



ISSN NO. 2320-5407

Journal homepage: <http://www.journalijar.com>  
Journal DOI: [10.21474/IJAR01](https://doi.org/10.21474/IJAR01)

INTERNATIONAL JOURNAL  
OF ADVANCED RESEARCH

## REVIEW ARTICLE

### Overview of zoonotic porcine *Sarcocystis* infection in India

Mandeep Kaur<sup>1</sup> and M S Bal<sup>2</sup>

1. Veterinary Public Health & Epidemiology, KCVAS, Amritsar.
2. Animal Disease Research Centre, GADVASU, Ludhiana.

#### Manuscript Info

##### Manuscript History:

Received: 22 April 2016  
Final Accepted: 14 May 2016  
Published Online: May 2016

##### Key words:

*Sarcocystis*, zoonoses, pigs, transmission, diagnosis, molecular techniques.

##### \*Corresponding Author

Mandeep Kaur.

#### Abstract

Pig farming is very common throughout the world and large numbers of people are associated with it directly or indirectly. Pigs are also source of many zoonotic diseases especially parasitic diseases. *Sarcocystis* is one of the important coccidian protozoan parasite of pig. This parasite has broad host range and can induce infection and clinical signs in both carnivorous and herbivorous host. Humans acquire infection by ingestion of cyst or eating raw and undercooked infected meat. Three species of *Sarcocystis* viz. *S. suihominis*, *S. miescheriana* and *S. porcifelis* are found in pigs. Among these *S. suihominis* is zoonotic in nature and causes intestinal infections in humans because of consumption of raw infected pork. Pigs usually become infected by contact with contaminated human faeces. The objective of this review is to highlight importance of *Sarcocystis* in pigs and its zoonotic potential, along with recent advancement in the diagnosis from meat samples.

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#### Introduction:-

A large number of farmers in developing countries depend on animal husbandry for their livelihood. Pig farming has a higher potential to contribute to more economic gain because pigs have higher fecundity, higher feed conversion efficiency, early maturity, shorter generation interval and relatively smaller space requirement. Moreover, pig farming provides employment opportunities to rural people/farmers and help in up gradation of their living standards. It is estimated that in the coming next ten years, the total consumption of meat in India will double from its present value. Pork is one of the major meats consumed throughout the world. In India, 70% of the pig population is reared under traditional small holder, low-input demand driven production system, except for limited number of semi-commercial pig farms in Kerala, Punjab and Goa (DARE, 2014). Major drawback in the growth of the pork industry, particularly in developing countries is the health status of the pigs. In developing countries because of backyard farming, pigs tend to eat garbage and acquire many diseases. Zoonotic parasites especially; *Taenia* sp., *Trichinella* sp. and *Sarcocystis* sp. are very common in pigs. *Sarcocystis* spp. are a group of tissue cyst-forming coccidia which infect a vast range of animals (including pigs) and people as well.

*Sarcocystis* is an obligate intracellular protozoan parasite in the phylum Apicomplexa. It was first reported in 1843 by Meischer, who reported thread like cysts in striated muscle of house mouse. Life cycle is indirect, involving a definitive and an intermediate host. This parasite has a wide host range. In pigs three species of *Sarcocystis* are reported namely *S. suihominis*, *S. miescheriana* and *S. porcifelis* with their definitive hosts as man, dog and cat respectively (Dubey and Fayer, 1983). *S. miescheriana* is the most prevalent and pathogenic among these three species (Lindsay et al., 1995). *S. suihominis* is of zoonotic nature and is responsible for causing human intestinal sarcocystosis which occurs because of consumption of raw infected pork. High prevalence of *Sarcocystis* particularly of *S. suihominis* have been reported from India (Saleque & Bhatia 1991; Solanki et al 1991; Prasanth 1995; Avapal et al 2004; Kaur et al 2016) which is possibly due to unhygienic slaughter and backyard rearing of pigs with easy access to human faeces (Shah, 1995).

### Mode of Transmission:-

*S. suis* is transmitted from pigs to humans (definitive host) when human eat the infected sarcocyst containing pork. Sexual stages of parasites develop in the infected human intestine and ultimately excretion of oocysts in the faeces occurs and pig get the infection on consumption of food and water contaminated with human faeces containing oocysts. Development of asexual stages occurs in the intermediate host pig after it ingests the oocyst stage from definitive host faeces and end with the formation of intramuscular cysts in the pigs (Fayer, 2004). It has been anticipated that environmental contamination with human faeces is responsible for high prevalence of zoonotic sarcocystis infections in pigs (Kaur et al., 2016).

