 http://dx.doi.org/10.14500/aro.10150

Prevalence of *Sarcocystis* Species (*Sarcocystis ovicanis* and *Sarcocystis capricanis*) in Tongue Muscle of Sheep and Goats in Duhok Province, Kurdistan Region, North Iraq

Ihsan K. Zangana[1] and Shivan N. Hussein[2]

[1]Department of Animal Production, College of Agriculture, University of Duhok, Duhok, Kurdistan Region - F.R. Iraq

[2]Department of Medicine and Surgery, College of Veterinary Medicine, University of Duhok, Duhok, Kurdistan Region - F.R. Iraq

***Abstract*–*Sarcocystis* species are coccidian protozoan parasites of the phylum apicomplexa. The prevalence of *Sarcocystis* species (*Sarcocystis ovicanis* and *Sarcocystis capricanis*) in tongue muscle of sheep and goats that collected from abattoir and outside of abattoir (at Butchers Markets) in Duhok province was determined for the first time during the period of 4 months from September 01, 2014, to January 03, 2015. Three techniques were applied for this purpose. Direct scotch cellophane adhesive tape test, muscle mincing and squash method, and pepsin digestion technique. The overall prevalence was 97% in sheep and 100% in goats. No significant difference (p>0.05) was obtained between male and female of both species. Histopathological analysis revealed different size and shape of microcysts with thick cyst wall, in addition to mild histological changes.**

***Index Terms*–Microcysts, *Sarcocystis* species (*Sarcocystis ovicanis* and *Sarcocystis capricanis*), Tongue muscle of sheep and goats.**

I. Introduction

Sarcocystis species are cyst forming sporozoan parasites with an obligatory 2-host cyst cycle, involving carnivorous as definitive host and herbivorous and omnivorous as intermediate hosts (Dubey, et al., 1989a). *Sarcocystis* species are common parasites with worldwide distribution in man and many species of animals. They infect skeletal muscle, cardiac muscle, and smooth muscle (Fayer, 2004). Four species of *Sarcocystis* have been identified from domestic sheep including *Sarcocystis ovicanis (Sarcocystis tenella)* and *Sarcocystis arietcanis* are pathogenic species, form microscopic cysts of sarcocystis and are transmitted through canids, as well as *Sarcocystis ovifelis (Sarcocystis gigantea*) and *Sarcocystis medusiformis*, are non-pathogenic species of *Sarcocystis*, form macroscopic cyst and are transmitted through felids (Dubey, et al., 1989b; Hosseini, et al., 2012; Hamidininejet, et al., 2012). There are three reported species of *Sarcocystis* in domestic goats; *Sarcocystis capricanis* and *Sarcocystis hircicanis* produce microscopic pathogenic and *S. caprafelis* produces macroscopic non-pathogenic cysts (Dubey, et al., 1989). Merogony and cyst formation (A sexual stage) take place in the intermediate host. Gametogony and sporogony (sexual stage) take place in the definitive host. Most pathogenic *Sarcocystis* spp. causes disease only in their intermediate hosts not in their definitive hosts (Dubey and Lindsay, 2006).

In general, the *Sarcocystis* species that are transmitted via canids or primates are more pathogenic than those transmitted by felids. In intermediate hosts, the reduction of wool quality and milk yield, abortion, central nervous system signs, and death may result (Fukuyo, et al., 2002). This paper planned on a study of prevalence and histopathological changes of *Sarcocystis* species in infected tongues of sheep and goats slaughtered inside and outside of abattoir in Duhok governorate for the first time.

