Republic of Iraq
Ministry of Higher Education
& Scientific Research
University of Al-Qadisiyah
College of Veterinary Medicine

Tradisional and Molecular Detection of Sarcocystis species infection in slaughtered Cattle and Imported Beef meet

A Graduation Project Submitted to the Department Council of the Internal and Preventive Medicine-College of Veterinary Medicine/ University of Al-Qadisiyah in a partial fulfillment of the requirements for the Degree of Bachelor of Science in Veterinary Medicine and Surgery.

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2021 A.D. **1442 A.H.**

بسم الله الرحمن الرحيم

"يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا

الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ"

صدق الله العظيم

"سورة المجادلة-ايه 11"

Certificate of Supervisor

I certify that the project entitled **(Tradisional and Molecular Detection of Sarcocystis species infection in slaughtered Cattle and Imported Beef meet)** was prepared by **(Ameen Abd-al-hassn.owed)** under my supervision at the College of Veterinary Medicine / University of Al-Qadisiyah.

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Certificate of Department

We certify that **(Ameen Abd-al-hassn.owed)** has finished his/her Graduation Project entitled **(Tradisional and Molecular Detection of Sarcocystis species infection in slaughtered Cattle and Imported Beef meet)** and candidate it for debating.

list of content

abscrat

The lifecycle of a typical member of this genus involves two host species, a definitive host and an intermediate host. Often, the definitive host is a predator and the intermediate host is its prey. The parasite reproduces sexually in the gut of the definitive host, is passed with the feces, and ingested by the intermediate host. There, it eventually enters muscle tissue. When the intermediate host is eaten by the definitive host, the cycle is completed. The definitive host usually does not show any symptoms of infection, but the intermediate host does.

Route of infection:

Infection occurs when undercooked meat is ingested. The incubation period is 9–39 days

Diagnosis:

Definitive diagnosis by biopsy of an infected muscle. Sarcocysts are identifiable with hematoxylin and eosin. The PAS stain may be helpful, but variable uptake of stain is common. Along with the sarcocysts, inflammatory cells may be found. Other findings include myositis, myonecrosis, perivascular and interstitial inflammation, vasculitis, and eosinophilic myositis**.**

Prevention:

Infection can be prevented by cooking the meat before eating. Alternatively, freezing the meat at −5°C for several days before ingestion kills the sporocysts.

chapter one : interoduction

1.1 History

Sarcocystis, the genus were "milky white threads" parasite first described in skeletal muscle of house mice in Switzerland by Friedrich Miescher in 1843 **(Dubey etal., 1989)**.

This parasite with no scientific name at the following 2decades, were termed as "Miescher's Tubules". Also, in 1865 Kuhn found same muscular cyst in pig which named it Synchytrium mieschеrianum, and then, the name is changed to Sarcocystis meischeriana **(Savini, 1994).** oduced in 1882 by Lankester (sarx = flesh and kystis bladder) and multiple species were discovered

The genus name ***Sarcocystis*** is intr according to the host species **(Obijiaku, 2012)**. Several Sarcocystis species were named according to present of parasite in the host at the time between 1885 and 1972. In spite of the identification of different species. It was not possible on that time due to unclear life cycle of parasite until 1972. At this time Moulé (1888) and Hasselmann (1926) named S. cruzi and S. hirsute as two species infected cattle **(Dubey ef al., 1989).**

Inoculation of bradyzoites stage from infected bird muscle in to cultured mammalian cells, and succumb development into sexual stages and oocytes, thus guide to deseription the life cycle and all other stages which are unknown until 1970

(Fayer, 1976). Three species of ***Sarcocystis*** in cattle, first improved by Heydom ef al. (1972) were cyst found in the cattle muscle, while the sexual stages in man, dog and cat respectively. The Sarcocystis of the species were structurally diferent from one another **(Fayer, 1972; Tong et al., 2018).**

The biology of this parasite can be clarified by studies of the transmission of S. fusiformis, when the sarcocysts were fed to different possibility definitive hosts such as cats, dog. and humans.

T he new species names ***S. bovicanis*** (old name: S. cruzi) bovifelis (old name: S. hirsuta) and ***S. bovihominis*** (old name: S. hominis). Moreover, Heydorn and Rommel (1972) were the first providing conclusive evidence of the existence of three structurally different Sarcocystis species in cattle, with dogs, cats and man as definitive host, Additionally, they found more than one species can be infected both the intermediate and definitive host **(Dubey et al., 1989; 2010).**

Nevertheless, Levine in 1977 stated that the old names S. cruzi, ***S. hirsute*** and ***S. hominis***, first described by Railliet and Lucet in 1891 **(Dubey, 1976; Fayer, 2004)**. Currently, S. cruzi).(dog), ***S. hirsuta*** (cat) and ***S. hominis*** (man) are the only valid names according to theInternational Code of Zoological Nomenclature (ICZN), **(Dubey et al., 1989; 2016).**

1.2 Classification

The genus ***Sarcocystis***, is a genus of tissue cyst-forming coccidia Sarcocystis was reviewed and over 200 species have been characterized, and only fifty six species are known the definitive and intermediate hosts **(Levine, 1986; Frenkel and Smith, 2003)**. Examination the phylogenetic relationships of ***Sarcocystis*** species to each other, and to other cyst-forming protozoa, such as Toxoplasma gondii and Neospora caninum molecular techniques have been applied **(Ellis et al., 1995; Ferreira et al., 2018; Selene et al., 2019)**. Genomic analysis is start to provide more insight and new taxonomic schemes are proposed. However, relatively few studied in detail of these species have been done and for now, most descriptions are still based on the morphology (Figure 2.1), **(Frenkel and Smith, 2003)**.

Kingdom	Chromalveolata
Phylum	Apicomplexa
Class	Conoidasida
Order	Eucoccidiorida
Family	Sarcocystidae
Genus	*Sarcocystis*

Figuer(1) classification of sarcosystis

1.3 cycle

Sarcocystis have different species, and it have a characteristic feature by its intermediate host specific relation, such as the sporocyst of ***Sarcocystis hominis*** when infect cattle but not pig, on other hand sporocyst of ***Sarcocystis*** suihominis when infect pigs but not cattle **(Fayer, 2004; Dubey et al., 2015a).**

Multiple livestock (herbivores) can serve as intermediate host of many ***Sarcocystis species***, such as cattle and buffaloes and also these protozoan parasite which may infect numerous than one intermediate hosts. Additionally, carnivores like, cats and dogs have been classified as adequate definitive hosts for a wide range of Sarcocystosis **(Vangeel et al., 2012; Duby et al., 2015a).** The life cycle is characterized by alternation of asexual and sexual generation and can be divided into three distinct phases: sporogony, schizogony and gametogony (Figure 2.2), **(CDC, 2008).**

Sporogony involves the formation of sporozoites which might initiate infection in the intermediate host. Schizogony is a sexual reproduction by multiple fissions while gametogony is the sexual reproduction where the fertilization Of female gametes are takes place in the definitive host **(Fayer, 1972; Dubey, 1976; Dubey etal.,2015a).**

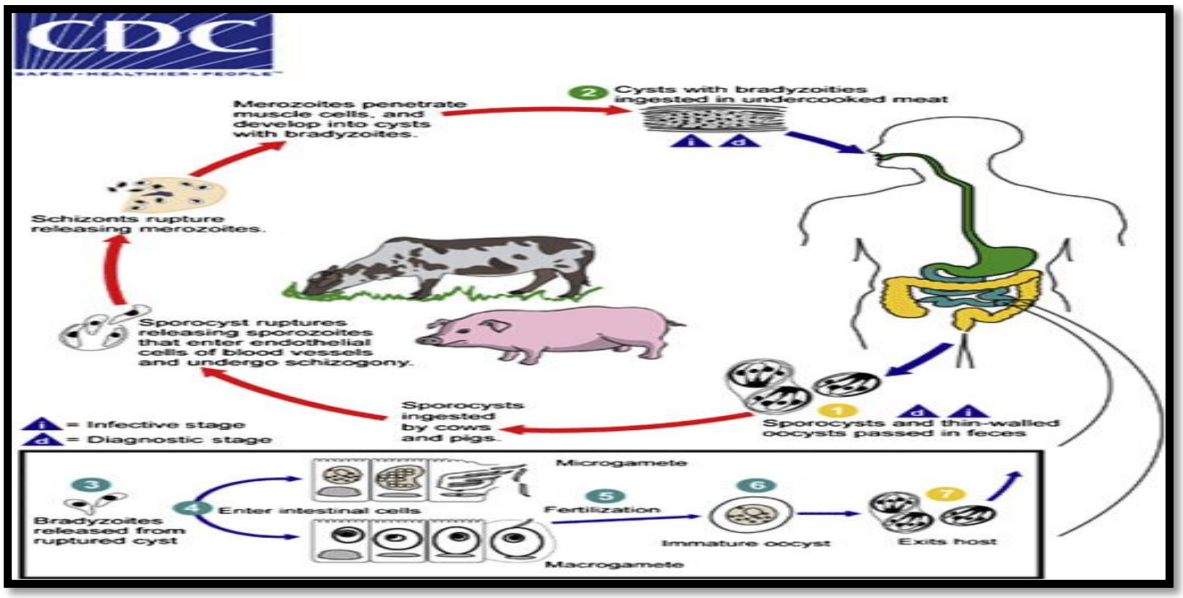


Figure (2):life cycle of sarcocystisspices(CDC,2008)

