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Cytopathological and histopathological studies on *Sarcocystis* in oesophagus of goat

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Abstract
Sarcocystosis is a silent zoonotic parasitic disease caused by an obligatory intracellular coccidian protozoan of the genus *Sarcocystis* with a worldwide geographic distribution. The present investigation was carried out to record the prevalence, cytopathological and histopathological findings of Sarcocystosis in oesophagus of goat in Jaipur, Rajasthan. In this study, a total of 1115 of digestive tract samples were collected from goats, irrespective of age groups, breeds and sex. Out of the total 1115 samples, 152 representative samples of oesophagus were processed for subsequent cytopathological and histopathological examinations. The results of the present study revealed an overall prevalence of sarcocystosis as 13.63 per cent. After investigated esophagi, the goat had been infected with both types, macroscopic and microscopic cysts for the infection. During gross examination, only in four cases (0.35%) macroscopic sarcocysts noticed, in the form of many white to pale, oval fat (macro), grain sized, multifocal nodules on outer surface and in the majority of cases microsarcocysts were evident in oesophgeal muscles. In cytopathology, giemsa stained impression smears, collected from the oesophagi were found positive for microscopic sarcocysts and banana-shaped bradyzoites in clusters as well as individually. Histopathologically, the study depicted the presence of several forms of microscopic cyst, including spindle, circular, elliptical, cylindrical and twisted form in esophagus. Microscopically, these appeared as blue colour sarcocysts contained bradyzoites in tunica muscularis including both thin-walled and thick-walled. Individual microcyst was surrounded by single layer of muscle fibers and degeneration and necrosis of muscle bundles were prominent. Hence, this study is one of the very few studies which have investigated the cytodiagnosis of zoonotic disease in veterinary sciences. It contributes in the existing literature on cytohistopathological diagnosis of the Sarcocystosis not only in goats but also in other meat producing animals. In present investigation, the prevalence was primarily related to the presence of microsarcocysts in muscles. Thus, microscopic examination of slide impression smears collected from the meat is essential to diagnose the Sarcocystosis for screening of the disease. It is recommended that meat should be cooked sufficiently or frozen before use to prevent health hazards to the consumer.

Keywords: Cytodiagnosis, bradyzoites, sarcocysts, prevalence, goat

Introduction
Sarcocystosis caused by the genus *Sarcocystis*, is widespread in livestock and has significant economic impact on production of domestic animals as well as on health, may cause mortality in many species of domestic and wild animals. *Sarcocystis* species are intracellular protozoan parasites with a requisite two host life cycle based on a prey-predator (intermediate- definitive) host relationship (Narnaware *et al.*, 2016) [17]. This genus consists of more than 189 species with global distribution (Poulsen *et al.*, 2014) [19]. The majority of the known *Sarcocystis* spp. appear to be intermediate host-specific (Gjerde., 2016) [8]. A wide range of vertebrates, including mammals, birds, and fish, are infected by *Sarcocystis* spp. Merogony and cyst formation (asexual stage) occur in the vascular endothelial and striated muscle cells of the intermediate host (Herbivores and omnivores, such as humans serve as both intermediate and definitive hosts) whereas gametogony and sporogony (sexual stages) occur in the intestine mucosa of definitive host (Fukuyo, *et al.*, 2002 [7], Hoeve-Bakker *et al.*, 2019) [10]. Definitive hosts are infected through the ingestion of *Sarcocystis* in muscle tissues, which include carnivorous predators, scavengers, as well as humans. Intermediate hosts become infected through the ingestion of sporocysts present in feed or water contaminated by fecal materials of definitive hosts (Gjerde, 2016) [8]. The multiplication within striated muscle cells leads to the formation of mature sarcocysts, which have a characteristic morphology for each species, and

