

TROCARIZATION FOR DIAGNOSIS OF SARCOCYSTOSIS IN SHEEP

Barwary , Mwafaq S. Q.* , Al-Bayati , Saad M. H.** , Hussain , Nadheem J.*

Hussain , Yosra Y*

- College of Agriculture, University of Duhok, Duhok, Iraq.
- ** College of Veterinary Medicine, University of Duhok, Duhok, Iraq.
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ABSTRACT

Trocarization was used for diagnosis of sheep sarcocystosis for the first time with current study by using human biopsy needle. The obtained samples exposed to recommended sarcocystosis diagnosis techniques (Trichinoscopy, Post trichinoscopy drop examination , Peptic digestion method and histopathological method) .

Out of eleven biopsy samples (taken from the oblique muscles of the left flank) Trichinoscopy was the less sensitive (7/11) , Post trichinoscopy drop examination (9/11 and lately the most efficient methods which are Peptic digestion (11/11) and histopathological method (3/3) .

The biopsy technique showed to be suitable for sarcocystosis diagnosis in sheep without any effects on the live of the animals which make it the preferable method for advance researchs.

INTRODUCTION

Sarcocystosis is caused by species of *Sarcocystis* , an intracellular protozoan parasite in the phylum Apicomplexa (1) . These parasites have an indirect life cycle , cycling between a definitive (final) and an intermediate host (2) . Intestinal infection occur in the definitive host , and tissue invasion is seen in the intermediate host . More than a hundred species of *Sarcocystis* are parasites of domestic and wild animals (3). Many of these infections are asymptomatic , particularly in the definitive hosts (4) .

Sheep are infected with several species of *Sarcocystis* parasites which are *S.tenella* (*S. ovicanis*) , *S. arieticanis* , *S. gigantea* *S. ovifelis*) and *S. medusiformis* where dogs (for first two species) and cats (for last two species) are the final hosts (5) .

Sarcocystosis diagnosed through detection the sporocysts or oocysts in the feces of the final hosts , and in the intermediate hosts the diagnosis done by investigated the asexual stages as cysts or their contains (cystozoites) in the muscles by several techniques which are Trichinoscopy (6,7), Histopathological methods (6, 8) Peptic digestion technique for the muscles (9), post trichinoscopy drop exam (10), squeezing method (11) which all depends muscular samples taken after animals slaughtering and many immunological or molecular methods (12) .

Biopsy obtained by trocarization adapted in rare for human (14) or expansive animals like pony's (13,15) llowed by histopathological technique with H&E stain

(6,23).

The current study used biopsy samples obtained by trocarization for the first time in sheep followed by several diagnostic methods (Trichinoscopy ,Peptic digestion ,Post-trichinoscopy drop exam and H&E histopathological method).

MATERIALS AND METHODS

- 1- Animals : Eleven heads of native sheep over two years old choosing randomly from the agriculture college herd at Duhok university .
- 2- Trocarization : Trocarization done by using human biopsy needle (fig.1) under general surgical technique for the left flank of animal after clipping and shaving, disinfecting with Logols iodine (1%) . The needle entrance with oblique position (fig.2 , fig.3 and fig.4) and the collecting samples (fig.5) exposed to various diagnosed techniques which details lately.
- 3- Diagnostic techniques : Diagnostics techniques involved Trichinoscopy (6,7) ,which done by crush biopsy sample between two clean glass slides followed by microscopic examination (10X and 20X) looking for the intracellular cysts of the parasite . Post trichinoscopy drop test (10) done by left the crushed biopsy above test and covered the squash fluid left behind with cover slips , sometime adding a drop of distal water will be necessary to avoid dryness and then examine with microscope (X 40) for presence of parasite cystozoites. Peptic digestion of muscular biopsy (9) done by using digestion solution (Pepsin 0.3 g , HCl concentrate 7 ml and distilled water up to 1 Lt.) in about 5 ml / sample in test tube.
Lately , Histopathology used H&E stain done by routine laboratory procedure after fixing biopsy derived sample with 10% formalin as previously described (17,23).

RESULTS AND DISCUSSION

The biopsy operating shown to be consume 15 – 20 minutes / head including the examination except for histopathology and the animals have no any complication after 72 hrs of observation and that due to application of surgical procedure (fig.2 , fig.3 and fig.4), as well as just small blood drop may occur in some cases . This procedure by using left flank agreed with most recommended veterinary advice as this position not interfere with general physical status of the animal.

The samples obtained as biopsy (fig.5) measured in about 2 mm³ in size and that shown to be reliable for all other diagnostic tests. Trichinoscopy have good sense (7/11) to see the microcyst of the parasite (fig.6) which appear longitudinal and divided into apartments by internal septa and that resemble previous studies (10,11,18,19) in counter to the small number which involved with current study and also the targeted organ which are skeletal muscles that proved to be less sensitive to Trichinoscopy for sarcocystosis (10,16,18). Post- trichinoscopy drop test showed high sensitive (9/11) as it depend the presence of cystozoites of parasite (fig. 6) and that agree with previous studies (10, 18, 19) but adding distal water will be necessary as drying occur soon especially in hot climate which may stunt the diagnosis .