1.3.1 Stages of *Sarcocystis* inside the intermediate host (prey host)

Oocyte or two sporocysts are shed from the definitive host in feces **(Reissing et al., 2016)**. The shedding is usually low and intermittent, but can sometimes occur in large numbers **(Mehlhorn and Heydorn, 1978; Heckeroth and Tenter, 2007).**

Sporocysts can be infective immediately when shedding to environment, this characteristic feature of the genus Sarcocystis is because of other protozoan types of this family are shed unsporulated oocytes **(Mehlhorn and Heydorn, 1978; Vangeel et al., 2012).**

After ingested the sporocyst by their sensitive intermediate host, the cyst resist the stomach acidity and reach to small intestine then, plates forming the sporocyst walls disconnected to releasing the four sporozoites inside it **(Fayer, 2004; Dubey, 2015)**. Sporozoites are motile and migrate into the gut epithelium, then entering endothelial cells in small arteries throughout the body **(Dubey et al., 2015a)** First of four asexual generations of sporozoites (called schizogony or merogony), producing numerous merozoites (cells morphologically similar to sporozoites and bradyzoites) within 15 to 16 days after sporocysts ingested by intermediate host **(Fayer, 2004).**

Second generation occur in the endothelium as early as 15 days post inoculation in the capillaries, small arteries and virtually though out the body. The schizonts numerous were mostly inside kidney glomeruli. Also, the schizonts are differed on each other by their shape and size considerably. The schizonts in the skeletal muscles are longer than those in other tissues. Finally, both first and second generation schizonts are seen within the cell cytoplasm without surrounded by a parasitophorus vacuole **(Dubey et al., 1989).**

Merozoites released from these second-generation schizonts enter the blood stream and circulate extracellular or within mononuclear cells **(Dubey et al., 1989)**. They multiply in the blood stream by dividing into two progeny. These have been found six weeks after inoculation. The number of such generations in blood stream is unknown. A third generation of merogony within the blood has been described **(Fayer and Johnson, 1974; Speer and Dubey, 1981).**

Released merozoites from the second-generation schizonts that develop in the blood vessels penetrate muscle and nerve cells and cyst formation begins with the development of a parasitophorous vacuole **(Mehlhorn and Heydorn, 1978; Duby, 1989).** Merocytes forming due to divide of merozoites into pairs within the tissues of the cyst, these cyst was continue to there way for maturations, to forming mature, bananashaped bradyzoites. Finally the bradyzoites which formed begin to divide slowly and it will be infective to definitive hosts **(Tappe et al., 2013).**

The boarder of this vacuole is always a single unit membrane. This membrane soon becomes strengthened by an underlying layer of osmiophilic material. The complex formed becomes the primary cyst wall and has a thickness range of about 20-100B **(Mehlhorn et al., 1976; Olias et al., 2010).**

Bradyzoites maturation differ according species and may be average up to two months or more until bradyzoites form and the parasite cyst become infectious for the definitive host. Also, these cysts can persist for months or years **(Fayer, 2004).** Variation in the size from microscopic to macroscopic, also in length and circumference can be seen in mature ***Sarcocystis*** of each species.

Additionally, the developed structurally divers Sarcocystis walls that vary in thickness and organization of Villar protrusions, but all contain numerous bradyzoites **(Dubey et al., 1989; Fayer, 2004; Faraj and Kawan, 2012).**

Sarcocystis can be found mainly in all striated muscles of the body. These muscles can be seen in tongue, esophagus, diaphragm also it can be infected the cardiac muscle and lesser extent the smooth muscle. As well as ***Sarcocystis*** can found in small numbers in neural tissue like spinal cord and brain, as well as in Purkinje fibers of infected heart with Sarcocystis **(Fayer, 2004; Waheeb, 2018).**

1.3.2 Stages of *Sarcocystis* inside definitive host (Predator host)

Susceptible definitive host (Carnivores or omnivores) are eaten ***Sarcocystis***, the wall of the cyst was mechanically digested then ruptured in the stomach and intestine, lead to released bradyzoites which enter to the lamina propria of intestinal cells.

After penetration, the intracellular bradyzoites developed into a male (micro) or female (macro) gamonts which are found within parasitophorus vacuole of the goblet cells near Villi tip. **(Dubey and Lindsay, 2006; Yabsley et al., 2009).**

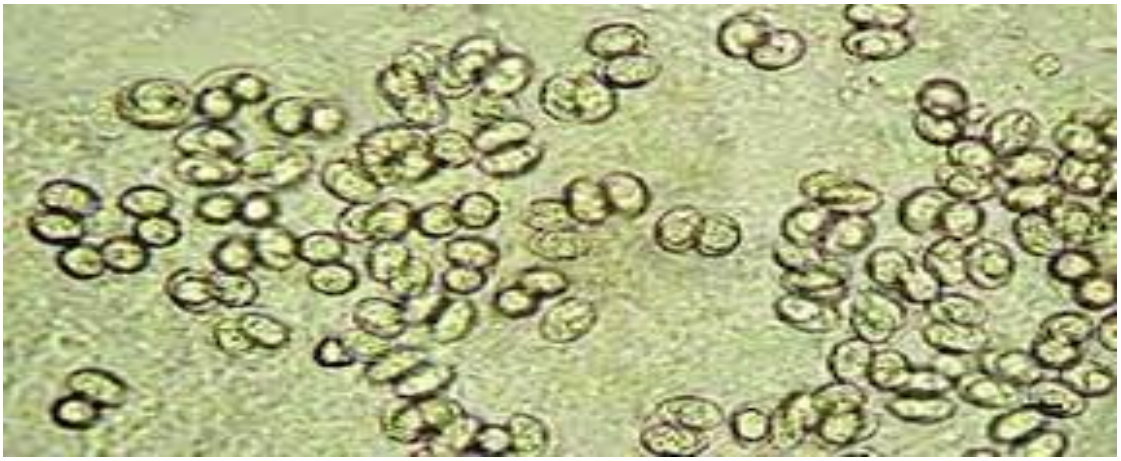
Macrogamonts usually rounded to oval in shape and contain a single large nucleus. They do not undergo cell division as the macrogamonts develop into macrogametes. Microgametes liberated from the microgamont actively moves up to the periphery of the macrogamont. When fertilization occur, a wall develops zygote circumference with develops wall, finally the oocyst is formed **(Sheffield and Fayer, 1980; Dubey et al,. 1989).**

Oocyst sporulated in the lamina propria, and it have one large nucleus contain one or two nucleoli with many Periodic Acid Shiff (PAS) - as positive granules **(Dubey al., 1989).**

The nucleus started to elongate and then divides into two nuclei one at each pole of the sporont. A second transversally division occur which giving a rise to two sporoblasts which the sporocyst become later. When the third division of nucleus occurred, the sporocyst will be having four sporozoites. The unsporulated and sporulated oocystes are found simultaneously because sporulation is happened asynchronous **(Dubey et al., 2015a).**

Sporolated oocyst appears as colorless, thin walled and have two elongated sporocysts each of them contained four sporozoites (Figure 2.3), **(Nimri, 2014; Calero et al., 2015)**. Sporulated oocysts release from the body in the feces.

Intact oocysts can be observed only in the first few days of patency, and appear as (2) adjacent sporocysts with the oocyste wall hardly visible, the thin oocyst wall often breaks, releasing individual sporocysts, which are mainly the only stage can be seen in the definitive host feces **(Gjered, 2014)**



Figure(3) individual sporocysts and oocysts, each containing two sporocysts wet mount (Nimri,2014)

1.3.3 Host specificity of *Sarcocystis*

The one of the specific characteristic of ***Sarcocystis spp.*** are strictly specific to the intermediate host, so a ***Sarcocystis*** spp. can developed and parasitize at only one species of intermediate host, as well as the specificity of these parasites to the definitive host is considerably smaller. It's clear that most of ***Sarcocystis spp.*** which transmitted from canids can't be transmitted from felids and vice versa. The only exception of this compatible pattern is S. wenzeli from chicken, which the final hosts of it are dogs or cats **(Odening, 1997; Dubey et al., 2016).**

1.4 Location of parasite

Sarcocystis have been observed in striated and smooth myocytes, nerves cells in brain and purkinji fiber of the heart, also the common location of this parasite is in the fibers of the striated muscle **(O Toole, 1987; Heckeroth and Tenter, 1999; Domenis et al., 2011).**

1.5 Morphology

The structure of the ***Sarcocystis*** wall may indicate phylogenetic relationships among hosts, for instance ***Sarcocystis*** species found in sheep and goats all possess similar cyst wall structure **(Heydorn and Rommel, 1972; Dubey et al., 1989)**. Currently there are 37 Sarcocystis wall types used to distinguish 6 species **(Odening, 1998).** It was register more than 200 species are composed of Sarcocystis genus **(Frenkel and Smith, 2003).**

