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Comparison the efficiency of different techniques for the diagnosis of *Toxoplasma gondii* infection in slaughtered ewes

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Abstract

Toxoplasma gondii is one of the most common parasitic infections of human and other warm-blooded animals causes toxoplasmosis. In the present study a total of 50 uterus samples collected from slaughtered ewes were investigated for detection of T. gondii. Several techniques have been used to diagnose the infection with this parasite. Firstly, the impression smears staining methods used for the all samples using giemsa stain. Secondly, uses of direct fluorescence technique by acridine orange method for staining the impression smears of the uteri. As well as the histological section technique was used to determine the developmental growth stages of the parasite of all uterus samples and finally the serological method by latex agglutination test was used for the detection of antibodies of parasite. The results showed that detection of T. gondii using these four methods was 100, 80, 80 and 50%, respectively. It was concluded that the impression smears of the uterus staining with Giemsa stain was more readily, effectively and efficiently, followed by the direct immunofluorescence technique and histological section stained with hematoxylin and eosin stain technique, and finally the serological method.

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Introduction

Toxoplasmosis caused by *T. gondii* is a zoonotic disease infecting human and warm blooded animals such as sheep, cattle, goat, horse, and other (1). The cats are the definitive host of the parasite that develops in the gut and later oocyst shed in the faeces to contaminate the pastures and enviroment (2). Animal such as sheep may be infected with sporulated oocysts by ingestion contaminated feed and water (3). If the infection occurs during early pregnancy, the fetus will be reabsorbed while the infection occurs in mid pregnancy the abortion will occur and late of pregnancy causes abortion, stillbirths, mummified fetuses or weak lambs at birth (4). Therfore, toxoplasmosis causes reproductive failure in sheep (5). For the detection of *T. gondii* there are different techniques used for this purpose .For example directl methods like preparing of impression smears from infected organs, histological examination and indirect method by serological methodes like latex agglutination test (LAT), modiffed agglutination test (MAT), ELISA (6,7). Several PCR-based methods and Real-Time PCR quantitative methods have been developed for the detection of *T. gondii* using various samples like blood and tissue biopsy (8). The aim of the present study was carried out to compare different techniques for the detection of *T. gondii* in tissues of uterus samples and sera of ewes.

Materials and methods

Sample collection

A total of 50 local breed ewes 2-4 years old slaughtered at Mosul abattoir were selected for diagnosis of naturally infected toxoplasmosis, some of ewes have case history of vaginal and uterine infections developed to abortion. Fifty

reproductive organs were collected after the ewes slaughtered from March 2018 to October 2018. Sample were placed on ice in a cooler and immediately to the Laboratory of Parasitology, College of Veterinary Medicine, University of Mosul for further analyses.

Impression smear method

Impression smears were prepared from different parts of uterus included horn, body and cervix, they stained with giemsa stain for detection of tachyzoite and tissue cysts stages of the parasite (9) and direct fluorescence stain acridine orang for detection of the tissue cysts of the parasite (10).

Histological methods

Uterus biopsy was fixed in neutral buffered 10 % formalin and paraffin embedded section were stained with hematoxylin and eosin, then microscopically examined for the detection of tachyzoite and tissue cysts of *T. gondii* (11,12).

Serological methods

During slaughtered of the animals the blood samples were collected from jugular vein. Sera was separated from each clotted blood sample and stored at -20ºC. All the sera samples were examined qualitatively based on latex agglutination test (LAT), visible clumps indicated agglutination and seropositive samples were subjected to semi qualitative test to obtain the titter of antibodies by using serial double dilutions of 1:4 up to 1:512 (13), also modiffed agglutination test (MAT) were applied to seropositive sample to determine the type of immunoglobulins, IgG or IgM as well as, detection the type of the infection *i.e.* active or non-active (14).

Statistical analysis

The data was analyzed using the χ square test at $P<0.05$ was considered statistically significant (15).

Results

Results of different techniques used for detection of toxoplasmosis are presented in table 1. From this table evident that impression smears stained with the Giemsa stain were positive in 100% of the examined slaughtered ewes. To an extent 80% were results obtained by using both direct fluorescence methods by acridin orange and histological method. The lowest result 50% was obtained by using serological methods. The intensity of tissue cysts in different location of urtri using impression smears are show in table 2 which reveled a some variation of tissue cysts. Distribution of these tissue cysts was deteced (Figures 1 and 2), also we demonstrated tachyzoite in some impression smears stained with Giemsa stain (Figure 3).

