## Natural heavy infection with immature sarcocysts of *Sarcocytis spp*. in sheep in Mosul city: A case report

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(Received September 27, 2019; Accepted November 24, 2019; Available online July 23, 2020)

#### Abstract

This study included recording the natural heavy infection with immature macrocysts (Sarcocysts) of *Sarcocytis spp.* in sheep. The sheep is one years old which is slaughtered at butcher shop at Mosul city in May 2018. This is the first case recorded of natural infection with immature sarcocysts of *Sarcocystis spp.* in Mosul city. Many of small nodules were observed during slaughter, these nodules are seen within esophageal muscles in different sizes and shapes, they were distributed randomly throughout esophageal muscles. Most of the sarcosystis were small in size the mean of size between 20- $28 \times 28-42$  µm they were histological examination showed that presence of only metrocytes. This confirmed the diagnosis that the sarcocysts were immature macrocysts (sarcocysts) for the *Sarcosystis spp.* In our study, heavy infected case with Sarcocystis reveals the fact that large numbers of cats(final hosts) in contact with sheep in pastures is considered the main risk factor for infection and feed with raw meat from infected sheep, which is very important for carcass condemnation when the meat inspection when abnormalities are found which indicate that the part of carcass, is unfit for human consumption it is condemned, which means the economic loss for livestock.

Keywords: Sarcocysts, Sarcocytis spp, Sheep, Heavy infection, Natural

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# حالة إصابة طبيعية شديدة بالأكياس العيانية غير الناضجة لطفيلي .Sarcocytis spp في الأغنام في مدينة الموصل: تقرير حالة نادية سلطان الحيالي'، منال حمادي حسن'، كرم هاشم الملاح' فرع الأحياء المجهرية، ' فرع الأمراض وأمراض الدواجن، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

#### الخلاصة

تضمنت الدراسة تسجيل حالة ولأول مرة في الضأن إصابة طبيعية شديدة بالأكياس العيانية غير الناضجة لطفيلي .Sarcocystis spp في ضأن بعمر حوالي سنة ذبح في احدى محلات القصابة في مدينة الموصل في شهر أيار عام ٢٠١٨ حيث تم ملاحظة أعداد كبيرة من الأكياس العيانية منتشرة بشكل عشوائي على طول عضلة المريء وبأشكال مختلفة بين البيضوي والكروي وبأحجام مختلفة ومعظم على خلايا العيانيات صغيرة الحجم حيث تراوحت بين ٢٠-٢٨ × ٢٨-٤٢ مايكرومتر ، وعند فحص المقاطع النسيجية لهذه الكياس تبين احتوائها على خلايا الأم العيانية منتشرة بشكل عشوائي على طول عضلة المريء وبأشكال مختلفة بين البيضوي والكروي وبأحجام مختلفة ومعظم الأكياس كانت صغيرة الحجم حيث تراوحت بين ٢٠-٢٨ × ٢٨-٤٢ مايكرومتر ، وعند فحص المقاطع النسيجية لهذه الكياس تبين احتوائها على خلايا الأم Metrocytes spp فقط مما يؤكد تشخيصها بانها اكياس عيانية غير ناضجة لطفيلي . الحالة الشديدة الإصابة بطفيلي .Sarcocystis spp بانه يوجد اعداد كبيرة من القطط (مضائف نهائية للطفيلي) مع الأغنام أثناء الرعي بالحقول وبالتالي يؤدي الى الحصول على لحوم مصابة واثناء الفحص العياني بالمجزرة سوف تزال الاجزاء الغير مرغوب بها للاستهلاك البشري و هذا يعني خسارة القصادية للثروة الحيوانية.

#### Introduction

Sarcosporidiosis that has high prevalence in sheep causes micro and macrocystis (muscular sarcocysts) in skeletal and cardiac muscle related to the final hosts (dogs or cats) respectively. The Sarcocystis protozoan parasite belong to the phylum Apicomplexa, Family Sarcocystidae and the Sarcocystis species follow an obligatory two hosts life cycle, alternating between an intermediate host (preysheep) and definitive host (predator - dogs or cats). Both *Sarcocystis gigantea* and *Sarcocystis medusiformis* are responsible for developmental of macroscopic cysts in muscles of sheep (1-3). Although the infection of sarcosporidiosis in sheep are not zoonotic but the importance of the infection lies in the carcass condemnation is justified on the basis of the negative visual impact that cysts may have on the consumer (4-7).

#### Material and methods

Sheep at the first year of age, slaughtered at one of butcher's shop, through the microscopical examination of carcass found large number of small nodules in esophageal muscles were randomly distributed with in the esophageal muscles. The portion of esophagus about 40 cm were resected and transported to lab of parasitology for macroscopic examination sarcosystis of sarcosystis were counted and collected about 30 cysts then measured the cysts by ocular micrometer (8). Cysts were prepared for light microscopically by then fixed in 10% neutral buffered formalin. Processing for histological examination the cysts were embedded in paraffin wax, sectioned at 5  $\mu$ m and stained with H&E (9).