The muscular cysts are easily visible by the naked eye. It's usually like cylindroid or spindle-shaped, running lengthwise in the muscles, but they may also be ellipsoidal or rather irregular. Size is vary according to the part on the host infected, also the cyst wall differ in appearance according the species **(Bucca et al., 2011).** The wall was a granulated and fine septa is project from its inner surface to divide the interior of the cyst into small compartments. Outer cyst wall surface is spongy, with a fine honeycomb structure. It sends numerous parallel, hollow, finger-like projections or Villi into the surrounding muscle tissue. These Villi may be as long as 8-10g in length, and are circular or ellipsoidal in cross section and about 0.7-0.8g in diameter **(Dubey et al., 1989; Nour011ahi-Fard et al., 2009).**

The mature trophozoites look like as banana-shaped, the anterior end slightly pointed and the posterior end rounded. They are 6-15 long and 2-4 wide, different in size according the species. They move by gliding or body flexion, twisting, turning, or following a spiral path (Figure 1.4), **(Fayer, 2004; Dubey et al., 2015a).**

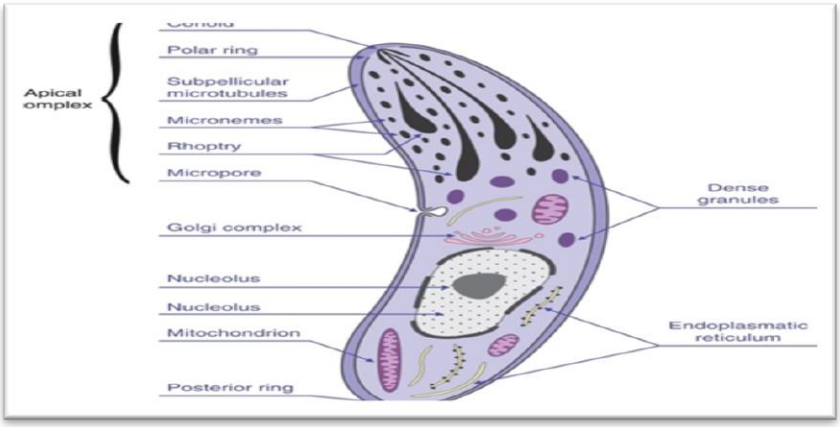


Figure (4): Structuer of sarcocystis trophozoite

At the anterior end, within the pellicle is a polar ring 0.4-0.5p in diameter, and within it is a hollow, truncate cone 0.3-0.4B long named as a conoid, while the polar ring 22-26p fine fibrils run backwards in the pellicle the full length of the body. Also, it can be observed in some individuals as short, club-shaped structures similar to the toxonemes of ***Toxoplasma*** in the cytoplasm beneath the pellicle **(Duby et al., 1989; Fayer, 2004)**. The posterior third of the body contains the nucleus. It is an ellipsoidal vesicle almost as wide as the body, and contains relatively small number of chromatin granules and endosome, the nucleus is surrounded by a large number of small vacuoles and granules, many of which contain glycogen, and these extend to the posterior end of the body, among them lie 1-3 serpentine mitochondria 0.15-0.2!1 in diameter and 2B or more long **(Gjerde, 2013).**

1.6 Transmission

1.6.1 Transmission in Animals

Definitive host infected with intestinal ***sarcocystosis*** when eating the raw or undercooked meat infected by mature cysts containing infective bradyzoites **(Dubey et al., 1989; Fayer, 2004; Smith, 2004)**. The liberation of bradyzoites from the cysts is done by digestive system enzymatic action. The sexual reproduction occur when the merozoites transform into male and female gametes and the microgamete inside the cells of the lamina propria of the small intestine; then, the merozoits fertilizes the macrogamete, giving rise to the zygote. Finally shedding the sporulated oocyst and liberated sporocysts in the feces **(lhsan and Shivan, 2015).**

Consumption of sporulated oocyst or sporocyst with contaminated food or water is virtually the mainly significant method to be transmitted the infection to intermediate host **(Hussein, 2015; Dubey, 2015).** Other mode to transmission the parasite could be para fly (Musca domestica) and cockroaches. Also, Sarcocystis can be transmitted on exoskeleton of the fly by mechanical transmission **(Calero et al., 2015).**

Infection by trans-placental is rarely documented in cattle and sheep, it has rarely occur in nature. Theory of transmission via milk colostrums has been postulated however the evidence of transmission was not provided **(Smith, 2004; Dubey and Lindsay, 2006)**. Also, the personal dealing at the meat inspector can causes the infection, these was lead to condemnation of the cattle carcasses at the beef industry in Municipal abattoir, Sri Lanka **(Hettiarachchi and Rajapakse, 2008)**.

1.6.2 *Sarcocystis* transmission from Animals to Humans

Different types of meat from many sources such as reptiles, birds, and species of wild mammals, which are consumed in various world parts, could be infected with Sarcocystis with unknown effect. Therefore, they remain many possibility but unknown sources of human infected with intestinal type of ***Sarcocystosis*** **(Hoeve-Bakker et al., 2019).**

So, human acquiring intestinal ***Sarcocystosis*** is a result due to eating raw or undercooked beef or pork which carry the mature muscle cyst of S. hominis and S, suihominis respectively **(Bunyaratvej et al., 2007; Deuby et al., 2015a; Rie et al., 2018).**

The possibility of human infected due to eating food or drinking water contaminated with feces from a predator of non-human primates involving unknown species of Sarcocystis **(Fayer, 2004; lhsan and Shivan, 2015)**. Subsequently, the same similar conclusions were reached in reviews of human cases when cysts were infected the muscle tissues **(Beaver et al., 1979; Pathanathan and Kan, 1981; Soulsby, 1982).** Most human and non-human primates cases have been reported in tropical areas, one of these study registered 79 (21%) of 375 wild-caught monkeys examined, involve 14 species of Sarcocystis **(Karr and Wong, 1975).**

1.7 Epidemiology

Sarcocystis is one of the globally parasite which infected many species of animals **(Dubey et al., 1989; Roberts and Janovy, 2006)**. More than 50% of adult swine, cattle and sheep probably are infected with ***Sarcocystis spp***. Dogs and cats serve as definitive hosts for a variety of ***Sarcocystis species.***

They shed the sporocysts with in their feces over a several months after infection. The oocyte or sporocysts are known to be resistant to freezing and other harsh weather conditions **(Pritt et al., 2008).** The range of Sarcocystis prevalence in domestic mammals ranged from 10-100%. Cattle registered a highest prevalence as well as sheep and goats. Canids, felids and human are the definitive hosts of ***Sarcocystis species*** **(Odening, 1998; Dubey et al., 2015a).**

Unlike other species of coccidian oocyst which is shedding with feces recognized as the infective form of ***Sarcocystis***. Where, the oocyte sporulated when it passed in the feces, no dependence on weather conditions to sporulated **(Dubey et al., 1989; Urquhart et al., 2003; Fayer, 2004).** From the high prevalence of symptomless infections observed in slaughtered food animals, it is clear that where dogs or cats are kept close with farm animals or their feed, transmission is likely to occur **(Urquhuart et al., 2003; Al- Hasnawi, 2008)**. Studies on the prevalence of ***Sarcocystis*** species infected cattle in different countries show variable percentage of infection **(Singh et al., 2003).**

1.7.1 .Prevalence of Sarcocystosis

1.7.1.1 In Iraq

Latif et al. (1999) to detection the prevalence of ***Sarcocystis spp***. in Iraq by using different technique such as naked eye examination for macroscopic Sarcocystis, and pepsin digestion, muscle squash, squeezing methods and indirect fluorescent antibody test (IFAT) for microscopic types, Result show the prevalence percentage of the macroscopic infection were 4.1% in (605 sheep), 33.6% in (826 goats), 0.2% in (1080 cattle), 15.6% in (580 water buffaloes) and 0.3% in (36 camels),while the prevalence percentage of the microscopic type were 97.0, 97.4, 97.8,82 and 91.6 for the previous host mentioned respectively.

Serological study including detection of infection with ***Sarcocystis spp***. in Nineveh governorate, One hundred blood samples were taken from cattle, and the results revealed that the rate of ***Sarcocystis*** spp. infection is 45% **(Al-Taee et al., 2009)**. Mohammad, 2012 registered 66.5% that the prevalence rate of microscopic **Sarcocystis** from slaughtered cattle in Babylon abattoir which detected by trichenoscopy, while histological technique detected 70% was infected.

1.7.1.2 In Abroad

Mediterranean region, study by Ellah et al. (2011) compared conventional methods (macroscopic examination, histopathological examination) and ELISA for diagnosis of ***Sarcocystosis*** in buffaloes in Assiut governorate, Egypt. Result shows 23% the prevalence rate of macroscopic ***Sarcocystis***, while 94.44% registered by ELISA technique when examined animals which infected with Sarcocystis. Badawy et al. (2012) conducted a study in slaughtered cattle samples at sharkia governorate, Egypt to diagnosis Sarcocystis spp. by using both light and electron microscopy with random amplified polymorphic DNA (RAPD-PCR) technique.