Table 1: Detection of *T. gondii* in naturally infected and aborted slaughtered ewes

Sample	Giemsa stain	Direct fluorescent	Histology	Serology
50	100% a	80% b	80% b	50% c

Different letters indicate significant differences. The results were significant at the probability level $P<0.05$.

Table 2: Frequency of *T. godii* in the different parts of the uteri tissues of the ewe

Cervix	Uterus body	Uterus horn
169.7±36.38	185.28±36.37	195.14±31.53

Values represent the mean ±standard error rate.

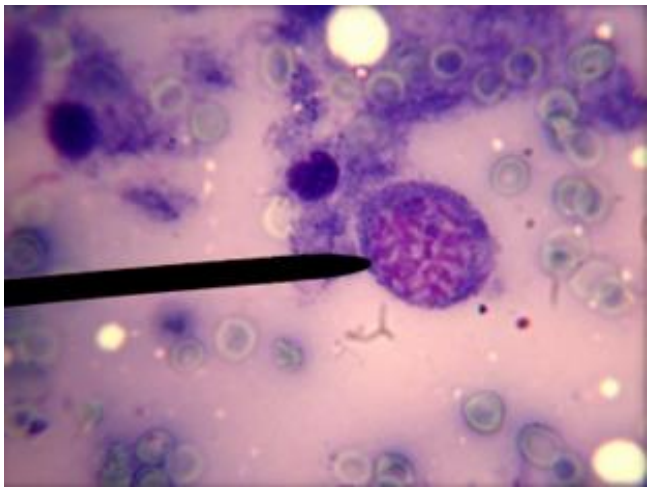

Figure 1: Microscopic image of tissue cysts of impression smears of the uterus of ewe stained with Giemsa stain (100x).

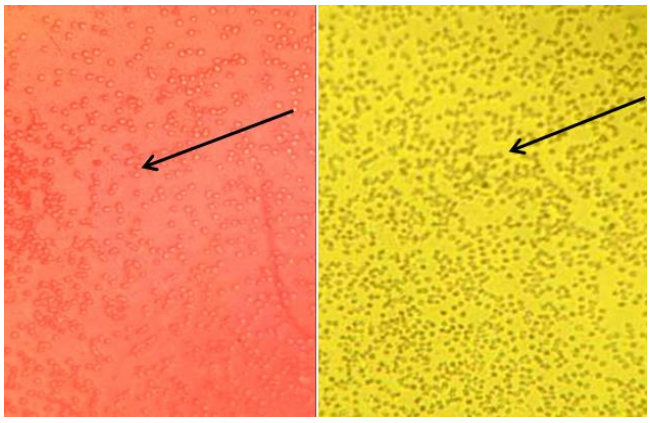

Figure 2: Microscopic image of tissue cysts of impression smears of the uterus of ewe stained with acridine orange dye (10x).

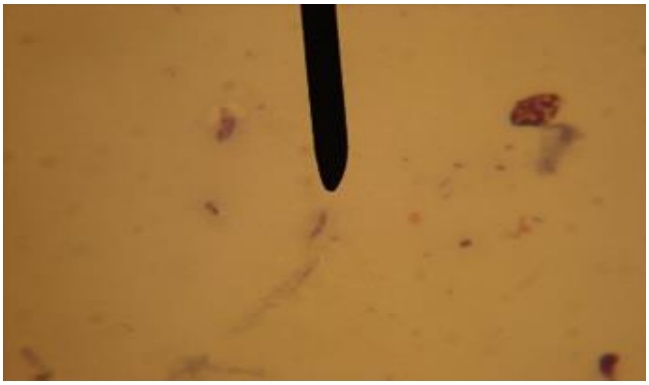

Figure 3: Microscopic image of tachyzoites of *T. gondii* of the uterus of ewe stained with Giemsa stain (100x).

The histopathological changes were manifested to a severe confined infiltration of the monocytes inflammatory cells (Figure 4). A rapid propagation of the tissue cysts of parasites was observed in the uterus tissues (Figure 5).

