the other hand, Gill et al. (1978) fed diaphragm muscles from buffalo naturally infected with *Sarcocystis* but observed shedding of only unsporulated *Isospora* oocysts and no sporocysts. Transmission studies carried out at Madras (Achuthan 1983) indicated that cat was the definitive host for at least one species in buffaloes. *Sarcocystis* from buffalo was fed orally to three cats which started shedding of sporocysts in the faeces 18–21 days onwards. A buffalo calf fed with these sporocysts became ill 38 DPI and died 77 DPI. The parasite was identified as *S. fusiformis*. Feeding of naturally infected bubaline tissues/sarcocysts of *S. levinei* was found infectious to pups (Deshpande et al. 1982; Jain et al. 1985) which shed sporocysts in faeces. In another study, gametogenous development of *S. levinei* in the small intestine of dogs (Jain and Shah 1986a) was described. Buffalo-dog-buffalo cycle was

further established by ensuing studies at Jabalpur (Ghoshal et al. 1987b, 1988). *S. levinei* was by far the commonest bubaline *Sarcocystis* species with predilection for oesophageal tissues and the patent period for sporocysts output in dogs was 15–74 days after being fed infected muscles (Ghoshal et al. 1988). Prevalence and intensity of *S. fusiformis* in Tarai region of U.P. was reported (Juyal and Bhatia 1989a). According to Dubey et al. (1989b), *S. fusiformis* forms large (up to 32 mm) sarcocysts in the oesophagus of buffaloes. In their opinion, the ultrastructure of sarcocyst wall is a useful criterion for distinguishing the species. As per Venu and Hafeez (1999), the prepatent period of *S. levinei* is 11–14 days. Large numbers of sporocyst output translates to enormous environmental contamination with this species in nature.

Goat

When Pethkar and Shah (1982) had reported the occurrence of two species in goats, the predominant microcysts were identified as *S. capracanis*, but the marginally present species which failed to develop in dogs and cats, was left unnamed. The assumption that man could be the definitive host of the species was tested experimentally (Chaudhary and Shah 1988) with the senior author volunteering to act as “guinea pig”, and swallowing, on two separate occasions, sarcocysts, but no sporocysts were recovered in his stools in 20 consecutive DPI. Thereby, it was concluded that the particular species did not utilize man as its definitive host. The prepatent period of *S. capracanis* in experimentally infected pups was found 9–15 (mean 12.25) days (Juyal et al. 1989a). Similar observations were recorded by Singh and Shah (1990b) on feeding offals from slaughter goats to dogs with 9 and 24 DPI as the prepatent and patent periods, respectively. Later, these authors (Singh et al. 1990) found high concentration of *S. capracanis* cysts in oesophagus and tail muscles of naturally infected goats, the latter site being suitable for detection in live animals. Diaphragm muscle was also commonly parasitized (Shastri 1990; Daya Shankar and Bhatia 1993; Wadajkar et al. 1994) and cardiac muscle next in frequency (Juyal et al. 1989a; Saha and Ghosh 1992). Evidence of occurrence of *S. hircicanis* in India was first provided by Srivastava et al. (1990) by morphological differentiation alone. More conclusive evidence came forth from Jabalpur (Sharma and Shah 1992) who not only reported *S. hircicanis* cysts having thin wall with hairy processes as against thick, radially striated wall of *S. capracanis*, but also on longer prepatent period (12–15 days as against 9 of *S. capracanis*) and larger size of sporocysts of *S. hircicanis*. Elsewhere, Daya Shankar and Bhatia (1993) from U. P. and Wadajkar et al. (1994) from Maharashtra, identified two species on the basis of cyst wall morphology viz.

S. capracanis and *S. hircicanis*. Venu and Hafeez (1999) recorded prepatent period of *S. capracanis* as 9–10 days. Dafedar et al. (2008) recorded prevalence of *S. capracanis* and *S. hircicanis* as 72.24 and 21.12 % respectively at an abattoir in Bangalore, Karnataka.