### Pathogenesis and clinical signs:-

*Sarcocystis* species infection is found to be maximum in adult pigs (Devi et al 1998). In general, *Sarcocystis* spp infections are considered of low pathogenicity and usually eosinophilic myositis is predominant finding in food animals including pigs (Avapal et al., 2004). Porcine sarcocystosis results in weight loss or reduced weight gain, muscle tremors, dyspnea, and abortions. Purpura of the skin, mostly on the legs and buttocks may be seen.

The common symptoms in human sarcocystosis including nausea, stomach pain and diarrhea, which depends upon the amount of cysts ingested (Fayer et al., 2015). Normally symptoms are limited to digestive disturbances but but occasionally may be life threatening (Juyal and Bhatia, 1989; Shah, 1984).

### Morphology of *Sarcocystis*:-

*Sarcocystis* are generally more host specific for their intermediate host than for their definitive hosts. The structure of the sarcocyst wall is considered as reliable criterion for distinguishing *Sarcocystis* species within a given host and *Sarcocystis* species are grouped into 24 types based on the structure of the wall (Dubey et al., 1989). Spindle shaped elongated sarcocysts, tapering at both ends with radial perpendicular papillomatous projections on the wall are seen in *S. miescheriana* (Raut and Saikumar, 2015) but in case of *S. suis* hair like villar projections on the outer surface of the cyst wall is observed (Saleque and Bhatia, 1991).

### Public health significance of *Sarcocystis*:-

Consumption of raw and partially cooked meat is the major contributing factor for human infections. Backyard pig rearing and unhygienic slaughter practices are also responsible for infections in people. *S. suis* infection is more prevalent than that of *S. hominis* in India because of non-consumption of beef due to religious beliefs (Singh et al., 2010). Intestinal sarcocystosis in humans was found more frequently in Europe than other continents because of limited surveys (Dubey et al., 1989). *S. suis* is pathogenic for man, if large dose of cysts are ingested.

Evidence of *S. suis* infection in humans was provided by Banerjee et al (1994) from Pantnagar where 14 out of 20 children were found positive for *Sarcocystis* infection. These children belong to families who slaughter pigs without any meat inspection procedure and consuming raw pork. Consumption of the raw offals with salt, including parts of the tail was the main reason of getting infection. 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.

The high prevalence of *Sarcocystis* and other intestinal parasites in the Thai laborers stool samples indicate the local habit of eating raw beef and pork, poor living conditions, and low levels of hygiene as the main cause (Wilairatana et al., 1996). In Tibet, *S. suis* were found in stools from 0 to 7% of 926 persons (Yu, 1991).

### Detection of *Sarcocystis* in pork:-

Rapid and reliable detection and identification of coccidian oocysts are essential for animal health and foodborne disease outbreak investigations (Lalonde and Gajadhar, 2011). History of consumption of raw or undercooked pork, along with signs of abdominal pain and diarrhoea are indicative of human intestinal Sarcocystosis. Detection of oocysts in the human faeces using floatation technique can be done in case of intestinal sarcocystosis. However, conventional parasitological techniques like intact cyst isolation method, pepsin acid digestion method and histopathology are used to diagnose sarcocyst in pork. Molecular techniques like conventional PCR and real time PCR are also used for diagnosis of *Sarcocystis* infection from meat samples. Serodiagnostic techniques for detection of *Sarcocystis* infection were also attempted in India but it did not give satisfactory results because of cross reactivity with other *Sarcocystis* species along with other genera like *Toxoplasma* and *Hammondia* (Avapal et al., 2002).

### 1. Rapid isolation of intact micro-*Sarcocystis* cysts from muscular tissues:-

Small pieces of 5-10 g suspected tissues are normally cut and are teased in normal saline solution (0.85%) with the help of needles and forceps for two minutes in a watch-glass. The slides are then visually screened under stereoscopic microscope (40 X) for the presence of microcysts. The prevalence of different species of *Sarcocystis* from the muscles of pigs has also been reported earlier in India from Bihar (Sahai et al 1982), Haryana (Gupta & Gautam, 1984), Western UP (Agnihotri et al., 1987; Prasanth 1995), Madhya Pradesh (Solanki et al., 1991), Assam (Devi et al., 1998), Andhra Pradesh (Srinivasa and Hafeez, 2002a) and Ludhiana (Kaur et al 2016; Avapal et al 2004). Cyst is mainly present in skeletal and cardiac muscles but occasionally, *Sarcocystis* cyst has also been detected from the brain of the pigs (Gupta and Iyer, 1984).