by which, definitive hosts become infected through ingestion of infected muscle tissues.(Gjerde *et al.*, 2020) [9]. *Sarcocystis* are generally nonpathogenic for the definitive host, several *Sarcocystis* species can cause many severe symptoms in intermediate hosts, like fever, weakness, inappetence, anemia, weight loss, pyrexia, hair loss, hemorrhagic diathesis, and encephalitis while in acute infections of prenatal females with *Sarcocystis* would bring about fetal death, abortion, premature labor and the muscles were full of gaps with widely separated fibers. Thus Sarcocystosis would cause inedible meat and massive economic loss further (Ren *et al.*, 2019) [21]. *Sarcocystis* spp. is a substantial economic zoonotic disease involving an individual host that may harbor more than one species of *Sarcocystis*. There are three common species of this parasite in domestic goats, *Sarcocystis hircicanis*, *Sarcocystis capracanis*; which produce microscopic sarcocysts that are transmitted by canids and wild predator while *Sarcocystis moulei* (*S. caprifelis*) produces macroscopic cysts transmitted by felids (Dubey *et al*., 2016 [5] and Olias *et al.*, 2010) [18] besides, the *Sarcocystis* species that are transmitted via canids or primates are more pathogenic than those transmitted by felids ((Dafedar *et al.*,2008 , Al-Waely and Abd AL-Amery, 2020, Swar and Shnawa, 2021) [4, 3, 25]. Despite the importance of worldwide goat production, in domestic goats (*Capra hircus*), in Rajasthan, scanty information is available regarding the prevalence of *Sarcocystis* spp. and about the use of cytodiagnosis of Sarcocystosis, therefore the utility of cytopathology as a rapid, cost-effective, and safe diagnostic procedure, was highlighted in this study.

Materials and Methods

1. Source and Collection of Samples

For present study, a total of 152 samples of the oesophagi were collected from goats of either sex, irrespective of age groups and breeds. The entire work was carried out in the Department of Veterinary Pathology, PGIVER, Jaipur. The organ/tissue samples for this study were collected from various slaughter houses, carcasses of goat submitted for postmortem examination to department of Veterinary Pathology, any mortality at Veterinary Clinical Complex, PGIVER, Jaipur and samples received from the field veterinarians in the department of Veterinary Pathology for the routine histopathological examination, included in this study.

2. Gross Examination

All the samples were examined grossly for alterations in morphology in terms of shape, size, colour, consistency, odour, location and type of the lesions in individual part of the oesophagus.

3. Cytological Examination

For cytological examination following technique was used –

3.1 Impression Smear / Touch Imprint Cytology

Impression smears from tissues were collected at necropsy. At least four slides were prepared from each specimens by using the technique described by Tribe (1965) and Tseng *et al.* (1999). The prepared smears were allowed to air dry. After drying, smears were immediately fixed using methanol for Giemsa staining. Then imprints were stained with Giemsa stain (Hi Media Laboratories, India) and thoroughly examined under light microscope for various cytopathological alterations.

4. Histopathological Examination

After thorough gross examination, representative pieces (approximately 0.5 cm thickness) of oesophagus were collected and fixed in 10% neutral buffered formalin. The formalin fixed tissues processed for paraffin embedding by acetone and benzene technique (Lillie 1965). The tissue sections of 4-5 micron thickness cut and stained with haematoxylin and eosin staining method (Gelberg 2012, Suvarna *et al*., 2008, Culling 1974, Luna 1960) for histopathological evaluation. Sections were thoroughly examined under light microscope for various histopathological changes.

Results

In the present study an overall prevalence of sarcocystosis was recorded in 152 cases (13.63%). Futhermore, out of 152 cases, oval fat macrosarcocysts were observed only in four cases (0.35%). Grossly, macrosarcocysts in the form of many white-pale to creamy colour, oval fat (macro), grain sized, multifocal nodules on outer surface of oesophagus were seen (Fig.1). In cytopathology, giemsa stained impression smears exhibited thin walled microscopic sarcocysts (Fig.2) and banana-shaped bradyzoites in clusters as well as individually (Fig.3&4). Histopathologically, the study depicted the presence of several forms of microscopic cyst, including spindle, circular, elliptical, cylindrical and twisted form in esophagus (Fig.5). These appeared as blue colour sarcocysts contained bradyzoites in tunica muscularis including both thin-walled and thick-walled. Individually microcysts were surrounded by single layer of muscle fibers and degeneration and necrosis of muscle bundles were prominent (Fig.6).