II. Materials and Methods

A total of 129 muscle samples of tongues including 81 sheep (48 male and 33 female) and 48 goats (25 male and 23 female) ranged their age from 1 to 3 years old were randomly collected from slaughterhouse and those animals slaughtered at outside of the slaughterhouse (from butchers markets) during the period of 4 months from September 01, 2014, to January 03, 2015, for the first time in Duhok province. All tongues before taking samples were inspected thoroughly for revealing macroscopic cyst. A small piece of each muscle from tongue was taken and kept in a

ARO-The Scientific Journal of Koya University
Volume V, No 1(2017), Article ID: ARO.10150, 5 pages
DOI: 10.14500/aro.10150
Received 11 June 2016; Accepted 30 January 2017
Regular research paper: Published 13 May 2017
Corresponding author's e-mail: shivan.hussein@uod.ac

clean plastic tube for histopathological section and further examination of microscopic cyst of sarcocystosis.

III. Methods for Detection of Microcysts

In the Laboratory of Clinical Pathology/College of Veterinary Medicine/Duhok University, the collected samples of tongue were prepared for examination. The following examinations were done.

A. Direct Scotch Cellophane Adhesive Tape

This method was simply performed and it was a newly modified method described by Hussein (2015). About 10 cm of scotch tape on sticky surface was put on the small pieces of cut surface of tongue tissue, and firmly pressed. Then, the tape applied on a clean microscopic slide and examined under light microscope using low and high power (×10 and ×40) for revealing different sizes of tissue cysts (bradyzoites).

B. Muscle Mincing and Squash Method

A modified method which described by Juyal, et al. (1989), was used in this study. A small piece about 2 g of tongue tissue was finely teared by sterile scissor and scalpel in a clean glass watch, then after mincing it mixed with 15 ml of saline buffer pH 7.2. The obtained suspension sifted and squeezed through three layers of surgical gauze. Several small drops of suspension were examined immediately under light microscope (×10, ×40). Dry smears from suspension were prepared and fixed with methanol then stained with 7% of Giemsa stain.

C. Acid Pepsin Digestion Test

A method which described by Dubey, et al. (1989) was used. 50 g of tongue tissue was minced then added into the digested solution (1.3 g pepsin, 3.5 ml HCL, and 2.5 g NaCl in 500 ml of D.W). It thoroughly mixed and then incubated for 30 min at 40°C. The digested muscle sifted via three layers of surgical gauze to remove undigestible particles. The filtrated solution centrifuged for 5 min at 3000 rpm. The sediment samples were re-suspended by adding 5 ml of buffer saline pH 7.2. Several drops were examined under (×10, ×40) light microscope and also dry smears fixed and stained with 7% Giemsa stain were prepared.

D. Histopathological Examination

The tongue muscles were fixed in 10% of neutral buffered formalin and then dehydrated by different concentration of ethanol. They embedded in paraffin and 5 µm in thickness sections were prepared and stained with hematoxylin and eosin (H and E), Giemsa stain and periodic acid-Schiff (PAS) stains then examined under light microscope (×40) (Boncroft and Stevens, 1999).

IV. Results

No macroscopic cyst of sarcocystosis was observed in all examined tongue samples in both sheep and goats by naked eye inspection. Microscopic type of *Sarcocystis* was revealed by different techniques. The overall prevalence of sarcocystosis was 97.5% in sheep and 100% in goats which recorded in this study (Table I). No significant difference ($p>0.05$) was observed in infection rate between male of sheep 95.8% and male of goats100% and female of sheep and of goats 100%.

The sensitivity of three techniques which applied to demonstrate the microscopic cyst of sarcocystis in sheep was appeared 100% by both acid pepsin digestion and mincing and squeezing method, while it was 92% by scotch cellophane adhesive tape. In goats, the sensitivity of the three techniques was 100% (Table II).

The intensity of microcyst was estimated by the number of cysts in each microscopic field. The mean number of the intensity calculated out of 60 microscopic fields (Table III). The number was higher in sheep 33.1 cyst for each field than in the goats 16.5 cysts for each field.

The different shapes of microcysts including oval, spindle, and elliptical shape found by scotch adhesive tape and by mincing and squeezing method (Fig. 1). Typical crescent shapes of bradyzoites observed by pepsin digestion technique in both animals (Fig. 2).

