

***Toxoplasma gondii*: Experimental infection of Isolated local strain in Sulaimani Province**

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Abstract

Background: *Toxoplasma gondii* infection in humans is widespread through out the world, approximately half a billion humans have antibody to *T. gondii*.

Objective: The present study aimed to isolate and identified the local strain of *Toxoplasma gondii* from diaphragmatic muscles of naturally infected farm animals (sheep&goat) through studying the localization of the parasite in different organs of experimentally infected albino rats.

Methods: The parasite was isolated from minced diaphragmatic muscles from naturally infected farm animal by digestion with acid –pepsin mixture .Fourteen albino rats were inoculated intraperitoneal with freshly prepared parasite , serodiagnosis was performed for all experimental rats after (6) weeks of inoculation using LAT. Post mortem examination and giemsa stained impression smears from internal organs were done at the end of the experiment.

Results: Crescent or arc shaped with pointed one end and rounded other end, with typical gliding

movements of tachyzoite was the important features of the parasite isolated from digested diaphragmatic muscles of farm animals .Impression smears from internal organs revealed presence of the parasite in,brain;lymph node; spleen; heart; liver; and kidney, and absent in,lung; peritoneum; uterus; and skeletal muscles.

Conclusion: The parasite *T. gondii* was isolated successfully by acid -pepsine digestion procedure ;and experimentally infection.The most affected organs are, ,brain;lymph node; spleen; heart; liver; and kidney.

Keywords: *Toxoplasma gondii*; Isolation; Sulaimani; Acid-pepsine

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Introduction

Toxoplasmosis is a zoonosis of world wide distribution ⁽¹⁾, occurring naturally in man, domesticated, wild animals and birds ,it occurs in most parts of the world and a high incidence may occur in particular areas ⁽²⁾.

Toxoplasma gondii is an intracellular parasite which attacks most organs with predilection for the reticuloendothelial and central nervous system ^(2, 3, 4). *T.gondii* destroys cells, and the explosive multiplication of tachyzoites of this organism is potentially devastating to the tissues of the intermediate host ⁽⁵⁾. Most infections are acquired via the digestive tract and so organisms are disseminated by the lymphatics and portal system with subsequent invasion of various organs and tissues ⁽⁶⁾

T. gondii can be isolated from patients by the inoculation of laboratory animals and tissue cultures with secretion, excretion, body fluids, tissue

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taken by biopsy and tissues with macroscopic lesions taken post mortem⁽⁷⁾. Several reports have demonstrated that tissue culture methods could be applied to the rapid isolation of *T. gondii* organisms from blood^(8,9) or infected tissues^(10,11), and could serve for diagnosis when serological tests are inconclusive⁽¹²⁾. But sample inoculation in cell cultures required specialized laboratories and fails if non-viable parasites are present due to tissue autolysis⁽¹³⁾. The laboratory animals are considered as the best method for the parasite isolation from the discovery time till now⁽¹⁴⁾, laboratory animals are injected either intraperitoneally, intracerebrally, intradermally route or through mouth. Each of these routes has advantages for example; intradermal route is best for isolation of the parasite from contaminated sample with bacteria, injection through mouth is used for the isolation of the parasite from cat feces and intraperitoneal route is considered as the best method for isolation of the parasite from uncontaminated samples and it's a sensitive method⁽¹⁵⁾. During the first and second weeks of inoculation *Toxoplasma* may appear in the peritoneal exudates, if the animals survive 4 to 6 weeks sera then are tested for anti *Toxoplasma* antibody⁽¹⁶⁾. The parasite may be observed in the body of injected laboratory animals in these locations: brain, heart, lung, liver, spleen, striated muscle, uterus, and ovary⁽¹⁷⁾.

Our study aimed to isolate and identify the local strain of *Toxoplasma gondii* from diaphragmatic muscles of naturally infected farm animals (sheep & goat) and study the localization of the parasite in different organs of experimentally infected laboratory animals, which were albino rats.