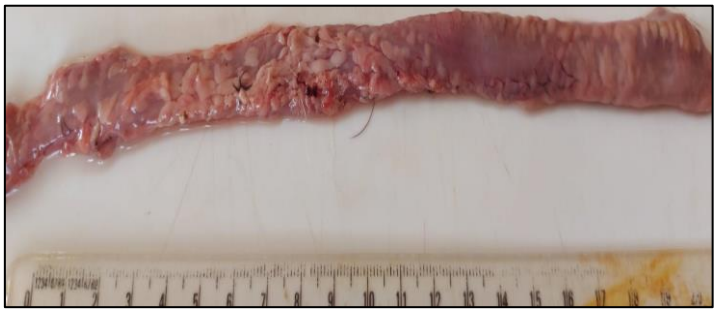

Fig 1: Photograph showing macro sarcocystis in the form of many white-pale to creamy colour, oval fat, grain sized, multifocal nodules on outer surface of oesophagus

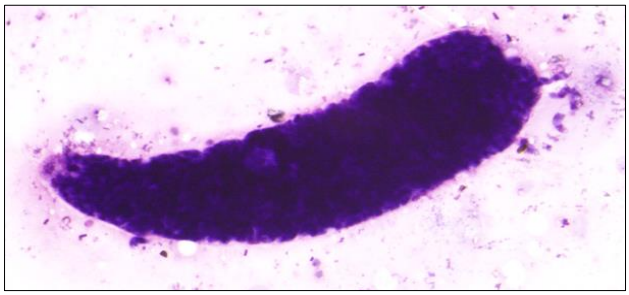

Fig 2: Touch imprint from the oesophagus depicting thin walled microscopic sarcocyst and few bradyzoites in background. (Giemsa stain, 400X)

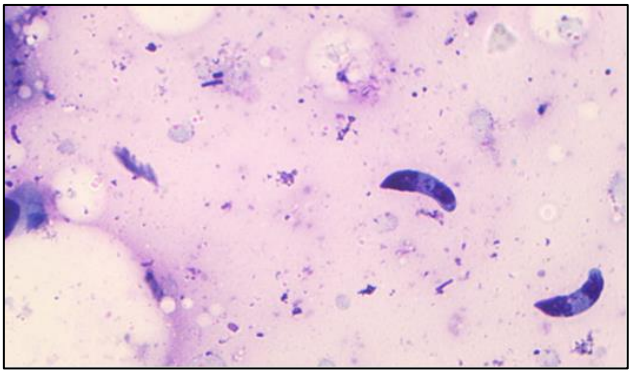

Fig 3: Impression smears from the cut surface of oesophagus revealing individual banana-shaped bradyzoites. (Giemsa's stain, 1000X)

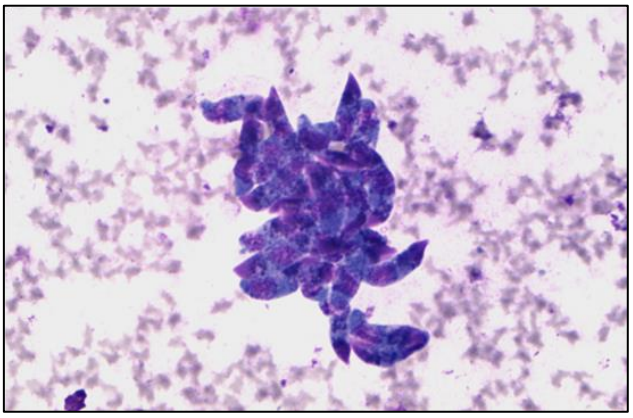

Fig 4: Impression smears from the cut surface of macrocyst revealing the cluster of banana-shaped bradyzoites.(Giemsa's stain, 1000X)