Pig

Pig has been shown to harbour three species of *Sarcocystis* utilising different definitive host species. Dog is the principal definitive host of *S. miescheriana*. Musculature from pigs fed to clean dogs resulted in excretion of sporocysts (Gupta and Gautam 1984) with a prepatent period of 12 days. These workers, however, did not name the species. It was left to a team at Jabalpur (Solanki et al. 1990) to work out the endogenous phase of the life cycle of *S. miescheriana*. In experimentally infected piglets, first generation schizonts were seen on 7th DPI in the endothelial cells of the capillaries of the liver only. Second generation schizonts were encountered on 12 DPI in the endothelial cells of arterioles and capillaries of heart, liver and kidney. Partially matured sarcocysts were seen on 90 DPI and fully mature on 120 DPI in muscles, maximum in the penis muscles and minimum in diaphragm; The maturity of sarcocysts was confirmed by feeding them to dogs which started shedding sporulated sporocysts in their faeces from 9 DPI. The gametogonous and sporogonous phase of the life cycle of *S. miescheriana* was elucidated in experimentally infected dogs (Solanki et al. 1991b); All these stages were located inside parasitophorous vacuoles in lamina propria of jejunum and ileum of small intestine. The prepatent and patent periods in dogs were 9–10 and 39–40 days, respectively. Comparative morphology of sarcocysts of *S. miescheriana* and *S. suihominis* collected from naturally infected pigs was described, along with their metrocytes and cytozoites (Solanki et al. 1991c). From slaughter pigs at abattoirs in U. P., incidence of both the porcine species was identified (Saleque and Bhatia 1991). Prasanth and Bhatia (1996) reported the species of *Sarcocystis* of the pig in western U.P. and described the sporocyst of a till-then unrecognized species. Devi et al. (1998) reported *Sarcocystis* spp. in carcasses of pigs in Assam by feeding sarcocysts to cats and dogs and observation of the sporocysts shed. They identified *S. miescheriana* and *S. suihominis*. The comparative morphology of these two species was later described by Avapal et al. (2003b).

Sheep

Hussain et al. (1986) observed infection in sheep which they designated as *S. ovisanis*. Later, Saleque et al. (1992) recorded prevalence of two species in U.P. and identified them on morphological grounds as *S. tenella* and

S. arieticanis. Swarnkar et al. (1999) found only *S. tenella* in sheep at an organized farm in Rajasthan. The most commonly involved muscles were diaphragm and oesophagus (Gupta and Gautam 1982; Saleque et al. 1992). Sporocyst output in dogs fed with sarcocysts of *S. arieticanis* and *S. tenella* was observed by Shrivastava and Jain (2001). In case of *S. arieticanis*, dogs started shedding sporocysts from day 10 to 12 PI, reached peak on 11 DPI and ceased from 21 DPI. Total sporocysts shed were estimated at 2.4×10^6 . In *S. tenella* infected dogs, prepatent period was 10–12 days and patency took, another 8–12 days.

Horse

Juyal et al. (1991) recorded *S. equicanis* from the oesophageal and diaphragmatic muscles of a naturally infected mare and confirmed the identity by feeding the muscles to dog which shed sporocysts from 7 DPI till it was killed on 19 DPI.

Clinico-pathology

In contrast to their high rate of prevalence, *Sarcocystis* spp. were initially considered to be of doubtful pathogenicity. This view has since changed and studies have shown it to produce clinical disease with morbidity and mortality in livestock (Achuthan 1983). This author found that experimentally infected buffalo calf became ill 38 DPI and died 77 DPI with impression smears/sections from heart, oesophagus and tongue showing developing forms of *Sarcocystis*. Biochemistry of *S. fusiformis* of buffalo, covering total lipids, phospholipids, cholesterol, fatty acids and glycerides, total proteins, acetylcholinesterase, glutamate oxalo-acetate transaminase and glutamate-pyruvate transaminase in sarcocyst was reported (Chaudhary et al. 1985). In a subsequent paper (Chaudhary et al. 1986), the glycogen content and activities of alkaline and acid phosphatases of sarcocysts of *S. fusiformis* from naturally infected buffalo were determined biochemically and histochemically. They also observed that sarcocysts are well demarcated from the host tissue by a thick wall. The presence of alkaline phosphatase activity on this wall suggested that it was metabolically active. Hussain et al. (1986) described pathological changes in 5–9 weeks old lambs experimentally infected with the highly pathogenic *S. ovisanis*, 0.5–1.0 million sporocysts orally. All the lambs died 25–31 DPI with widespread and severe hemorrhages in all internal organs. Before dying, the lambs became anorectic and weak. On the other hand, the only pathology noted by Rao and Rama Rao (1987) in infected cattle and buffalo, was inflammatory response appearing as mild degenerative changes of the cell cytoplasm at the cyst