Materials and Methods

Isolation of Parasite: Total of 25 diaphragmatic muscles samples were collected from 13 sheep and 12 goats from Sulaimani abattoir. These samples were collected in clean plastic and submitted immediately to the laboratory. Each sample was treated separately, and after removing adipose tissues from them they were cut into small pieces (7 - 26) gm in weight, and minced,

Acid – Pepsin Digestion Test: The minced meat was digested by acid - pepsin solution*, about 10 times of size of the meat added this solution in a clean dry beaker then stirred by magnetic stirrer for 30 minutes then left at room temperature^(18,19). The digested materials were filtered through several layers of sterile gauze; the filtered solution was put in clean test tubes (4 test tubes for each sample) and centrifuged at 3000 rpm for 15 min. For detecting the presence of the parasites in each sample, direct microscopical examination was done. The supernatant was discarded and a drop of sediment was placed on a clean glass slide by pasture pipette, mounted under cover slip, and viewed under microscope (X 40)⁽²⁰⁾. A drop of the same sediment was placed on a clean glass slide for preparing smear, left to dry and after fixation by methyl alcohol for two minutes, stained for 30 minutes with 10% Giemsa stain for further details.

*Acid - pepsin contents: Pepsin (5 . 2) gm, Sodium chloride (Na Cl) (10) gm, concentrated Hydrochloric acid (14) ml, all these were put in a glass (1000 ml) volumetric flask and the volume were completed to (1000) ml by adding of distilled water.

(after direct microscopical examination), were used for experimental infection in laboratory animals (rats) by performing the procedure described by⁽¹⁴⁾ briefly as following:-After centrifugation of filtered suspension for the first time, and discarding the supernatant, 10 ml of sterile normal saline (0.9 %) were added to the sediment, then centrifuged at 3000 rpm for 15 min, after that again the supernatant was discarded. This washing repeated for three times. After that, 10 ml of sterile inoculum was prepared by adding (100 µg) of Streptomycin and (1000 IU) of Penicillin per 1 ml of inoculum⁽²¹⁾. This solution was used for experimental infection in rats by intraperitoneal (ip) route, as it's considered the best method for inoculation of the parasite from uncontaminated samples and it's a sensitive method⁽¹⁵⁾.

Experimental Animals

Adult albino Wister rats *Rattus norvegicus*, of both sexes were used. Their age ranged between 6 - 8 weeks, and their weight range between 210 - 280 gm. Eighteen rats were distributed into two groups ,control (2 females and 2 males), while remained (7 females and 7 males) were used for the experimental infection .These rats reared under proper environmental conditions, in special cages, wood shaving was used for bedding, they fed on proper ration and clean water in adequate amounts.

Course of Infection

Rats were infected experimentally by intraperitoneal injection of 1 ml of prepared inoculums (50 parasites per ml) and the control group inoculated with 1 ml of normal saline⁽²²⁾, .For detecting early infection with *T. gondii*, the peritoneal washes done by using 2 ml of sterile normal saline after 10 days of injection. Smears prepared from washing peritoneal fluid, air dried, fixed with methyl alcohol and stained with 10

% Giemsa stain, and examined under (X 100) for detection the tachyzoite stage of the parasite.

Serodiagnosis: Serodiagnosis was performed for all experimental rats after⁽⁶⁾ weeks of inoculation according to⁽¹⁶⁾, 1.5 ml of blood samples were collected from experimentally infected rats by puncturing of retro orbital plexues, using capillary tubes (without anti coagulants) in clean plain tubes. After centrifugation at 3000 rpm for 15 min, the sera was separated in clean plain tubes, and then they tested immediately by LAT for detection of *T. gondii* antibodies.

Post mortem examination: Eight weeks after inoculation, rats were sacrificed⁽²⁾ by cervical dislocation⁽²⁰⁾. Post mortem examination was done for each one and removed their internal organs including heart, liver, spleen, lung, uterus, kidney, mesenteric lymph node and brain, Because *T. gondii* affects multiple organs⁽²³⁾.

Smear Preparation and Staining: Impression smears was prepared from all removed internal organs beside peritoneum and thigh muscle⁽¹⁷⁾, by cutting through the organ and the freshly-cut surface will eventually be imprinted (using a manageable size of the tissues). Imprinting is done simply by touching the prepared surface to a clean microscope slide, the imprints are allowed to air dry, after drying they were fixed in methyl alcohol and stained with 10 % Giemsa stain then examined under (X 100).