TABLE I
Overall Prevalence and Distribution of Infection between Male and Female of Sheep and Goats

Sex	Sheep			Goats		
	Number of exam	Positive	%	Number of exam	Positive	%
Male	48	46	95.8	25	25	100
Female	33	33	100	23	23	100
Total	81	79	97.5	48	48	100
Overall	129					

TABLE II
The Sensitivity Rate of the Three Techniques (APD, CAT and MSM)

Animal	CAT			MSM			PDT		
	N	Positive	%	N	Positive	%	N	Positive	%
Sheep	24	22	92	41	41	100	16	16	100
Goat	13	13	100	25	25	100	10	10	100
Total	37	35	95	66	66	100	26	26	100

CAT: Cellophane adhesive test, MSM: Mincing and squash method, PD: Pepsin digestion technique

TABLE III
The Intensity Rate (Number of Cysts/Field) of Microcysts in Sheep and Goats

Animal	Tongue	
	Number of cysts/60 fields	Number of cysts/field
Sheep	1986	33.1
Goats	992	16.5

TABLE IV
The Size of Microcysts (Mean±SD) of Tongue in Sheep and Goats

Animal	Number of cysts examined	Mean±SD	
		Length	Wedth
Sheep	65	12.9±1.4	7.4±0.7
Goats	65	11.3±1.2	7.2±0.8

SD: Standard deviation

http://dx.doi.org/10.14500/aro.10150

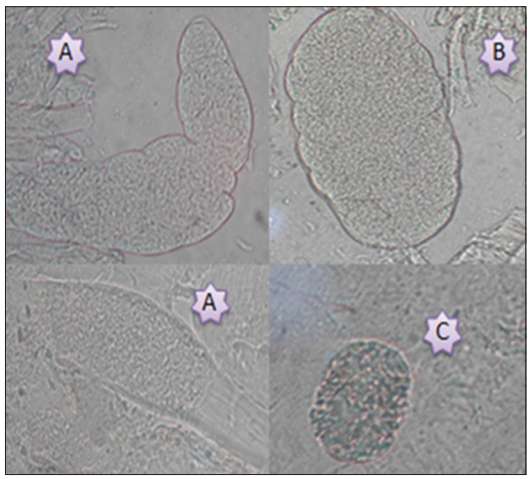

Fig. 1. Scotch adhesive tape method. (A) Spindle cyst, (B) elliptical cyst, (C) rounded cyst

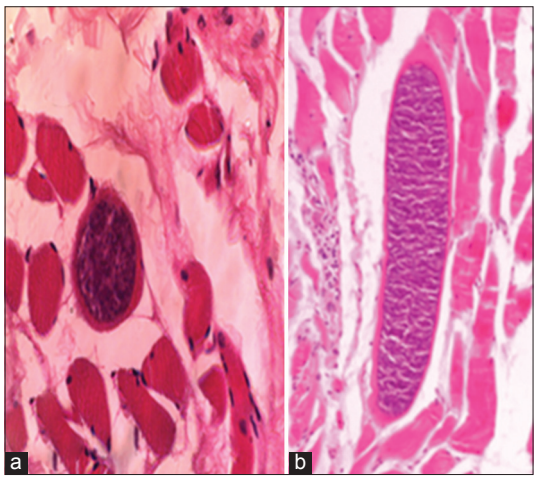

Fig. 3. Histopathological sections. (a) Oval shape, (b) spindle shape of microcysts (×40)

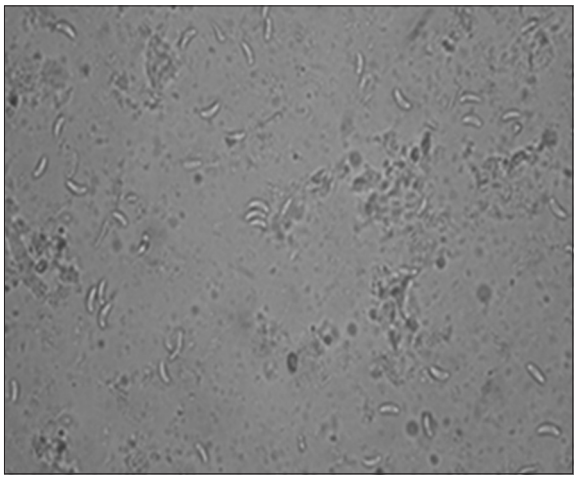

Fig. 2. Crescent shape of bradyzoites by peptic digestion and Giemsa staining (×40)