#### Result

Macroscopically, the immature macrocysts were detected by gross examination in the muscles of esophagus of sheep. The morphological randomly features of macrocyts were white, rounded or oval cysts heavily distributed throughout esophageal muscles. (Figure 1). Immature sarcocysts were approximately measured 33  $\mu$ m in length and 25 $\mu$ m in width (Table 1). Histologically, most of sarcocysts appeared to contain only metrocytes (Figure 2). According to the morphology and size of the cysts as well as microscopical examination most of cysts were immature sarcocysts in the esophagus.

Table 1: The size of immature macrocysts in esophagus of a sheep (n=30 cysts)

Width		length	
Mean ±D	Rang	Mean ±SD	Rang
25 ±2.7	20-28	$33 \pm 5.1$	28-42

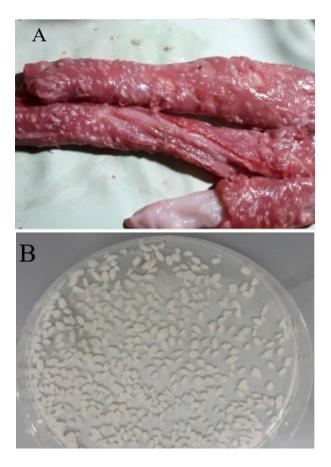


Figure 1: Esophagus with heavy infection macrocystes (A), white rounded and oval cysts (B).

#### Discussion

Due to difficulty of diagnosing of Sarcosporidiosis in living sheep and the difficulty of examining whole carcass by veterinarians in private butcheries and in our study the only heavy infected sample (esophagus) is difficult to be obtained in other infected muscles.

Most species of Sarcocystis that infect domestic animals are specific species for the intermediate host (10). Sheep are intermediate hosts for the Sarcocystis, *Sarcocystis gigantea* and *Sarcocystis medusiformis*, which are transmitted by felids (11). In our study, heavy infected cases with Sarcocystis reveals the fact that large numbers of cats in contact with sheep pastures is considered the main risk factor for infection and supply of raw meat from infected sheep carcasses which contributes significantly to the propagation of Sarcosporidiosis by Sarcocystis (12).

Our study also showed that the infection only with heavy immature macrocysts in young sheep at one year old might probably occurred by either ingested (high dose) of oocysts or sporocysts of Sarcocystis at the same time or at short exposure time with grass contaminated grass with faeces of the infected cats; or due to slow or gradual development of immature macrocysts (13,14) these results confirmed by (15-17) who mentioned that immature Sarcocystis developed in the esophagus and tongue from 1.3 to 4 and 6 months post infection in their experimental study with *Sarcocystis gigantea* and *Sarcocystis medusiformis*. Our morphological study revealed that immature cysts vary in shape and size it appears at the round or oval and size ranging from 20-28 x 28-42  $\mu$ m, this is identical to study done by (14-19).

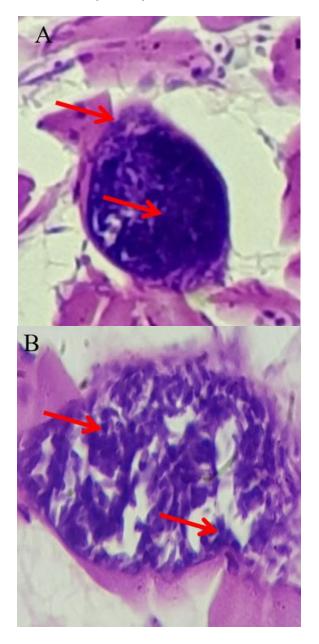


Figure 2: Immature macrocysts of *Sarcocystis spp.* contain only metrocystes. H&E, 200x (A), 560x (B).

While our histological study of macrocysts approved that all of macrocysts contain only meterocytes similar to observations by (15) his result revealed that firstly in the cross section of immature cysts contain only metrocytes, while mature cysts containing both metrocytes and merozoites and he added that metrocytes which appeared are closely packed, vesicular cells with definite basophilic nuclei in hematoxylin and eosin stained section, additionally the cyst wall of the immature macrocysts was not measurable at light microscopy of which is important for the differentiation between the species of sarcocysts (10,14,20).

According to (21) the veterinarians working as official meat inspectors at the abattoir, who systematically examined the external surfaces of the carcass, esophagus, heart, diaphragm, peritoneum and pleura to detect macrocysts Sarcocystis species. According to the abovementioned legislation, partial carcass condemnation when one or more cysts in one organ in this case it is trimmed off. While total execution performed when macrocysts were found in two or more organs (20).

#### Conclusion

The natural heavy infection with immature macrocysts (Sarcocysts) of *Sarcocytis spp*. in sheep. Most of the macrocysts (Sarcosysts) were small in size the mean of size between 20-  $28 \times 28$ - $42 \mu m$  they were histological examination showed that presence of only metrocytes. This confirmed the diagnosis that the sarcocysts were immature macrocysts (sarcocysts) for the *Sarcosystis* in sheep. It should be the veterinarians working as official meat inspectors at the abattoir, who systematically examined the external surfaces of the carcass, such as esophagus, partial organ condemnation when one or more cysts in one organ in this case it is trimmed off.