lodgement site. Clearly, the pathogenic effects are dose-related, as observed by Juyal et al. (1989b) in experimentally induced *S. capracanis* infection in pregnant goats. A dose of 5,000 sporocysts produced no clinical signs, 10,000 produced clinical disease characterized by two peaks of fever 12 and 20 DPI, pale mucous membrane and premature delivery in one of the three goats, 25,000 sporocysts caused more pronounced clinical effects as well as stillbirth in two of three goats. In the goats given 50,000 sporocysts, severe disease with premature expulsion of dead foetus or weaklings that died within 24 h, resulted. Cyst extracts of *S. fusiformis* of buffalo were toxic to inoculated laboratory rabbits and mice (Saleque et al. 1991). In experimentally infected pigs, *S. miescheriana* caused pathogenic effects mainly associated with 2nd generation schizonts (Solanki et al. 1992). Inflammatory response accompanied with gross and microscopic lesions were observed in different organs viz. heart, liver, lung, brain and spleen. A special feature was the inflammatory changes in the penile urethra of the male animals. Further clinico-haematologic and serum biochemical changes in *S. miescheriana* infected piglets were reported by Dey et al. (1995). The piglets suffered acute post-oral inoculation with $10/15 \times 10^5$ sporocysts and 4 of 12 died 16–19 DPI. The clinical symptoms observed were fever (up to 41.5 °C), anorexia, dullness, staggering gait, lacrimation and severe anaemia. Haematologically, decreased PCV and TEC, neutrophilia, lymphopenia and monocytosis were observed between 7 and 35 DPI. Total protein and albumin values were decreased whereas globulin values increased. The values of creatinine, blood urea nitrogen and bilirubin increased markedly. Wadajkar et al. (1995) observed clinical signs in kids orally inoculated with graded doses of *S. capracanis* which included two febrile peaks and death of all the six kids in 13–23 DPI. At necropsy, extensive petechial haemorrhages were observed on skeletal muscles, serosal surface of the alimentary canal, lymph nodes, urinary bladder and epicardium. Haemorrhagic lesions on trachea, aorta and auricles were consistent features in all the kids. Microscopic lesions consisted of severe congestion of blood vessels including capillaries, sinusoids and sub-endothelial haemorrhages. Pathology of *Sarcocystis* in natural infections in cattle, buffalo, sheep and goat, was reported by Mohanty et al. (1995b). In general, heavily infected animals were weak, debilitated and emaciated with pale mucous membranes, ascites, lymphadenopathy and in some cases, loss of hairs from switch of tail, particularly in cattle and buffalo. Histopathology in cattle revealed numerous cut cysts in cardiac and tongue muscles with loss of striation of some spots and hyalinization of infected muscle fibres considered confirmatory of *S. cruzi*. In buffaloes, the muscle around immature cysts showed loss of striation, vacuolation and fragmentation. The