Result identified thin walled microscopic ***Sarcocystis*** in 29.6% (24/81) of the cattle when examined, which later identified as Sarcocystis cruzi. Also, a thick wall cyst observed in (81/3) %4.9and identified as ***Sarcocystis hominis***

Another study in Egypt, in Assiut province was done to determine the prevalence and species composition of ***Sarcocystis*** in buffaloes. A total of 90 buffalo's esophagi were examined. The prevalence rates were 25.5%, 27.7% and 94.4% by gross examination, microscopic examination and using ELISA respectively. S. fusiformis reported the species caused macroscopically infection of buffaloes, but three species were recognized as microscopically infection S. levinei, S. cruzi, and S. hominis. Also, its first report improved that S. cruzi can infected the buffaloes in Egypt **(Metwally et al., 2014).**

Other study on the occurrence of ***zoonotic Sarcocystis spp***., which isolated at Giza governorate, Egypt from different abattoirs, they examined 103 slaughtered animal (61 cattle, 42 buffaloes) by naked eye, pepsin digestion method and histological examination to isolation the bradyzoites, overall prevalence of ***Sarcocystis*** infection were 60% in cattle and in buffaloes it were 69% **(Nahed et al., 2014)**. The prevalence differed according age of animal, study in Egypt show higher infection rates with Sarcocystis species in old-aged animals 5 years and above, than those occurred in younger animals (2-3 years), Additionally mixed infection with Sarcocystis spp. could be found. Mixed infection will be found in 83.8% (311/371) for S. fusiformis and 29.9% (47/157) for S. buffalonis which affected buffaloes **(El-Seify et al., 2014).** Prevalence study

according the ***Sarcocystis*** infection in abattoir of El-Kharga, Egypt were registered 127/1790 (7.09%) in male and 32/330 (9.69%) in females in examined bovine carcasses, and macrocytic form in cattle referred to S. hirsuta, while S. fusiformis was characterized as macroscopic one in buffalo.

In the same study found the side of infection show, the highest detection rate of Sarcocystis lesions was the esophagus 76.3%, and then in throat muscles 35.3% followed by tongue 33.8%, and finally the diaphragm muscles 18.71% **(Ahmed et al., 2016).** Alexandria markets, Egypt study done on 55 different tissue sections of meat from the imported frozen buffalo samples were collected from different localities, showed that frozen buffalo meat has multiple ***Sarcocystis*** infections with S. fusiformis and S.

cruzi **(Mohamed et al., 2016).** Other study on 384 samples, of imported frozen beef referred to 280 Indian buffalo and 104 Brazilian cattle meat were collected from Alexandria province, Egypt. Result shows a low prevalence of macroscopic ***Sarcocystis spp***. in buffalo beef (3.9%) with no detection of macrocysts in cattle beef. Only 50 samples were taken from both buffalo beef for microscopic detection by used pepsin digestion, histopathology and PCR resulting in 24%, 38% and 34% positive samples respectively. Simler number of samples were taken from cattle beef and the result appeared lower infection rates as 6%, 2% and 2% **(Hussein et al., 2017).**

The prevalence ranging between 24-100% according to the morphological and molecularly identification of Sarcocystis as a first study done in cattle at the Qena Governorate, Upper Egypt **(El-kady et al., 2018).** Other study was performed at Tunisian cattle in North-West Tunisia (Béja governorate) to determine the prevalence of ***Sarcocystis*** spp. infection in slaughtered cattle.

The molecular test for 150 DNA extracted from beef meat samples, appeared the infection rate was 38% **(Safa et al., 2016)**. A supplied meat for hamburger in the Iran, prevalence rate of ***Sarcocystis*** was detected in this meat was 6.25% **(Khaniki and Kia, 2006).** Eslami et al. (2014) collected beef sample from Yazd slaughter house, Iran extracted DNA and used 18SrRNA gene as a target gene for ***Sarcocystis*** species detection by molecular technique and then Restriction Fragment Length Polymorphism (RFLP) analysis identified.

It was the first report of molecular identification of S. hirsute in Iran. Also, other RFLP study was performed in slaughtered cattle and water buffaloes in Ahvaz, Khuzestan province, Iran to inspection of meat. In this study, meats were collected from 124 cattle and 147 buffaloes, and 50 gm of each animal samples tissues was examined.

These tissues include esophagus, heart, intercostal muscle and diaphragm. Pepsin digestion was method used for samples examined. Genomic DNA of 80 positive samples was extracted and their 18S rRNA gene was amplified. The results detected S cruzi in cattle, and S. fusiformis in water buffaloes **(Hossein et al., 2013).**

A collection of different skeletal muscles were collected from fifty cattle slaughtered in abattoir of Iran. All sample of this study showed infected by Sarcocystis cruzi with the percentage rate 100% **(Mahmoud and Tahereh, 2017)**. For determine which Sarcocystis spp. present in the meat product from beef by the study includes 150

samples divided to 50 samples of minced meat, meatballs, and fermented sausages respectively. These samples were collected in the city center from butcher's shops, from businesses that produce such items, and from general stores. Results show the rate of infection was 28% in 14 samples of minced meat, 68% in 34 samples of meatballs and 2% in one sample of sausage **(Ufuk et al., 2018).** Many studies were done in India and the first research of Sarcocystis prevalence by Ramanujachari and Alwarin (1951) results, show that the

prevalence rate of ***sarcocystosis*** is higher in buffaloes than cattle. A high prevalence of ***sarcocystosis*** in the muscle of both cattle and buffaloes in Bihar, India 71.5% and 69% respectively **(Juyal et al., 1981).** Another study on 238 carcasses of which examined in Madhya Pradesh, India, over 80% contained Sarcocystis **(Jain and Shah, 1987).**

Result of examined 86 cattle, 102 sheep, 120 goats and 60 buffaloes by Mohanty et al. (1995) at Bhubaneswar, India, found 296 were positive, and the highest prevalence was in buffaloes (86.6%) then sheep (81.37%) followed by cattle (80.23%) and lowest one were goats (76.6%).

Venu and Hafeez, (2000) considered that the close association of dogs as a factor in the transmission of ***Sarcocystis*** infections in ruminants which examined 143 cattle and 169 buffaloes in Tirupati, India, indicated the prevalence rate as 58.74% and 79.9% in cattle and buffaloes respectively.

A prevalence study on the ***Sarcocystis*** infection in the slaughtered cattle of Chittoor district, India by examined 150 samples were examined both macroscopically and microscopically for the presence of parasite infection, ***Sarcocystis*** infection in cattle was 91.33%, while the prevalence of macroscopic and microscopic cyst was 6.57 and 93.43% respectively **(Mounika et al., 2018)**. In other world Obijiaku et al. (2013)

conducted a cross sectional study in Zaire by collected esophagus and diaphragm samples from two-hundred slaughtered cattle and analyzed by pepsin digestion and histological examination of tissues section where 85 samples were found positive with a prevalence rate of 42.5%. All detected ***Sarcocystis*** were microscopic in nature and 99% had thin cyst wall while 4% had thick cyst wall and the identified species were ***S. cruzi*** and ***S. hominis***.

Examination of 50 samples of kibbe which prepared from row beef collected from 25 Arabian restaurants in San Paulo, Brazil, the result found all samples were have Sarcocystis infection **(Pena et al., 2001)**. Sequence analysis of PCR products, which isolated from thick-walled cysts composed from minced beef in Belgium registered that

S. hominis was infected 97.4% of the samples, these highest percentage due to the common consumption of raw minced beef in Belgium and other European countries **(Vangeel et al., 2007).** Water buffaloes in Philippine were commonly reported infected with both macroscopic and microscopic types of ***Sarcocystis,*** the S. fusiformis revealed to the macroscopic species, while the S.

levinei revealed to the infection with microscopic species **(Florencia et al., 2000)**. Domenis et al. (2011) setup one diagnostic protocol to determine the prevalence of ***Sarcocystis*** in the breed of semi-intensively cattle at north western, Italy. They used histological examination of the esophagus, diaphragm, and heart to detected ***Sarcocystis spp***. the prevalence rate with type of species isolate from cattle were S. cruzi 74.2% followed by ***S. hominis*** 42.7% and S. hirsute 1.8%. Latif et al. (2013) investigated the prevalence of ***Sarcocystis*** in 102 cattle and 18 water buffaloes from abattoirs of Selangor state, Malaysia. Samples collected from the

skeletal muscle, tongue, heart, esophagus and diaphragm. Result show the positive samples rate of cattle was 36.2%, while positivity rate in water buffaloes was 66.7%, all samples examined by light microscopic and histopathological analysis. The first diagnosis of Sarcocystis infection in Lithuania domestic animals especially cattle and pigs was earlier in beginning of 1976 **(Prakas and Butkauskas, 2012).** Investigation of slaughtered cattle and pigs have been found the raised of infection with S. hominis and S. suihominis in Japan **(Saito et al., 1999).** Also other study described the thick wall cyst of Sarcocystis which isolated from

slaughtered cattle in Saitama Prefecture, Japan which detected the cysts which was 7-10 gm thick and supplied with finger-like Villar protrusions, also ***S. cruzi*** were detected in 60 beef sample. Hungary have registered a high prevalence rate with S.

cruzi in there slaughtered cattle followed by the zoonotic species ***S. hominis***. However, rate of infection with both species was differed according cattle breeds **(Sandro et al., 2015).** From 210 samples collected from Korean native cattle, which examined in Korea to identified the rate of infection with ***Sarcocystis***, only 31 samples with (14.8%) have positive for ***Sarcocystis*** **(Tong et al., 2018).**

Using the molecular study in Netherlands slaughtered cattle by PCR amplicons of sequence identities of 97% were infected with S. cruzi (65.4%) while, S. hominis registered (12.5%) followed by S. bovifelis (8.7%) and both S hirsuta, S. heydorni have (l .0%). Also, mixed infections were detected in 17.3% of the samples **(Hoeve-Bakker et al., 2019).**

1.8 Pathogenesis and clinical singes of *Sarcocystis* infection

1.8.1 Clinical singes and pathology of *Sarcocystis* in the intermediate host

Generally, ***Sarcocystis*** species are not all pathogenic for intermediate hosts. Clearly, ***Sarcocystis*** species transmitted by canids are more pathogenic than those transmitted by other definitive hosts **(Dubey et al., 1989).**

Severity of the infection in the domestic animals depends on species of parasite, number of digested oocyst, also the age and immune state of animal. Incubation period is usually one month. Experimental infection of cattle by Fayer et al. (1976) summarized that acute infection of cattle don't developed acute ***Sarcocystosis*** unless digested 200,000 sporocysts or above at a given time.