Results

Isolation of the Parasite: Out of 25 diaphragmatic muscle 12 (48 %) were showed presence of *Toxoplasma gondii* tachyzoite stage, 7 samples (58.33 %) of sheep and 5 samples (41.67 %) of goats were carried this parasite. No significant differences was found between both animal species at (P

≥ 0.05), as illustrated in (Table 1) .Upon the direct microscopic examination of the digested diaphragmatic muscle of sheep and goats by acid-pepsin solution revealed the free tachyzoite stage, which appeared as crescent or arc shaped with pointed one end and rounded other end, with typical gliding movements of tachyzoite, as shown in (Figure 1- a & b) and (Figure 2- a & b).This morphological feature was identically to those described by ^(15,23).

Infection of experimental animals.

All experimentally, intra-peritoneally infected rats with digested diaphragmatic muscle, were stayed a live throughout of the study. Out of 14 inoculated rats 5 (35.7 %) rats, which were of both sexes 2 males and 3 females, appeared the presence of motile tachyzoite stage of *T. gondii* in peritoneal exudates, (Figure 3).

Serological test by LAT was done on 14 sera samples of infected rats (6) weeks after intraperitoneal inoculation, only 7 (50 %) sera positive by this test.. At the end of experiment, all rats were killed and impression smears was prepared from internal organs, striated muscle, peritoneum and brain, and stained by 10 % Giemsa stain for detection of the parasite from these organs, (Figure 4, 5, 6, 7, 8 and 9) were revealed the parasite from organs: brain , lymph node, liver, heart, spleen and kidney respectively, but no parasites were observed from lung, peritoneum, uterus and muscles impression smears. Post mortom examination showed no gross pathological lesions in the internal organs.

Table 1: Prevalence of *T. gondii* in diaphragmatic muscle samples of sheep and goats

Type of the animals	No. of examined Samples	No. of positive Sediment suspension	(%)
Sheep	13	7	58.33
Goat	12	5	41.67
Total	25	12	48

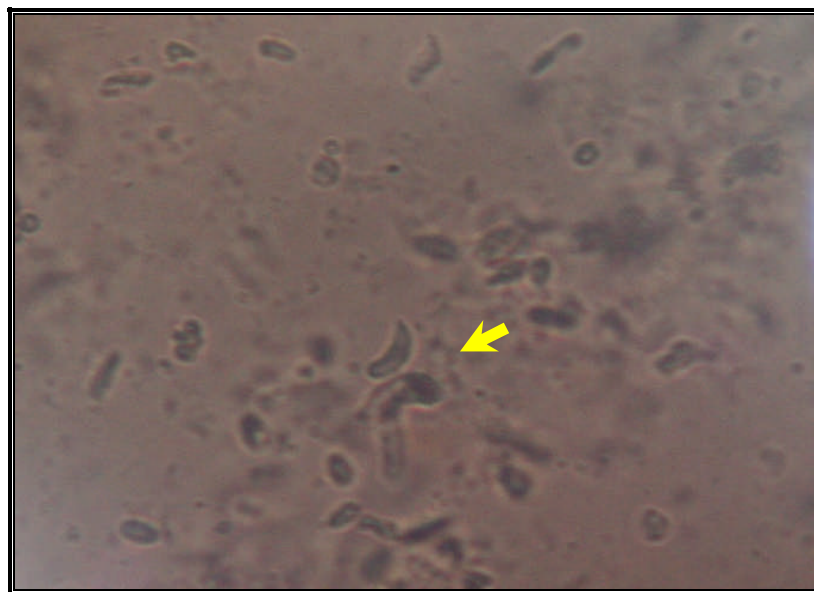


Figure 1: Tachyzoite of *T. gondii* from acid-Pepsin digested diaphragmatic muscle,, wet preparation (X 40).