affected muscles were also infiltrated with aggregation of inflammatory cells predominantly lymphocytes. Intramuscular oedema was also present. The findings indicated the high pathogenicity of *S. fusiformis*. In sheep, infected tongue and oesophagus showed loss of striation, oedema and infiltration of inflammatory cells along with rupture of some muscle fibres contrary to the earlier report of Hussain et al. (1986), severe haemorrhages in internal organs, were not observed in any of the sheep. In a later study on pathogenesis of *S. tenella*, Banerjee (1998) observed clinical signs of fever, severe anaemia and neurological symptoms in infected sheep. Haemorrhagic lesions were seen in the sites of development of schizonts viz. endothelial cells of internal organs, including liver, kidney, CNS, cardiac and skeletal muscles, spleen, mesenteric lymph nodes and lungs. These changes were coupled with significant rise in the total protein concentration and cell counts in CSF were constant features in acutely infected lambs. The LD₅₀ was 25,000 sporocysts. Pathological changes in natural *Sarcocystis* infected pigs were essentially similar viz. hidebound condition, emaciation and haemorrhages on the serosal surface of the large intestines and viscera including lungs (Avapal et al. 2004). Heart surface also showed haemorrhages, congestion and atrophy of fat which imparted a grey gelatinous appearance in a number of cases. In a slaughter house based study on cattle (Patra et al. 2006), infected muscles showed inflammatory reaction with necrosis, congestion, myositis and disorganization of muscle fibres. Histopathologically, villar projections of the cyst wall were observed. The chambers were uniform in size with a clear cord like structure. Sporozoites were aggregated at the junction of the central and peripheral area. Although not recorded so far in India, the discovery of *S. neurona* as the primary cause of equine protozoal myeloencephalitis (EPM) is a major recent development that has captured the imagination of researchers abroad (Dubey 2001).

Diagnosis

Since no stage of these intracellular protozoa comes out of the infected/diseased intermediate host, diagnosis in the living animal is not easy. Clinical signs of *Sarcocystis* are vague and do not allow more than a presumptive diagnosis. In the acute phase, these are anaemia, fever, anorexia, excessive salivation and abortion. In chronic phase, growth is retarded and there is loss of hair especially from the switch of the tail, neck and rump (Dubey et al. 1989b). Therefore, biopsy from tail muscle has been suggested as a means to monitor the development of sarcocysts in *S. capracanis* in live animals (Singh et al. 1990). At post-mortem, various techniques viz. peptic digestion for 2 h,

histological examination and teasing out of cysts from musculature in NSS, have been variously assessed and compared (Juyal et al. 1988, 1989c; Avapal et al. 2003a). Like in many other parasites, immunodiagnosis has also been tried. Evidence of cell-mediated immunity was found in kids experimentally inoculated with *S. capracanis* sporocysts (Juyal et al. 1989d). Delayed type of hypersensitivity as well as antigen induced inhibition of migration of leucocytes was noticed on 28 DPI. Double immunodiffusion and single radial immunodiffusion for detection of antibody responses against cystic antigen of *S. fusiformis* (Juyal et al. 1990), preparation and assessment of partially purified antigens of *S. cruzi* and *S. hirsuta* for immunodiffusion, immuno electrophoresis (IE) and precipitation tests to detect serum antibodies (Pandit et al. 1993), and attempts to detect seroprevalance in swine (Avapal et al. 2002) have not been satisfactory due to cross-reactivity, not only with other *Sarcocystis* spp. but even related genera like *Toxoplasma* and *Hammondia*. In serodiagnosis, high sensitivity of dot-ELISA test as compared to double immunodiffusion and counter immunoelectrophoresis (CIE) was reported in serum sample of cattle in Punjab (Singh et al. 2004). Serodiagnosis in buffaloes was performed (Rohini and Hafeez 2005) with three tests viz. gel diffusion, IE and CIE, of which CIE gave the highest (90 %) sensitivity. Persistence of serum antibodies to *S. neurona* could be detected in horses many years after their relocation from the endemic North America to India (Brown et al. 2006). Recently a molecular technique PCR has been used for species specific identification of *Taenia* spp. cysticerci and *Sarcocystis* cysts from infected pigs and cattle (Gonzalez et al. 2006) opening up potential new approach for species specific diagnosis of sarcocystosis.

Control and treatment

The key to control is in the interruption of the life cycle (Bhatia et al. 2010) by preventing the carnivore definitive hosts from: (a) eating raw meat of offals of slaughtered/dead animals, and (b) contaminating the feed and water of livestock with their faeces. For chemotherapy, anticoccidials are recommended in a general way and have been tried in a few instances. Sulphadiazine, Bifuran (a combination of nitrofurazone and furazolidone) at 10 mg/kg and pyremethamin at 1 mg/kg b. wt. were ineffective in treating pups experimentally infected with *S. levinei* (Deshpande et al. 1983b). Salinomycin at 5 mg/kg b. wt. was found effective in experimental sarcocystosis (*S. capracanis*) in goats (Kumar et al. 1988) as well as at 4 mg/kg b. wt. in dogs. On the other hand, dogs treated with sulphamethoxazole and trimethoprim showed no response. Singh et al. (1990) used 20 % amprolium in experimentally