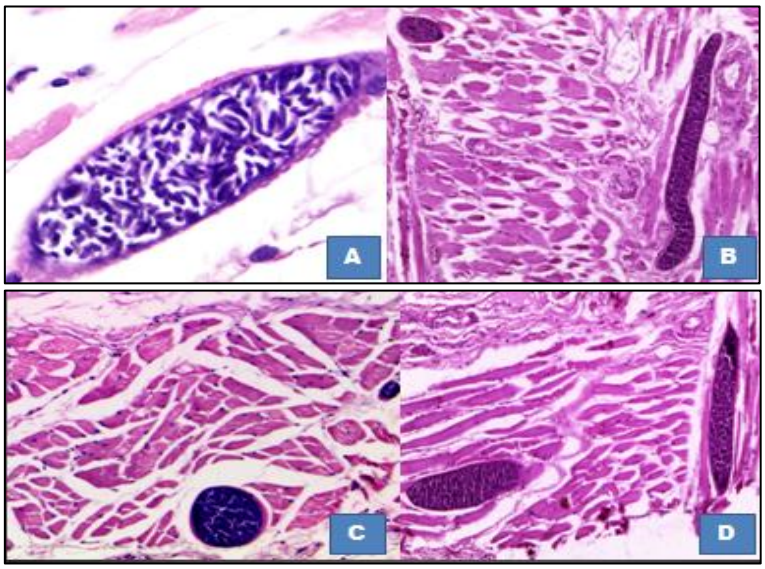

Fig 5: Photomicrograph of oesophagus depicting the presence of several forms of microscopic sarcocysts including spindle shape, containing bradyzoites (A), oval and twisted (B) circular (C), elliptical and cylindrical (D) forms in esophageal muscles. (A - H&E, 1000X and B,C,D - H&E, 100X)

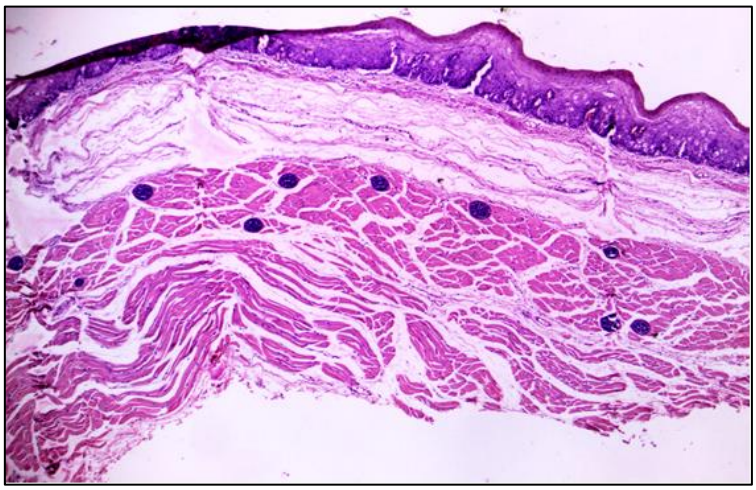

Fig 6: Photomicrograph of oesophagus depicting the presence of many blue colour sarcocysts including both thin-walled and thick-walled, degeneration and necrosis of muscle bundles. (H &E, 40X)