Peptic digestion method occupied the more sensitive test (11 /11) with current research as cystozoites of parasite is the target of this test which liberated easily due to digestion of muscles by Pepsin which not affect the parasite , and that resembles previous studies (11,,21,22,24) .

Lately , H&E histopathology method gave the known details of sarcocystis cyst (fig. 7) as the outer capsule with pink color and internal basic contains with transparent septa with it efficiency (3 / 3) with mention the difficulty and time cost of this method (11 , 22) .



Fig. 1 :Human biopsy needle which is used for Trocarization of sheep.



Fig. 2: The site of penetration (left flank) for biopsy .



Fig.3 : The oblique penetration of biopsy needle.



Fig 4 : The skin of animal after biopsy operation (observe the clipping , shave and disinfecting).

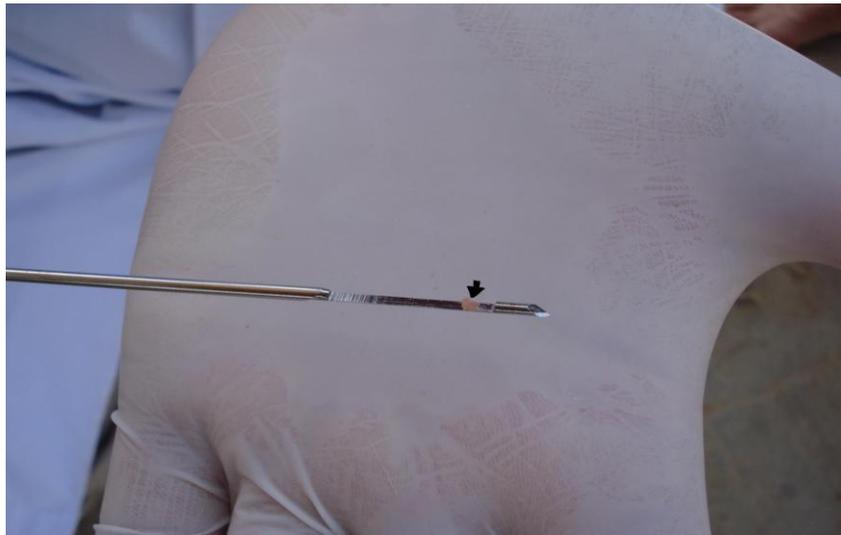


Fig. 5 :The sample collected by biopsy.



Fig. 6 : The microcyst of *Sarcocystis* spp. by Trichinoscopy method (X20).

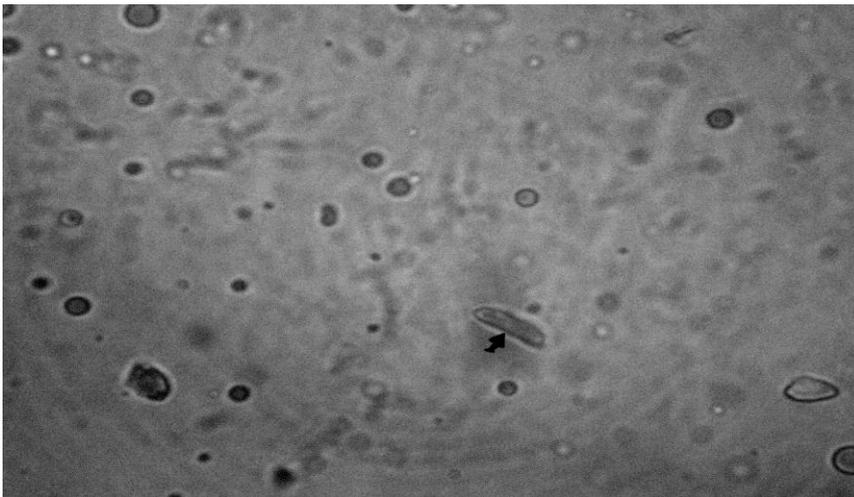


Fig. 7 : The cystozoites of *Sarcocystis* spp. (arrow) of sheep by Post trichinoscopy drop examination (X40).



Fig.8 : The cystozoites of *Sarcocystis* spp. (arrow) by peptic digestion method (X40).