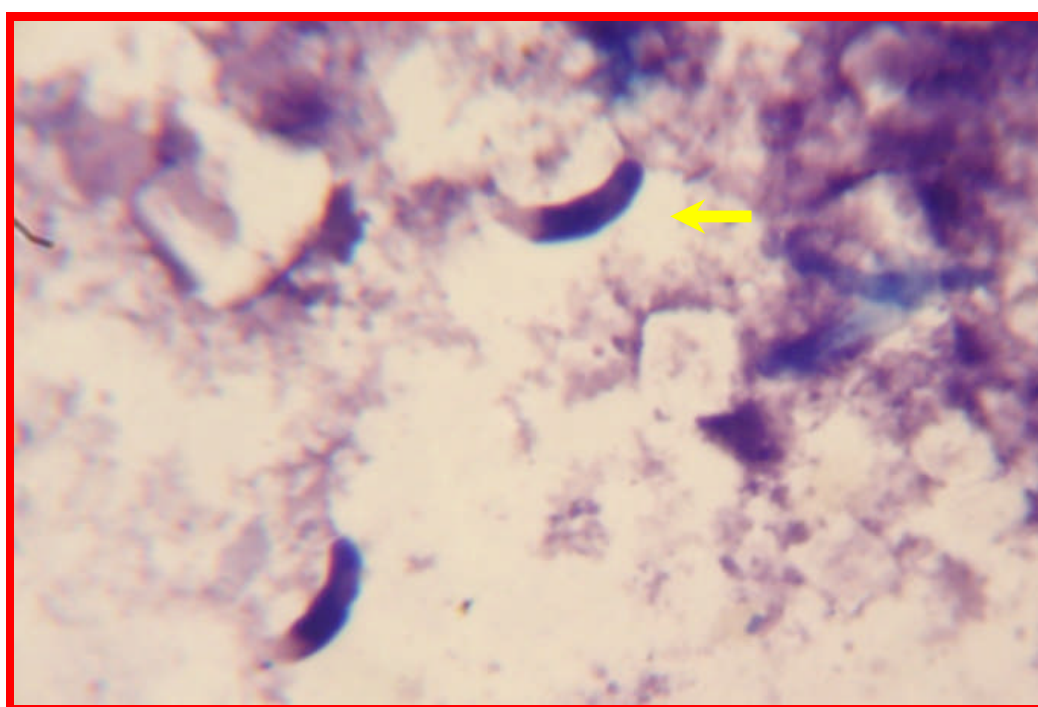


Figure 2: Tachyzoite of *T. gondii* from acid-Pepsin digested diaphragmatic muscle, Giemsa stain (X 100).

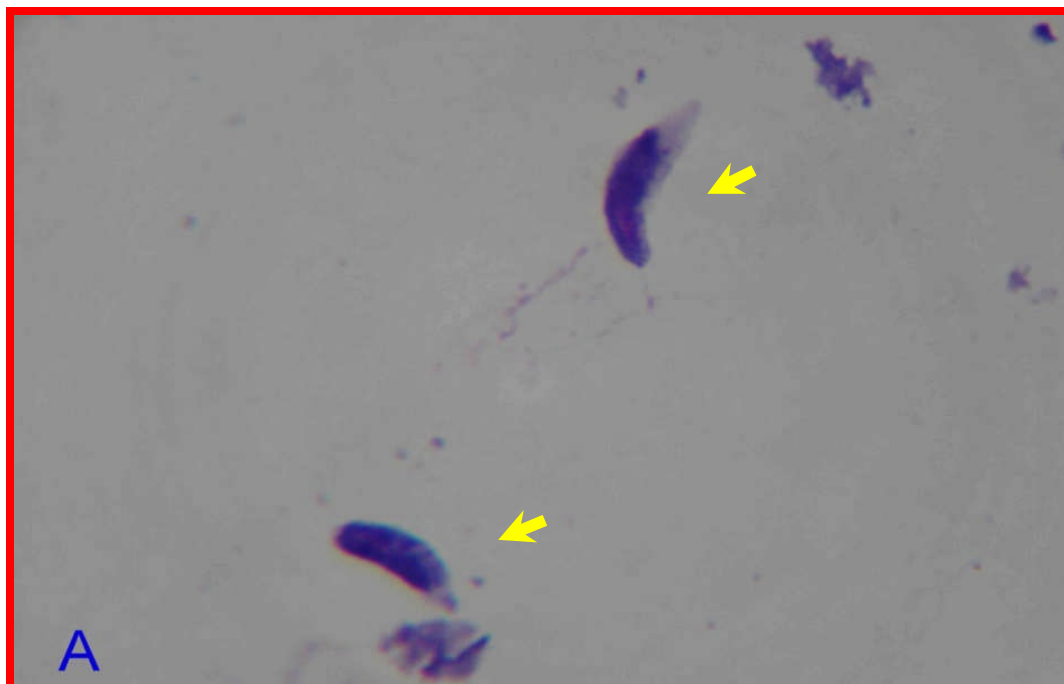


Figure 3: Tachyzoite of *T. gondii* from peritoneal exudate of rat (10 days) post inoculation. Giemsa stain (X 100).