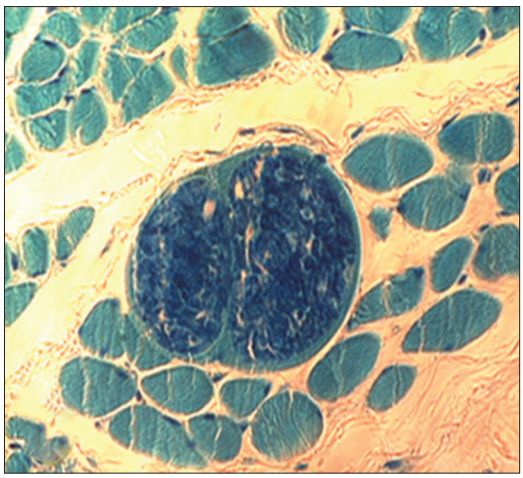

Fig. 4. Microcyst impacted with bradyzoites stained by giemsa (×40)

The size of microcyst was measured using ocular micrometer. There was no statistically difference ($p>0.05$) in mean±standard deviation (SD) of length and width of cysts in both sheep and goats. The measurement size of cysts was 12.9±1.4 µm in length and 7.4±0.7 µm in width in sheep, while the length 11.3±1.2 µm and the width 7.2±0.8 µm of cysts was in goats (Table IV).

Histologically, high intensity of different shape and size of cysts were found between muscle fibers of tongue by H and E technique (Fig. 3). The intact cysts were impacted with numerous bradyzoites seen by giemsa technique (Fig. 4). Obvious degenerated muscle fibers surrounded the tissue cysts with slightly infiltration of mononuclear cells were seen in Fig. 5. The intact thick wall around the cysts was positively stained with PAS technique (Fig. 6).

V. Discussion

Sarcocystis occurs either as microscopic or macroscopic cyst in striated muscles and sometimes in non-striated muscles (Dubey, et al., 1983). Through inspection of all tongues, no macrocyst was observed in this survey. Similarly, no data recorded the presence of macroscopic cyst in tongue, while this type of cyst prevalent on esophagus and diaphragm of several animals. Beyazit et al. (2007) recorded the highest prevalence of macrocyst in esophagus in all age groups of sheep and goats in Turkey, and also Barham et al. (2004) found the highest rate of infection 99% in esophagus and lowest rate 3% in diaphragm of goats in Sulimania province. Latif, et al. (1999) found the rate of 4.1% and 33.6% in goats and sheep, respectively, in Baghdad region. In Duhok province, Hussein (2015) found the rate 1.2% and 2.6% in sheep and goats, respectively. Another type of *Sarcocystis* (microscopic cyst) was revealed in a high frequency in the tongues in this study. The rate of infection was 100% in goats and 97.5% in sheep. This high prevalence may have diversity with definitive host (dogs) and sporocysts contamination of water and food. Furthermore, various studies were reported the presence of microscopic cysts in organs of different animals mainly tongues. Morsy, et al. (2011) found the infection rate of microcyst 43% in Egyptian goats tongue. Dafedar, et al. (2008) found the prevalence rate 12.5% in tongue of goats in Bangalor, Karnataka state. Various diagnostic methods