Discussion

Sarcocystis is considered as one of the most prevalent livestock parasites, with potential public health importance due to the consumption of undercooked or raw meat. The results of the present study revealed a moderate prevalence of *Sarcocystis* infection among goats. In the present study an overall prevalence of sarcocystosis was recorded in 152 cases (13.63 %) and it was closely related to the results reported by Al-Waely and Abd AL-Amery (2020) [3] as 13.30% in Iraq. Diversity in sarcocystosis prevalence in oesophaus has been reported recently, in different geographical areas of Rajasthan such as lower prevalence was observed as 0.89% by Singh (2019) [22] in Udaipur region, 7.94% by Pushpa (2013) [20] in Bikaner region of Rajasthan, India and in other places *viz* Hussein (2015) [12] found 2.6% in Iraq. Present findings were lower as compared to that reported by Latif *et al.(*1999) [15] as 33.60 % in Iraqi goats, 34.62 % in Nagpuri goats by Jumde *et al.* (2000) [14] and very lower that recorded by Dafedar *et al.* (2008) [4] as 72.00 % in Indiain goats, Bangalore, Karnataka State, Daya Shankar and Bhatia (1993), Chhabra and Samantaray (2013) as 73.33 % in Uttar Pradesh, India , 77.30 % by Hu *et al.*(2016) [11] in China and 79.40 % in Egyptian goats reported by Morsy *et al.* (2011) [16]. Futhermore, out of 152 cases, oval fat macrosarcocysts were observed in four cases (0.35%). The results of this research were almost in agreement with findings of Al-Waely and Abd AL-Amery (2020) [3] in the esophagus as 1.66% while this percentage disagreed with Swar and Shnawa (2020) [24] reported as 8.8% in Iraq goats. This finding may be due to the variation in risk factors associated with the distribution of *Sarcocystis* spp. Infection. In the present study the prevalence of sarcocystosis comparatively higher than recent studies in Rajasthan, may be due to improper disposable of affected carcasses, the free movement of large number of stray dogs and access to infected offal accounts for the higher prevalence of the infection. However lower prevalence recorded by other authors may be due to screening, lesser number of samples. Grossly, sarcocysts in the form of many white – pale to creamy colour, oval fat (macro), grain sized, multifocal nodules on outer surface of oesophagus seen and these study findings were in association with the previous literature (Pushpa, 2013 [20], Sivajothi and Reddy 2017 [23], Singh, 2019 [22], Al-Waely and Abd AL-Amery, 2020 [3] and Swar and Shnawa, 2021) [25]. In cytopathology, giemsa stained impression smears exhibited microscopic sarcocysts and banana-shaped bradyzoites in clusters as well as individually. these results were nearly similar to the microscopic pictures described by the few authors in different parts of the world via different techniques such as Sivajothi and Reddy (2017) [23] in impession smears from meat muscles , Zangana and Hussein (2017) [26] in adhesive tape method from tongue mucles and Swar and Shnawa (2020) [24] in compression slides technique. Histopathologically, the study depicted the presence of several forms of microscopic cyst, including spindle, circular, elliptical, cylindrical and twisted form in esophagus, these findings were well in agreement with Al – Hasnawi (2008) [2]., Singh (2019) [22], Pushpa (2013) [20] and Al-Waely and Abd AL-Amery (2020) [3]. Sarcocysts appeared as blue colour contained bradyzoites in tunica muscularis including both thin-walled and thick-walled. Individually microcysts were surrounded by single layer of muscle fibers and degeneration and necrosis of muscle bundles were prominent, described previously El-Morsey *et al*. (2017) [6] , Januškevičius *et al*. (2018) [13] and Abdullah (2021) [1]. A large variation in the size and the shape of the sarcocysts was observed in the inspected esophagi of goats and this may be attributed to the age of the cyst as well as to the species of *Sarcocystis*. This variation may also depends on methods of animal farming and management, feeding in the open farmyard, the difference in the number of samples, the number of canine and feline predators and wild animals, contamination the feed and soil with sporocysts, house fly and cockroaches plays an important role in the mechanical transmission of *Sarcocystis*. [15, 9].

Conclusions

In nutshell, this study is one of the very few studies which have investigated the cytodiagnosis of zoonotic disease in veterinary sciences. It contributes in the existing literature on cyto histopathological diagnosis of the Sarcocystosis not only in goats but also in other meat producing animals and caution as a public health alert. This paper demonstrates the presence of *Sarcocystis* spp. that form microscopic cysts in domestic goats destined for human consumption in India. Since, the prevalence was primarily related to the presence of microsarcocysts in muscles, it is recommended that microscopic examination of slide impression smears collected from the meat, is essential to diagnose the Sarcocystosis timely for screening of the disease. Therefore, to pursue an effective management of parasitic zoonoses, awareness

raising should involve all figures in the supply chain from backyard slaughter practices to consumers. It is also suggested that meat should be cooked sufficiently or frozen before use to prevent health hazards to the consumer.

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