infected pups and observed reduction in sporocysts per gram (SPG) counts in the faeces. Oral amprolium at 100 mg/kg b. wt. and Maduramycin at 150 mg/kg b. wt. given to pups experimentally infected with *Sarcocystis* infected pork, for 5 days starting from the day when the faecal sporocysts were first sighted, resulted in 93.93 and 96.96 % reduction in SPG, in the respective treatment groups (Srinivasa Rao and Hafeez 2002b).

Zoonotic aspects

Only two species, *S. hominis* and *S. suihominis* infect humans as definitive hosts with cattle and pigs, respectively as the intermediate hosts (Dubey et al. 1989b). However, man is unique in being the definitive host for these species as well as intermediate host for some other. The relatively low prevalence of *S. hominis* in India can be attributable to human factors viz. non-consumption of beef on religious grounds by majority Hindu population and the habit of thorough cooking among the minority beef-eaters (Shah 1990). On the other hand, the prevalence of *S. suihominis* is high (Solanki et al. 1991c; Saleque and Bhatia 1991; Prasanth 1995), which is not unexpected given the backyard rearing of pigs with opportunity for scavenging of human faeces in the slums as well as unhygienic slaughter, handling and consumption of pork by slum dwellers (Shah 1995). Evidence of *S. suihominis* infection in humans was provided by a report from Pantnagar (Banerjee et al. 1994). These workers found positivity in stool samples of 14 out of 20 children belonging to families who slaughter pigs without any meat inspection procedure. Further, these children had the history of consuming raw pork and passed typical sporocysts in stools, with frequent diarrhoea and abdominal pain, which may not be exclusively due to intestinal sarcocystosis. However, such reports are isolated and human intestinal sarcocystosis appears under-reported in India. Cases of muscular or extra intestinal sarcocystosis among humans, as recorded, have been mentioned in the aforesaid but their clinical significance is unknown. Sarcocystosis has been regarded in India as an emerging zoonosis (Juyal and Bhatia 1989b) but has remained primarily of academic interest only.

Conclusion

Sarcocystosis has been regarded as an emerging disease largely because most of the information on this group of parasites, particularly the life cycles and association with disease, is recent. Much of what we know of sarcocystosis in India relates to prevalence which is high by practically all accounts. The species identification based on cyst

morphology and without transmission studies, remains empirical. The problem is further compounded by the knowledge that more than one species of *Sarcocystis* in an intermediate host can cycle through a given definitive host. For example, in sheep there are four species, two each transmissible via dogs and cats. As of present status, dog is the final host of at least one species each prevalent in cattle, buffalo, goat, pig and sheep in India and for some other, cat is the definitive host. This merits to be considered against the background that there are at least 21 species of *Sarcocystis* that are found in dog faeces, and at least ten in cat faeces (Dubey 2009). There are issues which need to be addressed by future research. It would be of interest to look for the species considered non-existent in India so far. In this context, looking out for *S. neurona*, the pathogenic species in the horse should be a priority. *Sarcocystis* of the dromedary camel (*Camelus dromedarius*) reported from other camel-rearing countries (Latif and Khamas 2007), has not been explored in India despite a sizeable camel population. There is variation in prevalence rate in the striated and cardiac musculature and to some extent, the CNS. Is this only due to predilection or more can be interpreted if such findings are consistent? Also, experimental work has indicated that only high dose of infection leads to clinical symptoms and/or mortality. How far that sort of situation prevails in nature, extent of soil/pasture contamination by sporocysts, morbidity and economic impact need to be worked out. As clinical diagnosis is difficult, application of molecular biology to devise specific diagnostic techniques is another fertile area for future development. In view of the zoonotic importance of related coccidians like *Toxoplasma* and *Cryptosporidium*, medical and public health authorities should be taken aboard to collaborate in the surveillance for the zoonotic potential of *S. suihominis*.

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