Clinical signs included fever, anorexia, tachypnea, tachycardia, encephalitis, encephalomyelitis, wasting, decreased milk production, diarrhea, muscle spasms, weakness, pneumonia, anemia, icterus and hemorrhage, prostration, and death in infected animals. These signs may be persist for several days to weeks. Additionally abortion or give birth to a still- born fetus occurred in pregnant mammals when ingest sporocysts **(Fayer, 2004; Dubey et al., 2015a; Cooper et al., 2016).**

In a general blood investigation for several elements show elevated in serum bilirubin, lactic dehydrogenase, alanine amino-transferas, sorbitol dehydrogenase and creatinine phosphokinase for brief periods during the anemic phase, blood urea nitrogen becomes elevated approaching terminal ***sarcocystosis*** **(Dubey and Bergeron, 1982; Frelier and Lewis, 1984; Mahaffey et al., 1986; Taylor et al., 2010)**. As infection becomes chronic, growth is more affected, animals become hyper excitable, they hyper salivate and they lose hair on the neck, rump and tail tip **(Fayer and Dubey, 1986).**

Emaciation become observed in some animal, while some eventually develop nervous signs including decumbency, nystagmus, cycling gait and sometimes lead to death **(Claire et al., 2014).** Domenis et al. (2011) described that cysts of ***Sarcocystis*** are common in the heart, esophageal and skeletal muscle of cattle but rarely cause disease. Cysts can vary greatly in shape and may be grossly visible or microscopic depending on ***Sarcocystis species***. Grossly visible cysts in mammals may look like tan-white grains of rice,

or they may be long and threadlike, or even round. Some animals that die from sarcocystosis will have hemorrhage throughout the body **(Fayer et al., 2015).**

Bovine Eosinophilic Myositis (BEM) is a histopathological descriptive term referring to a myositis in cattle in which the predominant inflammatory cell type is the eosinophilic granulocyte. Since cattle do not show any clinical symptoms, this pathological entity typically is observed in slaughterhouses and meat cutting plants **(Vangeel, 2012)** Grossly, edema with focal necrosis in digestive tract which associated with lymph-nodes is the first to be seen.

Hemorrhages later develop surface of cardiac and skeletal muscles and in the eyes sclera. Skeletal muscles mottled or striped with pale areas, interspersed with dark hemorrhagic areas are characteristic of acute ***sarcocystosis***. Hemorrhages vary from petechial to ecchymosis. Following acute infection, body fat becomes scanty and gelatinous. Body cavities contain straw colored fluid, and organs become icteric. In chronically affected animals, the most notable lesion is serous atrophy of fat especially pericardial and per renal fat, with white flecks of mineralization **(Johnson et al., 1975; Dubey et al., 1982; 2015a).**

1.8.2. Clinical singes and pathology of *Sarcocystis* in the definitive host

Typically the definitive hosts do not show any clinical signs of ***sarcocystosis*** **(Dubey and Odening, 2001).** When fed the tissues infected with numerous ***Sarcocystis spp***.to dogs, cats, coyotes, foxes and raccoons there is no illness observed on these animals, although sporocysts were being shed. In spite of that, a few dogs have non diagnostic clinical singes such as vomiting or become anorexic for 1 -2 days **(Dubey et al., 1989).**

When human serve as final host with infected of intestinal ***Sarcocystosis***, the infection start often asymptomatic, then it will be clear spontaneously, with symptoms include mild fever, chills, vomiting with diarrhea, and respiratory problems also observed **(Lau et al., 2014)**. Experimentally infected by S. hominis in Chinese volunteers when are consumed 567 to 740 Sarcocystis from infected buffalo meat.

Then registered the clinical singes appear which include abdominal pain, distension, watery diarrhea, and eosinophilia in the first week of infection, clinical singes will be ending in the four weeks after ingesting the ***Sarcocystis***. All volunteers were spontaneously cured without treatment **(Chen et al., 1999)**.

Accidentally human infected of non-human Sarcocystis species, in this case the infections are not intestinal but rather result in muscle cysts. Clinical singes in acute case like fever, muscle weakness, musculoskeletal pain, rash, cardiomyopathy, bronchospasm and subcutaneous swelling, chronic myositis, and eosinophilia was also reported in patients with muscular ***sarcocystosis.***

The human will be a dead-end intermediate hosts **(Van den Enden et al., 1995; CDC, 2017).** Recent studies have shown ***Sarcocystosis*** as one of an opportunistic parasite in the patient infected with HIV-infection **(Velasquez et al., 2008).**

1.8.3 Pathogenesis of *Sarcocystis* Infection

Tissues and cells necrosis in the infected organ occurred due to schizonts effect where depending on the species of ***Sarcocystis***, location and multiplication potential.

Moreover, localized tissue necrosis does not appear much enough to cause the extensive illness or death seen in domestic animals such as cattle, buffaloes, sheep, goats and pigs **(Xiang et al., 2009; Valentine and McGavin, 2012).**

The perivascular mononuclear cell infiltration observed in experimental infection around ***Sarcocystis*** cruzi due to antigenic reaction of host, which liberated from sporozoites or immature schizonts or the expression of parasite antigens by host cells **(Dubey et al., 2015a).** Probably stimulated by similar parasite antigens lead to infiltration of mononuclear cells in the kidney, liver, lungs and other organs are hypoproteinemia and vasculitis may probably produce ascites and edema in tissues **(Smith et al., 1987; Dubey et al., 2015a).**

Release pyrogens from mature rupturing schizonts directly on the hypothalamus or indirectly by stimulating the release of prostaglandins, which related with fever, and fever reach to the peak when the schizonts maturation and release of merozoites into the bloodstream **(Fayer and Dubey, 1986; Dubey et al., 1989).**

Most clinical finding of acute ***Sarcocystosis*** in cattle, pigs, sheep and goats are Anemia, but the mechanism of occurring is unknown **(Mahaffey et al., 1986; Gajadhar et al, 1987).** The appearance of reticulocytes post the infectious stage which indicate an intact condition of type of anemia were either normocytic or normochromic and primarily hemolytic **(Fayer, 2004).**

When animals become infected with pathogenic species of ***Sarcocystis*** during pregnancy, abortion and fetal death can result. Most domestic animals that have appeared clinical singes of sarcocystosis from experimental infections induced in mid to late gestation aborted, whereas most infected animals without signs of infection carried their fetus to term **(Leek and Fayer, 1978; Dubey et al., 2015a).**

Brain lesions include non-suppurative meningitis or encephalitis, in addition to gray and white mater of the cerebrum, cerebellum have a central necrotic area surrounding by small foci of glial cells, with infiltration of perivascular mononuclear cells and sometimes microthrombi occur in the vessels in reaction foci affected the brain, it leads to acute brain inflammation which end to meningitis, as well as its presence in some area such as cerebellum and cerebrospinal cells in the brain **(Dubey et al., 1989; Cooper et al., 2016).**

Lesions in other organs include non-suppurative hepatitis and myocarditis. Infection also occurs in the lung lead to pneumonitis and Kidney lead to renal glomerulus inflammation accompanied by focal necrosis and hemorrhage **(Dubey et al., 2015a; Ar'aoz et al., 2019).**

Striated muscles have specific inflammatory singes, which is called eosinophilic myositis (EM), which occurs due to accumulation of eosinophil in the infected area. This condition is mainly seen in cattle **(Gajadhar et al., 1987; Fayer, 2004).** Chronic cases of ***Sarcocystosis*** are little known Sarcocystis about infected animals. Although Sarcocystis affected the muscles or central nervous system without any host reaction because of host adaptation, but some ***Sarcocystis*** probably rupture from time to time releasing toxin **(Dubey et al., 1989).**

Sarcotoxin defined as hydrous extract of bradyzoites, it will be found caused toxic effect v,hen inoculated into rabbits **(Iliepe et al., 1981; Tadros and Laarman, 1982).** However, it is not known way release toxin

and its role play in chronic cases. Additionally, it has been postulated that substances released from ***Sarcocystis*** might stimulate tumor necrosis factor (INF) **(Fayer and Prasse, 1981; Al-Hyali et al,.2010).**