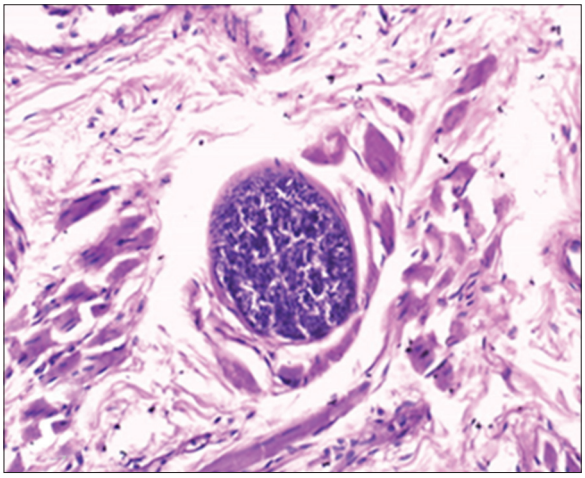

Fig. 5. Degenerated muscle fibers with mononuclear cells infiltration

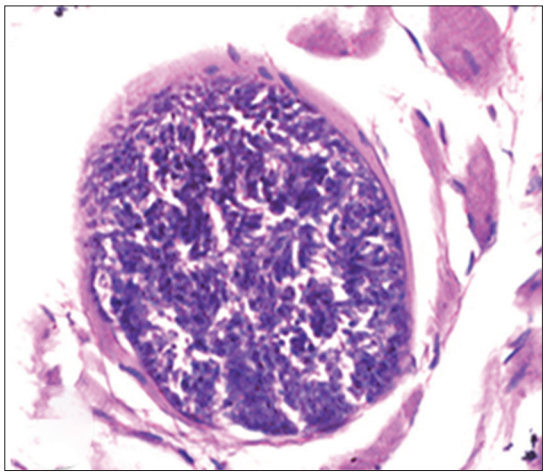

Fig. 6. Thick wall microcyst between muscle fiber stained with periodic acid-Schiff (×40)

were used for detection of sarcosporidiosis, which were had variability in sensitivity (Dubey, et al., 1988; Beyazit, et al., 2007). By pepsin digestion and muscle squash preparation, the high prevalence of microcyst was found 100%; this may be due to validity and reliability of these two tests; therefore, they considered as the gold standard tests in diagnosis of bradyzoites of sarcocystosis. Similar result was recorded by Dehaghi, et al. (2011), who found the prevalence of microcyst in goats 98% by impression smear and 100% by acid digestion technique. Histologically, analysis was revealed thick wall of microcyst in different intensity and in shapes with mild inflammatory infiltration. This indicated the presence of microcysts which is the most visible type by the procedures in chronic condition.

VI. Conclusion

This study considered the tongue of animals as a good sample in demonstration of microscopic cyst of *Sarcocystis* species in sheep and goats. The cellophane adhesive tape was a newly developed technique used in this study. It was simple, rapid and inexpensive but its sensitivity was lower than peptic digestion which considered as a golden standard test. There was no significant difference in the prevalence of sarcocystosis among intensity, measurement size and cell wall of microcysts between tongues in sheep and goats. For the first time, the estimation of intensity of infection (cysts/field) and measurement size of microcysts after staining with 7% giemsa was used in this study. Depending on the morphology, two suspected species of *Sarcocystis* were found including; *S. ovicanis* (*Sarcocystis tenella*) in sheep and *Sarcocystis capracanis* in goats which produce microscopic cysts of sarcocyst.

References

Barham, M., Stützer, H., Karanis, P., Latif, B. and Neiss, W., 2004. Seasonal variation in *Sarcocystis* spp. Infections in goats in northern Iraq. *Parasitology,* 130(02), pp.151-156.

Beyazit, A., Yazicioglu, O. and Karear, Z., 2007. The prevalence of ovine *Sarcocystis* spp. In Izmir Province. *Ankara Üniversitesi Veteriner Fakültesi Dergisi,* 54, pp.111-116.

Boncroft, J.D. and Stevens, A., 1999. *Theory and Practice of Histological Techniques*. 4th ed. Churchill Livingstone, London, pp.127-129.