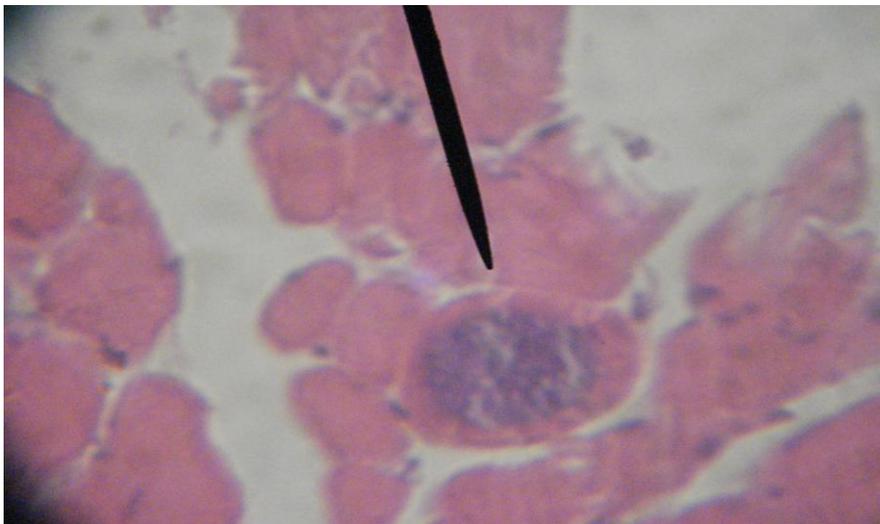


Fig.9 : A-The microcyst of *Sarcocystis* spp.(round) by histopathological test (H&E stain ,X20).

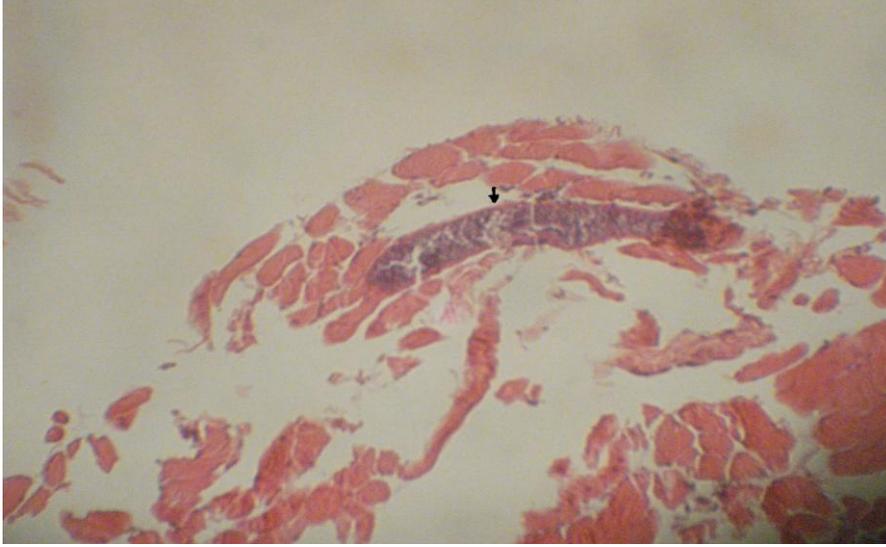


Fig.9: B-The microcyst of *Sarcocystis* spp.(longitydinal) by histopathological test (H&E stain , X10).

CONCLUSION AND RECOMMENDATION

The trocarization for obtained biopsy in the diagnosis of sarcocystosis is improved through this study with out need for slaughtering animals and in the same efficiency for further tests. That conclusion make this method recommended to use for future studies which may investigate other phases of the disease like pathophysiology or pharmaceutical research which mainly depend live animals.

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استخدام تقنية البزل في تشخيص داء الحويصلات الصنوبرية في الضان

يحيى ، يسرى حسين¹، جواد، ناظم حسن²، البياتي، سعد محي حيدر* ، پرواري، موفق سليمان قاسم¹

دهوك، العراق، جامعة دهوك، كلية الزراعة
كلية الطب البيطري ، جامعة دهوك، دهوك، العراق.

الخلاصة

تم استخدام تقنية البزل لتشخيص داء الحويصلات الصنوبرية بالضان اعتمادا على الخزعة التي تم الحصول عليها بتطبيق التقنية بوساطة ابرة الخزعة البشرية وذلك لأول مرة من خلال هذه الدراسة. ان الخزع المفحوصة باستخدام تقنيات تشخيصية خاصة بداء الحويصلات الصنوبرية(فحص منظار الشعريات و فحص القطرة ما بعد منظار الشعريات وفحص الهضم بمحلول الببسين واخيرا الفحص النسيجي)

تفاوتت في دقتها التشخيصية اذ كان منظار الشعريات اقلها حساسية (11/7) ثم فحص القطرة (11/9) فيما كانتا طريقتي الهضم بمحلول البيسين (11/11) و الفحص النسيجي (11 /3) هما الادق .
اظهرت الدراسة ان تقنية الخزعة بواسطة البزل ملائمة لتشخيص داء الحويصلات الصنوبرية في الضان مع عدم التأثير على صحة الحيوان وهذا يجعلها ملائمة لاجراء الابحاث المتقدمة.

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