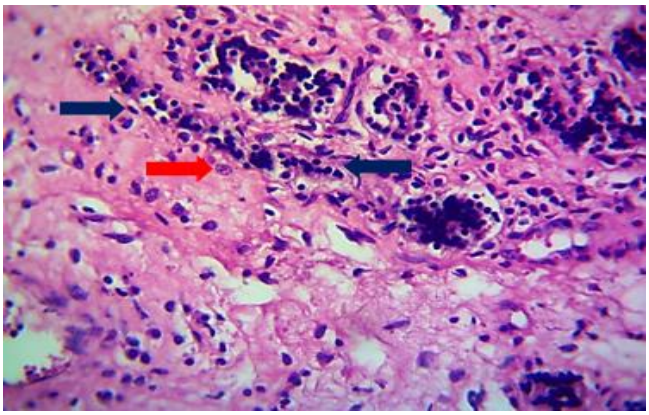

Figure 4: Microscopic section in a uterus of ewe, showing tissue cysts of *T. gondii* (arrow), with severe infiltration of mononuclear inflammatory cells (arrow). H&E, 600x.

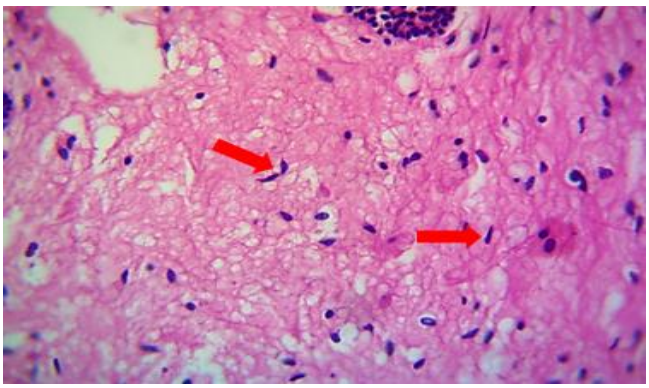

Figure 5: Microscopic section in a uterus of ewe, showing tachyzoites of *T. gondii* (arrow). H&E, 600x.

Table 3: Titration levels of anti -Toxoplasma antibodies in the sera of slaughtered ewes

Titration of antibodies levels				No (%) positive	Total examined
1:512	1:256	1:256	1:64		
3	6	6	7	25 (50%)	50

Discussion

Diagnosis of toxoplasmosis in the slaughtered ewes were depended up on impression smears stained with Giemsa stain, direct fluorescence stain by acridine orang, histological findings and serological tests. The impression smears stained with Giemsa stain was useful in exploring100% positive exploring ewes to *T. gondii* through either contaminated feed or water with Mature sporulated oocysts or by maternally acquired infection. Our finding was in agreement with those of Dubey (16) who recorded 95% infection rate in sheep in the United States, similarity Chabannes *et al.* (17) found that 92% of sheep were infected in France. Furthermore, our results were in accordance with those of Dubey and Schmitiz (13) who referred that 88.23% of aborted ewes of Oregon area in the United States had high infection rate, when use acridine orang stain in staining of impression smears indicated that the percentage of the infection with the parasite *T. gondii* was stained Acridine orange reach 80% and when compare between two stains observe that acridine orang stain shows a good diagnostic performance, with sensitivities of 81.3-100% and specificities of 86.4-100%, the most notable advantage of the Acridine orange method over Giemsa staining is its promptness; results are readily available within 3-10 min, whereas Giemsa staining may take 45 min or even longer .This is an important advantage for the organization of health services and the provision of effective treatment of toxoplasmosis (18). Hussein and Aghwan (10) used acridine orange in the diagnosis of nematode worms' eggs and recorded the total infection rate was 74% when we used acridine orange technique. Also Suleiman and Altaee (19) were used this stain in the diagnosis of parasite *Babesia spp.* in cattle. Acridine orange stain was more sensitive in detecting *Babesia* than standard Giemsa stain at low power. Parasites stained with Acridine orange fluoresced brightly against the dark background, and this greatly improved visibility, therefore increasing sensitivity even in low levels of parasitemia (18). The presence of tachyzoites and tissue cysts of the parasites in the uteri tissues stained with hematoxylin and eosin were reflected that these methods was very sensitive for diagnosis toxoplasmosis and also indicated that the real causes of abortion in these slaughtered ewes. These results were agreement with Dubey and Schmitz (13) recorded that *T. gondii* is the most important cause of abortion in New Zealand and in England. *T. gondii* antibodies were detected in the serum samples 50% by using LAT, they concluded that LAT is sensitive test and is efficient for screening purposes