1.9 Diagnosis

Acute infection of ***Sarcocystosis*** is difficult to diagnose, because the nature of disease is generally happened without specific signs. So the disease couldn't predictable in the intermediate host, because has no specific signs and finding of parasites in tissues of acutely infected. Although, there was a recent report of acute fatal Sarcocystosis in a pig breeding herd as well as S. cruzi as highly pathogenic for cattle **(Caspari et al., 2011 Dubey et al., 2015a).**

1.9.1. Traditional Methods

1.9.1.1. Morphological techniques

This technique is one of the most important one. It's also defined as basic diagnostic methods, because it's simple and inexpensive, gross inspection of muscular tissue cyst is not sensitive enough to be a reliable method for detection. Macroscopic cysts observed in infected area as showed in Figure (2.5), white or milky colored cysts embedded in the tissue and appear filamentous, globular, spindle shaped or like cooked rice grain **(Jehle et al., 2009).**

Figure(5): Bovine esophaguse showing macrocytic of sarcocystis spp.(panda, 2012)

1.9.1.2. Muscle Squash Method (Trichinoscopy)

Sarcocystis can be detected in unstained fresh squash preparations by trichinoscopy or stereoscopy at magnification of 10-40x. According to Mowafy (1993) microscopic Sarcocystis can be detected by cutting muscle to very small pieces then sample compressing between two slides and examining under microscope. Odening et al. (1995; 1996) and Motamedi et al. (2010) used this technique to identify Sarcocystis and cystozoites in the muscles of cattle and goat respectively, this method allows identify the intracellular cysts

of the parasite. Although the method is not suitable for identification, relatively more tissue can be examined in contrast to histological examination **(Yang et al., 2001).**

1.9.1.3. Squeezing method

This method is done by using garlic press, for detection the Sarcocystis and bradyzoites in the detection of ***Sarcocystis*** in bovine **(AL-Bayati, 1993).** This method used to detected Sarcocystis in sheep carcasses by Waheeb (2018)

1.9.1.4 .Muscle Digestion Method

The golden method for diagnosis ***Sarcocystis,*** It was stated that pepsin digestion was three times or more sensitive than histology in detection of parasite in muscle, pepsin digestion techniques with microscopic examination are used as practical and sensitive methods for bradyzoites detection **(Collins et al., 1980; Al-Hasnawi, 2008).**

Latif et al. (1999) investigated the prevalence of Sarcocystis spp. infection in different slaughtered livestock animals from the period 1992 -1996 in the Baghdad, Iraq. By using different technique such as naked eye for macroscopic ***Sarcocystis*** examination; while, pepsin digestion, muscle squash, squeezing methods used for microscopic ***Sarcocystis*** examination and finally indirect fluorescent antibody test (IFAT) used as serological examination.

Also the study include different organ examination. The highest infections with macroscopic cysts were found to be in the esophagus, while the lowest foundin the heart. Among the microscopic methods, pepsin digestion method gave the highest rate 93.3% then by IFAT with 88.6% followed by squeezing 81.3% and finally the muscle squash 81.2%.

Hamidinejat et al. (2010) collected different organ from slaughtered cattle Ahvaz, Khuzestan, South-West of Iran which include skeletal muscle, esophagus, heart, tongue, diaphragm and abdomen, He found that the prevalence of ***Sarcocystis*** infection was 100% by digestion method, and it considered as the perfect method for diagnosis. These

methods are used in epidemiological studies, but they can also be used for purification of bradyzoites for antigen and nucleic acid preparations. To purify the bradyzoites, a subsequent step of density gradient centrifugation is needed. The density of Sarcocystis bradyzoites varies with the age of the tissue cyst and with the species, therefore optimizing the composition of the discontinuous density gradients is always needed **(Heckeroth and Tenter, 2007).**

1.9.2. Histopathological Examination

This method used frequently but it is not a sensitive detection method due to restricted or small amount of sample tissue can be obtained; histology permits to study the Sarcocystis morphology, routine identification depend on morphological features of the cyst wall due to the low resolution by light microscopic examination Figure (2.6). Also differentiation between thin and thick-walled species is possible according this method **(Al- Hasnawi, 2008).**

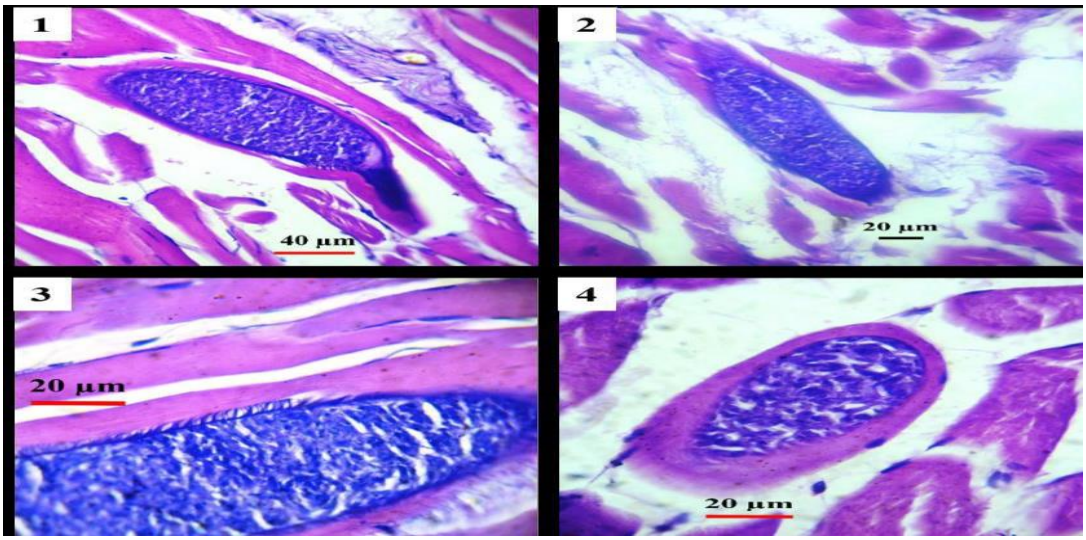


Figure (6): sarcocystis cruzi in the esophagus of cattle with H&E(X100)

Histological methods are used to determine the regional prevalence and identification of Sarcocystis spp. Examination 48 samples of retail beef by histology and compare with other detection methods such as PCR, the result in parallel by histology and PCR where 16 and 26 samples were positive respectively **(Pritt et al., 2008)**. Inspection of 128 beef cans divided to 64 cans from animals bred in Argentine and 64 cans from animals bred in Brazil, by Ghisleni et al. (2006) to evaluate the prevalence of ***Sarcocystis spp***. and the presence microscopic lesions in the muscular tissue.

Result show the Brazilian beef positive rate was 6.25% prevalence was lower in average infection compared to Argentinean beef which there positive rate was 23.44%. This study improved the confirmative histological examination to assessment parasitic prevalence in canned meat. Meistro et al. (2015) aimed to specify the prevalence of ***Sarcocystis spp***. infected the 25 bovine minced meat samples, intended for raw consumption which collected from butcher shops and retail stores in Turin's province, Italy. Samples examined by both histology and PCR, result of Sarcocystis spp. prevalence to be 64% and 88% respectively.

1.9.3 Serodiagnosis

Numeral serological tests have been used to determine the antibodies of ***Sarcocystis*** infection such as complement fixation test (CFT), (Munday, 1975); tube agglutination test (TAT), gel diffusion test (GDT), **(Shukla and Victor, 1976)**; double immune diffusion (DIDT), and single radial immune diffusion (SRIDT), **(Juyal et al,.1990)**; immune electrophoresis (IE), counter immune electrophoresis (CIEP), **(Pandit et al., 1993; Rohini et al., 2005)**; ELISA **(Gasbarre et al., 1984; Liang-Zhang et al., 1987; Savini et al., 1994; Metwally et al., 2014)**; dot-ELISA **(Singh et al., 2004; Panda, 2012),** and immunofluorescent antibody test (IFAT**), (Collins et al., 1980; David et al., 1990; Dasma Bai, 2012).** These tests were found to be simple and accurate, additionally sensitivity and specificity will be differs, because it depending upon the antigenic types.

1.9.3.1 Complement fixation test (CFT)

Simple and sensitive test, when randomly examined 50 buffaloes and 25 cattle serum samples collected from Madras / India, slaughter house for ***Sarcocystis*** infection by Complement Fixation Test (CFT). The results showed positive reaction in 50 (100%) buffaloes and 24 (84%) cattle **(Shukla and Victor, 1976)**.

