SARCOCYSTIS INFECTIONS IN PIGS OF LUCKNOW; INDIA AND OBSERVATION ON ITS TRANSMISSION TO DOGS AND CATS

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Tissues from 195 mature pigs obtained from Slaughter house/ Meat markets of Lucknow Town, adjoining Suburb Areas and Rural Areas were examined for infection with *Sarcocystis* spp. Digestion techniques revealed zoites in 28 (16.5%) sows. Infected meat was fed to laboratory-reared dogs and cats. Dogs shed sporocysts (8.3 mum X 11.2 mum) 12 to 14 days after infection. Cats were refractory to infection. This is the 1st time in which a definitive host has been demonstrated for species of Sarcocystis occurring in Lucknow swine. In contrast to previously reported low prevalences of Sarcocystis infections in swine, the relatively high prevalence reported here indicates that *S. suicanis* may be of importance to swine producers in Lucknow.

Key Words: Sarcocystis, Digestion techniques, Merozoites, Ficoll-Hypaque Media, Sugar floatation.

Introduction

Sarcocystis is a genus of protozoa. Species in this genus infect reptiles, birds and mammals. The name is dervived from Greek: *sarx* = flesh and *kystis* = bladder. The organism was first recognized in a mouse by Miescher, 1843. His findings were not recognized as a protest initially and the literature referred to the structures he described as "Miescher's Tubules". Incidentally Miescher's son — Johann Friedrich Miescher — discovered DNA. There are about 130 recognized species in this genus. Revision of the taxonomy of this genus is ongoing, and it is possible that all the currently recognized species may in fact be a single species or much smaller number of species that can infect multiple hosts. While the majority of the species in this genus infect mammals, about a dozen are known to infect snakes. Within the genus a number of clades have been identified. These include one that contains *S. dispersa*, *S. lacertae*, *S. mucosa*, *S. muris*, *S. neurona* and *S. rodentifelis* (Elsheikha *et al.*, 2005). Infection with this parasite is known as sarcosporidiosis. Because of initial confusion over the taxonomy of this parasite it was originally referred to as *Isospora hominis*. The older literature may refer to this organism. Human infection is considered rare with less than one hundred published cases of invasive disease. These figures represent a gross underestimate of the human burden of disease. Stool examinations in Thai laborers showed that *sarcocystis* infection had a prevalence of 23%. Virtually all cases appeared to be asymptomatic which probably explains the lack of recognition. A study of 100 human tongues obtained at postmortem in Malaya revealed an infection rate of 21%. There was no sex difference and the age range was 16 to 57 years (mean 37.7 years) as reported by Wong and Pathmanathan, 1992.

The first report of human infection was by Lindemannl in 1868. Although several additional reports were subsequently published, these early descriptions were not considered definitive. The first generally agreed definitive description of this disease was published in 1894 by Baraban and Saint-Remy. This species was named by Rivolta after Lindemannl in 1898.The pathology is of two types: a rare invasive form with vasculitis and myositis and an intestinal form that presents with nausea, abdominal pain, and diarrhea. While normally mild and lasting under 48 hours, the intestinal form may occasionally be severe or even life threatening. The invasive form may involve a wide variety of tissues including lymph nodes, muscles and the larynx.

The invasive forms were considered to belong to a single species - *S. lindemanni* - and the intestinal form due to *S. hominis* (from undercooked beef) or *S. suihominis* (from undercooked pork). The description of *S. lindemanni* has since been considered to be unsatisfactory and has been declared a *nomem nudum* (a name without a recognised species). Two species currently considered to be capable of causing human infection: *S. bovihominis* (*S. hominis*) and *S. suihominis*. Infection occurs when undercooked meat is ingested. The incubation period is 9–39 days. Human outbreaks have occurred in Europe. Rats are a known carrier. Because infection is rarely symptomatic, treatment is rarely required. There have been no published trials so treatment remains empirical. Agents that have

been used include albendazole, metronidazole and cotrimoxazole for myositis. Corticosteroids have also been used for symptomatic relief.Infection can be prevented by cooking the meat before eating. Alternatively freezing the meat at 5^0C for several days before ingestion will kill the sporocysts.