Dafedar, A.M., D'Souza, P.E., Ananda, K. and Puttalakshmamma, G., 2008. Prevalence of sarcocystosis in goats slaughtered at an abattoir in Bangalore, Karnataka state. *Veterinary World*, 1(11), pp.335-337.

Dehaghi, M.M., Fathi, S. and Asl, E.N., 2011. Survey of *Sarcocystis* infection in slaughtered goats in Kerman Abattoir, Southeast of Iran. *Journal of Animal and Veterinary Advances*, 10(9), pp.1205-1208.

Dubey, J. and Lindsay, D.S., 2006. Neosporosis, toxoplasmosis, and sarcocystosis in ruminants. *Veterinary Clinics of North America: Food Animal Practice*, 22(3), pp.645-671.

Dubey, J.P. and Fayer, R., 1983. Zoonosis in practice. Sarcocystosis. *British Veterinary Journal*, 139, pp.371-377.

Dubey, J.P., Lindsay, D.S., Speer, C.A., Fayer, R. and Livingston, C.W., 1988. Sarcocystis arieticanis and other *Sarcocystis* species in sheep in the United States. *Journal of Parasitology*, 74, pp.1033-1038.

Dubey, J.P., Speer, C.A. and Charleston, W.A.J., 1989b. Ultrastructural differentiation between *Sarcocystis* of *Sarcocystis hirsuta* and *S. hominis*. *Veterinary Parasitology*, 34, pp.153-157.

Dubey, J.P., Speer, C.A. and Fayer, R., 1989. *Sarcocystosis of Animals and Man.* CRC Press Inc., Boca Raton, Florida, pp.113-120.

Dubey, J.P., Speer, C.A., Munday, B.L. and Lipscomb, T.P., 1989a. Ovian sporozoan encephalomyelitis linked to *Sarcocystis* infection. *Veterinary Parasitology*, 34, pp.159-163.

Fayer, R., 2004. *Sarcocystis* spp. In human infections. *Clinical Microbiology Reviews*, 17(4), pp.894-902.

Fukuyo, M., Battsetseg, G. and Byambaa, B., 2002. Prevalence of *Sarcocystis* infection in meat-producing animals in Mongolia. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 33(3), pp.490-495.

Hamidininejet, H., Jalali, M.H.R. and Nabavi, L., 2012. Survey on *Sarcocystis* infection in slaughtered cattle in South-West of Iran. Emphasized on evaluation of muscle squash in comparison with digestion method. *Journal of Animal and Veterinary Advance*, 9(12), pp.1724-1724.

Hosseini, S.R., Shakerian, A. and Tahamtan, N., 2012. Survey of *Sarcocystis* infection in slaughtered sheep in Isfahan, Qom and Shahre-Kord, Iran. *Journal of Animal and Veterinary Advances*, 11(15), pp.2683-2686.

Hussein, S.H.N., 2015. Prevalence of *Sarcocystis* Infection in Small Ruminants (Sheep and Goats) and Experimental Infection in Dogs and Cats in Duhok Province. MSc Thesis Submitted to Council of College of Veterinary Medicine, University of Duhok.

Juyal, P.D., Ruprah, N.S. and Chhabra, M.B., 1989. Rapid isolation of intact micro-sarcocystis (*Protozoa-Apicomplexa*) cysts from muscular tissue. *Indian J Animal Health*, 28, pp.69-70.

Latif, B., Al-Delemi, J., Mohammed, B., Al-Bayati, S. and Al-Amiry, A., 1999. Prevalence of *Sarcocystis* spp. In meat-producing animals in Iraq. *Veterinary Parasitology*, 84(1), pp.85-90.

Morsy, K., Saleh, A., Al-Ghamdi, A., Abdel-Ghaffara, F., Al-Rasheid, K., Bashtar, A.R. and Mehlhorn, H., 2011. Prevalence pattern and biology of *Sarcocystis capracanis* infection in the Egyptian goats: A light and ultrastructural study. *Veterinary Parasitology*, 181(2), pp.75-82.