1.9.3.2 Agglutination and participation test (AT and PT)

Indirect hemagglutination of sera from 19 cattle in Kentucky, United state revealed a mean antibody titer of against ***Sarcocystis bovicanis*** antigen **(Giles et al., 1980)**. Pandit et al. (1993) prepared and assessed partially purified antigen of ***Sarcocystis cruzi*** and ***Sarcocystis hirsute*** for double immune diffusion (DID), immune electrophoresis (IE) and precipitation tests to detect serum antibody in cattle sera. Counter immune electrophoresis (CIEP) proved to be the most rapid and easy to perform test for diagnosis of field cases of ***bovine Sarcocystosis.***

Study of ***bovine Sarcocystis*** antigen are investigated the possibility of cross reaction with Toxoplasma gondii due to their molecular weight, also found no cross reaction could be either in immune blot or latex agglutination test (LAT) **(Hettiarachchi et al., 2008)**. Bayani et al. (2014) diagnosed the parasite in the serum of patient was 47 years old, she was admitted to Rohani Hospital, Babol, Iran. The Sarcocystis infection

diagnosed by test serum with direct agglutination test (DAT) was positive with titer of 1 : 640 and 1:1280. ***Sarcocystis*** hirsuta was used as antigen, the preparation of this antigen done by killed whole bradyzoites in formalin; the prepared antigen used for diagnosed the antibody in infected animals. So, the detection of 88.33% grazing cattle antibody for this parasite done with Modified Agglutination Test (MAT), **(Kalita et al., 2015).**

1.9.3.3 Indirect immunofluorescence antibody technique (IFAT)

Fluorescence microscopy of 4 calves were breed referred to Holstein-Friesian calves, feed of ***Sarcocystis bovicanis***, with 200,000 sporocysts. The results showed presence of anti-bovine IgG and IgM, additionally three animals also ill and died at the days of 35 and 59 of a 63-day experiment **(Fayer and Prase, 1981).** Cattle in Argentinean were tested 380 serum for microscopic infection of ***Sarcocystis*** by IFAT in samples collected from five slaughterhouses.

IFAT results showed that 379 samples were positive and had titers 25 or higher **(Moré et al., 2011).** Study reported immunofluorescence antibody technique as the best for diagnosing sarcocystosis with a positive result of 82% then muscle digestion 52%, gross examination 16% and finally squash techniques with positive rates 8% **(Dasma Bai, 2012).**

The study from neighborhood abattoirs in Egyptian water buffaloes (Bubalus bubalis), in the Sohag, Egypt by collected 145 samples of slaughtered buffaloes to specified the incidence rates of ***Sarcocystis spp***. Result show the incidence rate by using IFAT was 64.8% **(Adel et al., 2017).**

1.9.3.4 Enzyme linked immunosorbent assay (ELISA)

Evaluated for the diagnosis of S. cruzi infection by used an enzyme linked immunosorbent assay (ELISA) doing when use crude antigens of cystozoites and merozoites. A high linkage was found between the parasitological data and results of the serological assessment. Assessment done with the merozoites antigen will be gave best result with examined animals if infected or none.

Also it was observed cross reaction with the heterologous species of Sarcocystis and T. gondii **(Savini et al., 1994)** Blood serum samples were collected from the 300 water buffaloes. Aged ranged between 5 months to 7 years, and were slaughtered at the Ahvaz abattoir, Iran.

One hundred and seventy-one (57%) animals were found to be positive for Sarcocystis bradyzoites by microscopic examination, while One hundred and sixty-three (54.3%) serum samples were positive for Sarcocystis antibodies in the ELISA **(Masoud et al., 2007).** Ocular muscle, esophagus, diaphragm and heart samples collected from 100 slaughtered cattle at Assiut abattoir, Egypt were examined grossly, microscopically and serology.

The total infection rate was found to be 94% by microscopically methods, but serological examination of sera from the same animals by (ELIZA) show that the infection rate was 98% **(Fatema et al., 2008).** Other study in Assiut province, Egypt on the prevalence of Sarcocystis in buffaloes by using 90 buffalo's samples, revealed the prevalence of Sarcocystis infection was 94.4% **(Metwally et al., 2014).**

1.9.3.5 Electron microscopy

Electron microscope has been used for multiple years to diagnosis ***Sarcocystis*** interspecies genus. Light microscopy is useless, because unable to determine the visible differences among species **(Dubey et al., 1989)**. Disadvantage of induced the electron microscopy, coming from the time need and the cost of this technique was expensive **(Pritt et al., 2008)**. Five hundred New-Zealand beef originated from slaughtered cattle was tested by electron microscope for identification of Sarcocystis infection. Result show that all cattle samples were positive, as well as both thin and thick walled cyst were detected with prevalence rate 98% and 79.8% respectively **(Böttner et al., 1987; zuo, 1992).**

Odening et al. (1995) found ***Sarcocystis cruzi***, ***S hominis*** and ***S. hirsuta*** were classified and compared between the two technique of light microscope (LM) and transmission electron microscopy (TEM); the result didn't observed morphological differences in the three forms of Sarcocystis from each of the three host species. The morphological criteria of the three species were discussed and, partly, newly defined. Additionally, a study find that S cruzi can be easily diagnosed and will be distinguished from other cattle ***Sarcocystis spp***.

1.9.4 Molecular diagnosis

Different molecular tools have been developed and used to detect and distinguished of Sarcocystis spp., and to assess the genetic variety among this protozoan at different population and host. Most common molecular marker used was based on Ribosomal DNA sequences, which determine the differentiation between ***Sarcocystis species***. Now a day the molecular diagnosis described as most significance method for diagnosis the ***Sarcocystis***, because PCR test assumed as high specificity technique **(Zhao et al, 2001; Hettiarachchi and Rajapakse, 2008; Rosenthal et al., 2008; Kia et al., 2011; Sisay et al., 2015; Tong et al., 2018).** The

excellent sensitivity suggested that rRNA based probes are capable of identification of individual protozoan, on other hand it can detectable low levels of coccidial infections, which couldn't done by other methods (Gajadhar et al,. 1992). Yang et al. (2001) extracted DNA templates by isolates of Sarcocystis hominis similar cyst collected from cattle and water buffalo, as well as from

S. fusiformis cysts and S. suihominis cysts. 18SrRNA genes were amplified, mutual with their morphological structure, these sequence data indicated that cattle and water buffaloes have the same species from 4 isolate.

Study in Yannan, China in 2002, for comparison the identity of S. cruzi isolate from 15 samples of water buffaloes and 10 samples from cattle examination done by PCR based REFLP analysis. The Result registered all 12 restriction enzyme from both isolate are identical, so this study improved that water buffaloes can able to be an intermediate host for S. cruzi **(Lie et al., 2002).** Other study based on RFLP-PCR and partial sequence analysis of 18S rRNA gene of Sarcocystis, which obtained from water buffaloes. Both of them revealed that the entire positive Sarcocystis sample represented S. fusiformis **(Oryan et al., 2011).**

Molecular biology of Sarcocystis investigated with RAPD-PCR (Random amplified polymorphic DNA polymerase chain reaction). After obtaining parasitic DNA and optimizing the PCR conditions, five primers were selected to amplify the DNA of each ***Sarcocystis species*** and various ***Sarcocystis species*** were detected and differentiated

(Gulcu et al., 2004). 18S rRNA gene found as a suitable target to classified ***Sarcocystis spp***. with closely related one and, also, to do the phylogenetic analysis **(Jeffries et al. 1997; Kia et al., 2011; Tamura et al., 2013)**. Additionally, the data based on phylogenetic studies of ***Sarcocystis species*** were increased at recent years, which supply a rich source of sequence that can be exploited to develop specific PCR **(Fischer and Odening, 1998).**

Gross examination conducted of both S. fusiformis and S. buffalonis by meat inspection in slaughter house, and then detected the parasite by histological test, followed by molecular technique based on analysis of 18S rRNA **(El-Seify et al., 2014).** Various molecular techniques such as PCR and its variants based on sequence changes have been used in recent times, for regarding the sensitivity and rapidity to determine the genetic variety among abundant parasites, phylogenetic and taxonomic studies and in epidemiological mapping **(Maurer, 2011)**

1.10 Prevention, control and treatment

Parasite control is difficult to impossible, but attempts have been made to prevent animal pasture / stables, water and feed to become contaminated by feces from dogs, foxes, cats, man and other definitive hosts **(Vangeel, 2012).** To reduce the spread of the disease and to control it the infected meat must be destructed and prevent its arrival to the final host, for humans freezing or food cooking of meat is considered the best means to eliminate the disease **(Dubey and Lindsay, 2006).**

Treatments with 2% chlorhexidine, 5% O-benzyl-p-chlorophenol, bleach (10%, 20%, and 100%), 12.56% phenol, 1% betadine, 10% formalin and 6% benzyl ammonium chloride, there were not effective in killing sporocysts. However, treatment with undiluted ammonium hydroxide (29.5% ammonia) for I hour killed sporocysts **(Dubey et al., 2003).** When animal dead, it should never be left for carnivores to eat, also

uncooked meat should never be fed to dogs and cats. Available vaccine is not discovered tell know in both intermediate and definitive hosts against sarcocystosis **(Dubey and Lindsay, 2006).**

Preventing this disease can be conducted by following:

- Exclusion of the final host of the herd.
- Final host prevent feeding on the row meat or dairy waste
- Burning dead herd
- Attention to cleaning and separation of infected animals to take treatment and return after confirmation of the final disposal of the disease (Shekarforoush et al., 2005).
- Examination of imported meat and slaughtered animals.