Four recognised species infect cattle: *S. bovifelis*, *S. bovihominis* (*S. hominis*) *S. cruzi* (*S. bovicanis*) and *S. hirsuta*. *S. cruzi* is the only species known to be pathogenic in cattle. Five species infect horses: *S. asinus*, *S. bertrami*, *S. equicanis*, *S. fayeri* and *S. neurona* (*S. falcatula*). All utilize canids as definitive hosts. Sheep may be infected by four recognised species of *Sarcocystis*: *S. arieticanis* and *S. tenella* (*S. ovicanis*) are pathogenic; *S. gigantea* (*S. ovifelis*) and *S. medusiformis* are non-pathogenic. Infection with these parasites is common in the US with over 80% of sheep examined showing evidence of infection as reported by Dubey *et al.,* 1988. *S. arieticanis* and *S. tenella* both produce extra intestinal disease. Anemia, anorexia, ataxia, and abortions are the chief clinical signs. Four recognised species infect pigs: *S. medusiformis*, *S. meischeriana* (*S. suicanis*), *S. porcifelis* and *S. suihominis*. *S. porcifelis* is pathogenic for pigs causing diarrhea, myositis and lameness. Infection by *S. tilopodi* of muscle tissue in the Guanaco has been reported by C. Michael Hogan, 2008.

Sarcocystis infection is common in pigs in several countries (Boch and Erber, 1981; Hindaidy and Supperer, 1979). In India Sahai *et al.* (1982) found *Sarcocystis* sp. in 53.33% of 170 pigs from Bihar, while Gupta and Gautam, 1984 reported infection in 68.8% of 157 pigs from Hisar. Khatkar *et al.,* 1992 reported 68.19% of 261 pigs from Haryana. This study reports prevalence of swine *Sarcocystis* infection in Lucknow (India) and its transmission to dogs and Cats.

Material and Method

Samples of thigh muscles from 195 indigenous and Yorkshire cross bred; healthy pigs were collected randomly in sterile disposable bags from slaughter house/meat markets of Lucknow town and its suburbs/ rural villages and were examined grossly for microscopic cysts of *Sarcocystis.* The tissues were processed immediately after procurement or preserved for 1-2 days at 4°C.

After trimming fat and fascia, about 2-4 mm of the muscular layer of the thigh muscle was taken randomly from three different places and pressed between two glass slides in such a way that a thin creamy layer was seen with the naked eyes. It was then examined microscopically at 100X magnification for Sarcocystis cysts. Those samples which were found negative by crush technique were further confirmed by muscle digestion technique.

Nine laboratory reared dogs were procured. Seven of them were fed Sarcocystis infected pork for three consecutive days @ 500 gm per dog daily in divided doses. Two dogs were kept as uninfected control. The controlled dogs were never fed pork before the experiment. The faeces of the dogs were collected daily and examined by the sugar floatation technique (Dubey *et al.,* 1972) one week prior to and 4 weeks after feeding infected meat. Flotation based on high-density solutions incorporating sodium chloride, cesium chloride, zinc sulfate, sucrose, Percoll, Ficoll-Hypaque or other such density gradient media is preferred to formalin-ethyl acetate or other sedimentation methods.

Result and Discussion

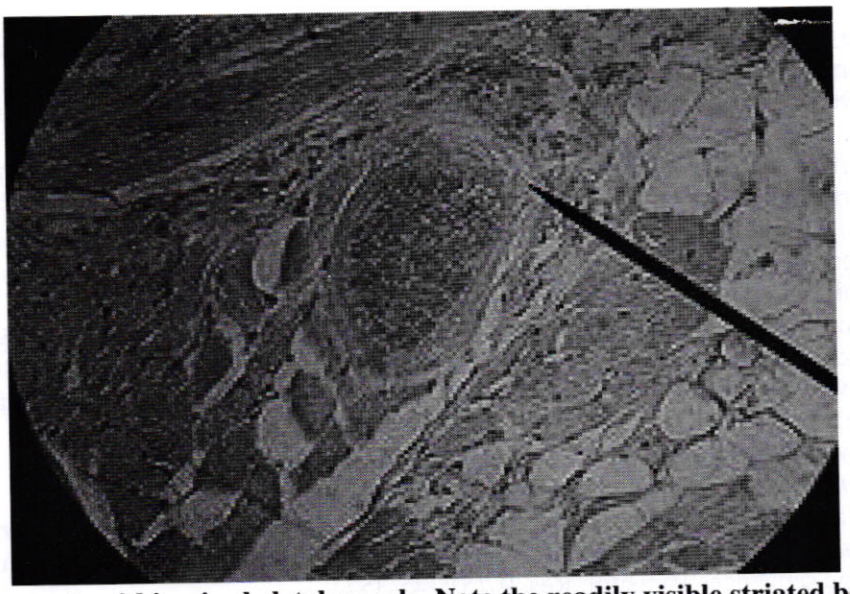
An overall prevalence rate of 68.20% of natural Sarcocystis infection was recorded. Age wise distribution indicated higher prevalence rate (79.31%) in adult pigs as compared to piglets (36%). Sexwise prevalence of Sarcocystis infection was found to be 72.5% in males and 67.50% in female pigs (Table 1).