#### Acknowledgments

The authors are very grateful to the University of Mosul/College of veterinary medicine for their provided facilities, which helped to improve the quality of this work.

#### **Conflict of interest**

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

#### References

- Dubey JP, Calero-Bernal R, Rosenthal BM, Speer CA, Fayer R. Sarcocystosis of animals and humans. 2<sup>nd</sup> ed. CRC press. New York: Taylor & Francis Group; 2016. 1-20 p.
- Al-Hayali NS, Daood MS. Study the occurrence of ovine sarcocystosis in the abattoir of Mosul city. Iraqi J Vet Sci.

2002;16(2):125-132.

- Al-Hyali NS. Seroprevalence and toxicity of *Sarcocystis gigantea* in different hosts in Nineveh province [PhD dissertation]. Mosul: College of Veterinary Medicine, University of Mosul, Iraq; 2006. 1-3 p.
- Fayer R, Dubey JP, Bovine Sarcocystosis. Compendium Food Animal. 1986;8: 130-142.
- Dubey JP, Leek RG, Fayer R. Prevalence, transmission, and pathogenicity of *Sarcocystis gigantea* of sheep. J Am Vet Med Assoc.1986;188(2):151-154.
- Tenter AM. Current research on Sarcocysts species of domestic animals. Int J Parasitol. 1995;25(11):1311-1330. https://doi.org/10.1016/0020-7519(95)00068-D
- Al-Hyali NS, Kennany ER, Al-Taei AF. Effect of lysate of Sarcocystis gigantea in rats. Iraqi J Vet Sci. 2011;25(2):81-85. 10.33899/ijvs.2011.5653.
- Al-Hyali, NS. Study the occurrence of ovine sarcocystis in Mosul city [MSc thesis]: Mosul: College of Veterinary Medicine, University of Mosul, Iraq; 1998. 20-21 p.
- Luna LG. Manual of histologic staining methods of the armed forces, Institute of pathology. 3<sup>rd</sup> ed. New York: Mc Graw - Hill Book company; 1968. 1-64, 236 p.
- Dubey JP, Lindsay DS, Speer CA, Fayer R, Livingston CW. Sarcocystis arieticanis and other sarcosystis species in sheep in the United States. J Parasitol. 1988;74(6):1033-1038. DOI: 10.2307/3282228
- Dehaghi MM, Sami MF and Radfar MH. Survey of Sarcosystis infection in slaughtered sheep in kerman Abattoir, Kerman, Iran. Com Clin Pathol. 2013;22(3):343-346. <u>10.1007/s00580-012-1414-9</u>
- Adriana T, Mircean V, Blaga R, Bratu CN, Cozma V. Epidemiology and etiology in sheep Sarcocystosis. Vet Med. 2008;65(2):49-54. http://dx.doi.org/10.15835/buasvmcn-vm:65:2:1522
- Mckenna PB, Charleston WAG. Recovery of Sarcocystis gigantea sporocystis from cat faeces. Vet Parasitol. 1988; 26:215-227. https://doi.org/10.1016/0304-4017(88)90090-8

- Beyazit A, Yazicioglu O, karaer Z. The prevalence of ovine sarcocystis species in Izmir province. Ankara Uni Vet Fak Derg. 2007;54:111-116.
- Munday BL, Obendorf DL. Morphology of Sarcocystis gigantea in experimentally infected sheep. Vet. Parasitol. 1984;16:193-199. https://doi.org/10.1016/0304-4017(84)90036-0
- Munday BL, Obendorf DL. Development and growth of Sarcocystis gigantea in experimentally infected sheep. Vet Parasitol.1984;15:203-211. <u>https://doi.org/10.1016/0304-4017(84)90072-4</u>
- Obendorf DL, Munday BL. Experimental infection with Sarcocystis medusiformis in sheep. Vet Parasitol. 1987;24:59-65. https://doi.org/10.1016/0304-4017(87)90130-0
- Svobodova V, Nevole M. Use of the muscle digestion method and indirect immunofluorescence reaction in the diagnosis of Sarcocystosis in sheep. Acta Vet Brno. 1990; 9:157-170. <u>10.2754/avb199059030157</u>
- Damboriarena PA, Silveira CS, Morais RM, Anjos BL. Natural Sarcocystis gigantea infection in sheep from southern Brazil. Ciencia Rural Santa Maria. 2016;46(7):1229-1233. https://doi.org/10.1590/0103-8478cr20151183
- Murtinez NB, Anastasio GB, Cano FM, Sanchez MP, Liopis MA, Perez CB, Goyena E, Berriatua E. Short communication: Sarcocystis infection a major cause of carcass condemnation in adult sheep in Spain. Span J Agric Res. 2012;10(2):388-392. <u>10.5424/sjar/2012102-523-11</u>.
- OJ, Regulation (EC) No 854/2004 of the European parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of oficial control son products of animal origin intended for human consumption. Official Journal of the European Union L 300.14/11/2009.p. 1-33.