Abouzeid *et al.* (20). Our results were disagreement with Asgari *et al.* (21), who recorded that 22.7% of sheep were infected in southern Iran. Our results were discrepant with those of Akhoundi and Youssefi (22), who explained and related the presence of antibodies to congenital constants in sheep using indirect immunodeficiency technique with the total infection rate of 29.5% Youssefi *et al.* (23) revealed 31.2% infection rate in sheep in Pabol, northern Iran, evidenced a rate of infection in the sheep may range between 17-18% (24), Lazim *et al.* (14) denoted that 26.5% of sheep where infected. Recently, Akhoundi and Youssefi (22) implied that the infection rate was 28.2% in sheep. However, our results were dissimilar with those of Al-Kappany *et al.* (1), who found a rate of infection in Egyptian sheep ranging from 26-41%. Modiffed Agglutination Test was indicated that the antibodies type was IgG which refers to the acquisition and exposure of these animal for infections for prolonged time, as indicated by the results, although the presence of the tachyzoites phases, the reason may be due to the rupture of old tissue cysts and the release of bradyzoites to induced reinfection, and period to form antibodies was 14 days, therefore the type of antibodies was IgG. Also, Kheezri *et al.* (25) was found the presence of IgG. It was concluded that the impression smears of the uterus staining with Giemsa stain was more readily, effectively and efficiently, followed by the direct immunofluorescence technique was more sensitive in detecting parasite than standard Giemsa stain at low power. Parasites stained with acridine orang fluoresced brightly against the dark background, and this greatly improved visibility, therefore increasing sensitivity even in low levels of parasitemia. The histological section technique was effective but consume time and finally the serological method was low sensitive due to shared antigenicity.

Conclusion

Different techniques used for detection of *T. gondii* in naturally infected slaughtered ewes. The lowest result 50% was obtained by using serological methods. To an extent 80% were results obtained by using both direct fluorescence methods by acridine orange and histological method and finally the impression smears stained with the Giemsa stain were positive in 100% of the examined slaughtered ewes.

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Conflict of interest

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

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مقارنة طرائق مختلفة لتشخيص الخمج بطفيلي المقوسات الكوندية في النعاج المجزورة

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الخلاصة

عد طفيلي المقوسات الكوندية من الطفيليات الشائعة التي تصيب الإنسان والعديد من الحيوانات من ذوات الدم الحار مسببة داء يسمى بداء القطط. تم جمع خمسين نموذجا من أرحام النعاج المجزورة وفحصت لغرض التحري عن الخمج بطفيلي المقوسات الكوندية وقد استخدمت العديد من التقنيات لتشخيص الخمج بهذا الطفيلي، حيث استخدمت تقنية اللطخات النسجية لجميع نماذج الأرحام وصبغت هذه اللطخات النسيجية بصبغة الكيمزا، كما استخدمت تقنية التألق المناعي المباشرة وذلك باستخدام صبغة الأكردين البرتقالية ،حيث صبغت اللطخات النسيجية للأرحام بهذه الصيغة ،فضلا عن استخدام تقنية التقطيع النسجي وتصبيغ هذه المقاطع النسجية بصبغة الهيماتوكسيلين - الأيوسين لتشخيص أطوار النمو المختلفة للطفيلي لكل نموذج من نماذج الأرحام المفحوصة وأخيرا استخدمت الطرق المصلية باختبار اللاتكس للتحري عن الأجسام المضادة لطفيلي المقوسات الكوندية. أظهرت نتائج البحث كفاءة هذه التقنيات في تشخيص الخمج بطفيلي المقوسات الكوندية فقد بلغت النسب المئوية للتشخيص ١٠٠ و٨٠ و٨٠ و٥٠% على التوالي. ونستنتج من ذلك إن تقنية اللطخات النسجية المصبوغة بصبغة الكيمزا هي أكثر الطرق فعالية في تشخيص الخمج بطفيلي المقوسات الكوندية وتليها طريقتي التألق المناعي المباشرة والتقطيع النسيجي وتأتي في المرتبة الأخيرة الطرق المصلية.

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