Macroscopic cysts were not seen in the thigh muscles of pigs. Elongated microcysts were found to have several compartments containing numerous zoites. A large number of banana-shaped bradyzoites with a few immature globular metrozoites were also noticed in the digest of thigh muscle. An intermediate host such as a cow or pig when ingests a sporocyst. Sporozoites are then released in the body and migrate to vessels where they undergo the first two generation of asexual reproduction. These rounds result in the development of meronts. This stage lasts about 15 to 16 days after ingestion of sporocysts. Merozoites emerge from the second generation meronts and enter the mononucleate cells where they develop by endodyogeny. Subsequent generations of merozoites develop downstream in the direction of blood flow to arterioles, capillaries, venules, and veins throughout the body subsequently developing into the final asexual generation in muscles. Merozoites entering muscle cells round up to form metrocytes and initiate sarcocyst formation. Sarcocysts begin as unicellular bodies containing a single metrocyte and through asexual multiplication numerous metrocytes accumulate

and the sarcocyst increases in size. As the sarcocyst matures, the small, rounded, noninfectious metrocytes give rise to crescent-shaped bodies called bradyzoites that are

Table -1

Age-wise animal	**Number examined**	**Number positive**	**Percent positive**
Adult	145	115	79.31
Piglet	50	18	36.00
Total	**195**	**133**	**68.20**
Sex -wise animal	**Number examined**	**Number positive**	**Percent positive**
Male	40	29	72.50
Female	155	104	67.50
Total	**195**	**133**	**68.20**



Sarcocysts within pig skeletal muscle. Note the readily visible striated border.

All the seven dogs started shedding sporulated sporocysts of *Sarcocystis suicanis* in their faeces 12-14 days after eating infected pork. Sporocysts were not seen in the faeces of the control dogs. Once the intermediate host is eaten by the definitive host such as a dog or human, the parasite undergoes sexual reproduction within the gut to create macrogamonts and microgamonts. Most definitive hosts do not show any clinical sign or symptoms. Fusion of a macrogamont and a microgamont creates a zygote which develops into an oocyst. The oocyst is passed through the faeces completing the life cycle.

The present study showed that 68.20% pigs had Sarcocystis infection in Lucknow. No *macrocysts* could be seen in the samples examined. Similar finding had been reported by Sanchez Acedo *et al.*, 1983 and Khatkar *et al.*, 1992.

In the present study higher rate of infection was seen in adult pigs than piglets. Seneviratana *et at.* (1975) have also reported higher prevalence of Sarcocystis infection in adult pigs. The prevalence of Sarcocystis were almost in same percentage in males and females which confirm the findings of Batistic (1965). The domestic dog was confirmed to be definitive host of *Sarcocystis suicanis*. This is in agreement with the findings of Prestwood *et al.* (1980) and Gupta and Gautam (1984). Rates in pigs vary: 18% in Iowa as reported by Dubey J.P.and Powell E.C. (1994), 27% in the Philippines as elicited

by Claveria, *et al.,* 2001; 43% in Spain as highlighted by Pereira A.and Bermejo M., 1988; 57% in Uruguay, as reported by Freyre, *et al.,* 2002 and 68% in India was narrated by Saleque A., Bhatia B.B. (1991). The infection rate in sheep is commonly above 90%.(Freyre, *et al,* 2002; Latif, *et al.,* 1999 and Woldemeskel M.and Gebreab F.,1996. Camels have a similarly high incidence of infection (Latif, *et al.,* 1999). Rates above 80% are known in cattle and goats (Latif, *et al.,* 1999 and Woldemeskel M.and Gebreab F., 1996). The incidence in water buffaloes, yak and hainag exceeds 80% (Latif, *et al.,* 199); while the incidence in horses, donkeys and chickens is lower (Woldemeskel M.and Gebreab F.,1996).

Conclusion

Infection with *Sarcocystis* is common. The diagnosis is usually made *post mortum* by examination of the skeletal muscle. In some species the cysts may be visible to the naked eye (ducks, mice, rabbits and sheep) but in most microscopic examination is required. *Ante mortum* diagnosis may be made with the use of dermal sensitivity testing or complement fixation tests. Muscle biopsy is also diagnostic but this is much less commonly used. The parasite's life cycle typically involves a predator and a prey animal. A single species may infect multiple prey or predator animals. No vaccines are currently known. Experimentally inoculated pigs appear to develop a persistent immunity so a vaccine may be possible.

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