Chapter Two
Discussion

2.1 Infection rate of *sarcocystosis*

sarcocystosis has been reported to be of worldwide in occurrence and known to cause abortion, reduce milk yield, neurological signs, loss of weight, and even death (fatal cases) in cattle depending on the species, animal immunity, environment and number of sporocysts ingested **(Soulsby, 1982; Dubey et al., 1989).** Intramuscular ***Sarcocystosis*** would be suspected based on various combinations of criteria including persistent myalgia, episodic weakness, subcutaneous nodules, dermatomyositis, eosinophilia, and elevated muscle creatinine kinase levels **(McLeod et al., 1980; Arness al., 1999).**

Some ***Sarcocystis spp***. are important pathogens of domestic and wild animals. These parasites are characterized by an obligatory two-host life cycle, the formation of ***Sarcocystis*** mainly in the muscles of the intermediate hosts and endogenous sporulation of oocyte in the intestine of the definitive hosts **(Moré et al., 2014; Dubey et al., 2015a)**. ***Sarcocystis*** can cause zoonotic infectious disease which is considered an important public health problem by infected with S hominis which considered to be as one of the most important species in the zoonotic perspective. Furthermore, another species, S.

heydorni with a cattle-human life cycle has recently been described **(WHO 1981; Dubey and Lindsay, 2006; Vangeel et al., 2007; Fayer et al., 2015; Dubey et al., 2015b).** So the identification of the presence of pathogenic Sarcocystis spp. in meat consumed by humans should be included in the monitoring systems seeking human health protection **(Dubey and Odening 2001; Taylor et al., 2010).**

Highest prevalence rate of ***Sarcocystis spp***. infected cattle registered at Baghdad in our study 70.5% in slaughtered cattle and 64% in imported beef which investigated by

traditional methods, while it registered 72.5% in slaughtered cattle and 69% in imported beef samples by molecular study. These similar to previously results which had been registered a high prevalence infection rate in other geographical locations of Iraq revealed that the rate of Sarcocystis spp. infection in the cattle 45% **(Al-Taai et al., 2009).** Earlier, Latif et al. (1999) record 97.8% ***Sarcocystis*** infected the Iraqi cattle. In Diala province study found the spread of both macrocystis and microcystis when they investigated the muscles tissue samples of 179 cattle and it was 2.23% and 81% respectively **(Al-Taai, 2002)**. Meanwhile, in other world countries have similar result, which registered 90% in Mongolia's cattle **(Fukoyo et al., 2002)**, 99.5% in cattle of Argentina **(More et al., 201 1),** 66% rate of Sarcocystis-infection among cattle in the Hungary **(Såndor et al., 2015),** also present that can be isolate **Sarcocystis** and / or cyst fragments (5-15 per sample) from each infected animal in three southwestern Romanian counties **(Imre et al., 2019).**

Both slaughtered cattle and imported beef have highly infection by microcytic type 67% and 51% respectively, these analogous with studies show, cattle mainly affected by microcystis type, 82.4% in the cattle of Australia was infected by microcystis **(Savini et al., 1992).** In Iran, the prevalence of Sarcocystis infections has been reported to be high in domestic animals including cattle **(Shekarforoush et al., 2005; Dalimi et al., 2008)**, also infection rate in cattle Sarcocystis were microscopically identified (90.47%) at Qena Governorate, Egypt **(El-kady, 2018).** Study finding in Zaire show high prevalence (42.5%) of ***Sarcocystis observed*** in cattle **(Obijaku, 2012).** Out of 120 cattle examined in Selangor, Malaysia 49 (40.8%) harbored the microscopic type of ***Sarcocystis spp.*** **(Latif et al., 2013).** Overall Sarcocystis spp., infection rate among

slaughtered cattle in Béja region (North-West Tunisia) was high 3640% **(Safa et al., 2016).** Also a high prevalence of infection was established in cattle (44.9—98.1%) in animals slaughtered for food in Lithuania **(Januskevicius et al., 2019).**

Usually the raw or undercooked meat containing tissue cysts are a source of the sarcocystis and related coccidian cyst-forming infections for humans in addition to carnivores animals, which shed large amounts of oocysts infecting human in addition to domestic animals and other herbivores. Therefore, many researchers attempt to isolate tissue cyst- forming coccidian parasites such as Sarcocystis spp. and Toxoplasma gondii from the meat samples because of several reasons include introducing some basic concepts about infectious diseases, cell biology of the parasites, teaching food safety and serological and molecular studies **(O'Donoghue and Rommel, 1992; Eggleston et al., 2008; Hussein et al., 2017).**

The explanation for the high prevalence of ***Sarcocystis*** infection could be due to animals management system that practiced by cattle owners where they allowed to roam about and scavenge for food, in this process they pick up the sporocysts of ***Sarcocystis*** shed by different definitive hosts such as dog, cat, humans and non-human primates thus resulting to Sarcocystis formation in the muscle of the cattle **(Dubey et al., 1989; Acha, and Szyfres, 2003; More et al., 2011).**

Water contamination with sporocysts of ***Sarcocystis*** from a carnivore or omnivore or foods washed or irrigated with contaminated water is the most likely source of sporocysts infection **(Fayer, 2004).** The SPOr0cysts can resist and retain their infectivity in the environment for different external factors (e.g., freezing, high temperature and several disinfectants), for a long period **(Duby et al., 2016)**

current findings showed that dogs play an important role in the distribution of the parasite cysts in livestock animals, because ***Sarcocystis*** in muscles of intermediated host, permanent existence of dogs in farms is very routinely in Iraq and the farms can be contaminated by a great amount of the parasite cysts which passed from infected dogs. Dogs are known as definitive hosts for some of the microscopic species of Sarcocystis **(Hilali et al., 2011; Hornok et al., 2015).**

Most species of ***Sarcocystis*** transmissible via cats have been found less frequently than those transmissible via canids, because cats are poor producers of ***Sarcocystis*** sporocysts, another reason may be that ***Sarcocystis*** of feline transmitted species require several months or years to become infective, another reason may be that some host species are inherently more susceptible to infection with some agents than others **(et al., 2010)**. The increased prevalence rate is due to the increased source of parasite sporocysts in pasture of cattle from definitive hosts. Similarly very high rate of prevalence between

%100-91was also reported across the globe such as United States **(Fayer and Dubey 1986),** South-West of Iran **(More et al., 2008),** Argentina **(Hamidinejat et al•, 2010),** Southern Italy **(Bucca et al., 2011; Chiesa et al., 2013),** Karnataka, India **(Dafedar et al., 201 1),** Germany **(Moré et al., 2014),** and Hungary **(Hornok et al., 2015)**. This is first study in Iraq which isolated Sarcocystis from imported beef meat; the IRIPorted beef in Iraqi market coming from different sources mainly India. Researchers have shown the cyst can persist for months or years in the tissues of the intermediate hosts tissues **(Fayer, 2004).** Many study on frozen beef used traditional methods for identification and characterization of Sarcocystis species based on isolated cyst

morphology using gross examination and light or transmission electron microscopy **(Nourollahi-Fard et al., 2009; Dubey et al., 2015a; Mohamed et al., 2016)**. Recently, molecular methods have been found very useful and sensitive in detecting Sarcocystis spp. in the samples **(Xiang et al., 2011; Stojecki et al., 2012; Hamidinejat et al., 2015; Gjerde, 2016)**. There are difficulties to calculate economic losses in present time, caused by microscopic Sarcocystis affected domestic animals, since it is difficult to diagnose clinical ***Sarcocystosis*** **(Chiesa et al., 2013).**

2.2 Traditional examination

Sarcocystis bradyzoites were microscopically detected in the current study by means of squeezing, pepsin digestion and blender technique. The bradyzoites presented as banana form with a spiked end of front and rounded rear end and slightly clear nucleus located near the rear end, bradyzoites were elongated and packed, so it was difficult to measure them. Additionally by trichnoscopy test, cysts show oval, elliptical Or elongated forms. Similar Fresh cysts were seen as fusiform-shaped microscopic cysts, Parallel to muscle fibers **(Fatma et al., 2008).**

All traditional methods (pepsin digestion, squeezing, blender and trichnoscopy) in our study had different sensitivities ($P<0.05$) like the findings of Latif et al. (1999) who compared these methods in different animals and agreed with all researches indicated that pepsin digesting method is sensitive test for this purpose **(Shekarforoush et al., 2005; Al-Hasnawi, 2008).**

Additionally, Mangas et al. (2015) compared two techniques of muscle digestion and scarification for detecting ***Sarcocystis species*** in beef cattle by collected 400 samples from tongue and heart of slaughtered cattle and analyzed them by pepsin digestion and scarification and described that the pepsin digestion was best than scarification for tongue samples and suggested that scarification of the heart can be used as an alternative to the digestion technique for the diagnosis of cattle infection by ***Sarcocystis*** with the advantage of being easy to perform and inexpensive.

Pepsin digestion method considered gold standard for diagnosis of ***Sarcocystis*** genus has been proven to be more sensitive than squeezing method and trichnoscopy, tissue digestion was found to be more efficient in detecting bradyzoites in tissues than histology, also is the most sensitive method to detect light ***Sarcocystis*** infection because several hundred or thousands of bradyzoites are released from Sarcocystis in the host tissue and ***Sarcocystis*** are digested making the detection of the parasites easier **(Mowafy, 2003; Hamidinejat et al., 2010; Ufuk et al., 2018).**

First use blender technique in our study to isolation the bradyzoites. This technique was identified as high sensitive technique for diagnosis the tissue parasite, (68% in slaughtered cattle and 61% in imported beef) as well as its less expensive COmpared with pepsin digested method because the technique lead to concentration of ***Sarcocystis*** in fresh sample **(Silva et al., 2002).**

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