### 2. Pepsin digestion technique for zoites:-

To view, clear zoites, tissue samples are digested in peptic digestive solution (Jacob et al., 1960) at room temperature for two to four hours by continuous stirring. The digestive material is then passed through a strainer to remove undigested fat and connective tissue. The strained material is then centrifuged for 10 minutes at 2,000 rpm. The supernatant is decanted and the sediment are resuspended in one mililitre of normal saline and observed under microscope.

### 3. Histopathological examination:-

Histopathology is helpful in the diagnosis of muscular sarcocystosis in animals and also in man, when it becomes intermediate host. Tissues like cardiac muscles etc. should be collected in 10 per cent formal buffered saline solution for histopathology. The formalin fixed tissues are then processed by acetone benzene method (Luna, 1968) and cysts are identified based on their cell wall characteristic.

Skeletal muscles show alternate dark and light band, giving tiger striped appearance along with congestion and haemorrhages (Avapal et al., 2004). The affected muscles become flaccid and yellow. Oedema along with mild to moderate haemorrhages together with sarcocysts are also observed in the muscles. Along with this, serous atrophy of the pericardial fat (Dubey et al., 1989; Avapal et al., 2004), hyaline, vacuolar degeneration and segmental necrosis accompanied by infiltration of inflammatory cells are also reported (Avapal et al., 2004). However, non-infiltration of inflammatory cells differentiate these lesions from the segmental necrosis of metabolic and toxic myopathies (Vegad and Katiyar, 1998).

Although conventional parasitological techniques and morphological techniques can identify sarcocysts, but they are often subjective and require parasitological expertise. So their molecular characterization becomes need of time for species differentiation mainly.

### 4. Molecular studies using conventional PCR:-

Conventional PCR technique can be used for detection of *Sarcocystis* cysts from the tissue samples. Polymerase chain reaction (PCR) has tremendous potential for rapid, specific and reliable detection of food borne pathogens. It is one of the most sought methods in parasitology in recent years. The PCR studies have mostly been carried out to detect the pathogen rapidly or to confirm the presence of the organisms. PCR is carried out to amplify 18S rRNA gene of *Sarcocystis* spp. Recently Kaur et al (2016) found the 72.8% prevalence of sarcocysts from pork meat samples using the conventional PCR technique.

### 5. Real-time qPCR Assay with melt curve analysis:-

A quantitative polymerase chain reaction assay with melt curve analysis (qPCR-MCA) can be applied for the detection of coccidian species in all the tissue samples. Recently Kaur et al (2016) applied qPCR-MCA technique for detection of sarcocysts from porcine meat samples and found 191 (76.4%) positive for sarcocysts out of 250 examined using the method as described by Lalonde & Gajadhar (2011).

### Treatment:-

Currently there is no vaccine or treatment available for intestinal sarcocytosis. Generally infection is asymptomatic and self-limiting. There is a case report of surgical resection of the small intestine of six persons, presented with segmental necrotizing enteritis because of sexual stages of *Sarcocystis* and gram positive bacilli in Thailand, followed by antibiotic treatment (Bunyaratvej et al 1982).

Srinivasa and Hafeez (2002b) reported 93.93 and 96.96 % efficacy of oral amprolium at 100 mg/kg b. wt. and Maduramycin at 150 mg/kg b. wt. respectively when given to pups experimentally infected with *Sarcocystis* infected pork.

### Prevention and control:-

Thorough cooking or freezing of meat should be done to prevent intestinal sarcocystosis in humans. Cooking of meat at 70 °C for 15 minutes or freezing at -4°C and -20°C for 48 and 24 hours respectively, make the pork safe for the consumption (Saleque et al; 1990). Pigs should not be allowed to ingest the sporocyst from definitive host i.e. human faeces contaminated water, feed and bedding (Fayer, 2004).

### Conclusion:-

*Sarcocystis sui hominis* is an important zoonotic parasite of pigs. Contamination from human faeces cause the disease in pigs and on consumption of the pork of infected pig, human gets the intestinal disease. There is need of strict inspection of meat at slaughter houses and also the access of pigs to garbage eating should be checked. Along with conventional method of diagnosis of cyst in pork, molecular methods can also provide good results. Proper cooking and freezing of meat should be done to avoid infection in humans.

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