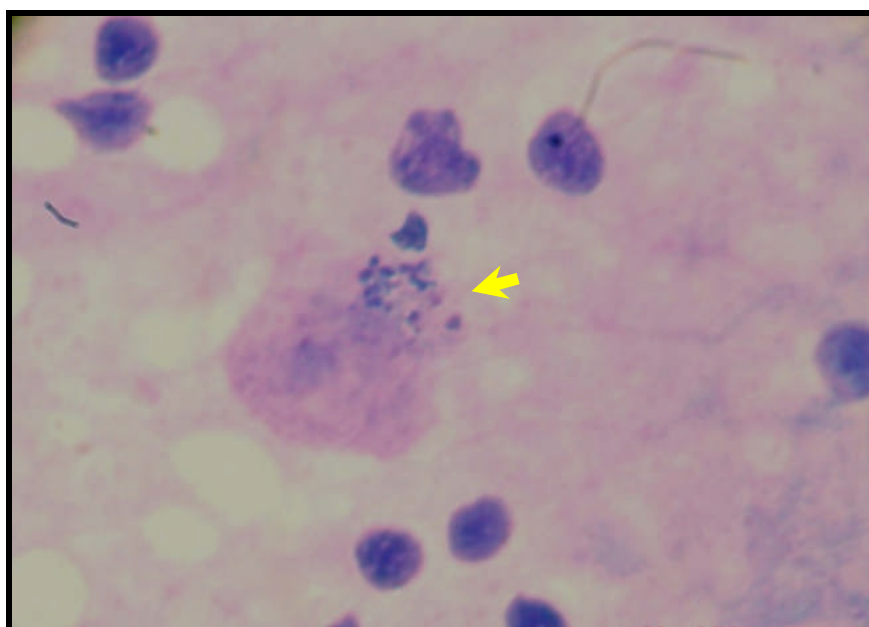


Figure 4: Brain impression smear of rat eight weeks post inoculation, showing presence of *T. gondii*, Giemsa stain (X 100).

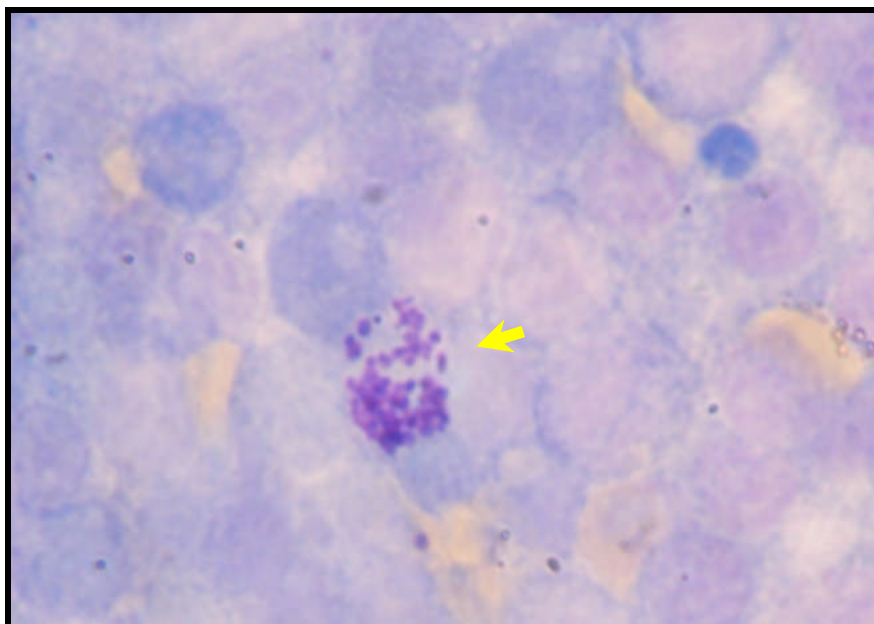


Figure 5:Lymph node impression smear of rat eight weeks post inoculation, showing presence of *T. gondii* , Giemsa stain (X 100).

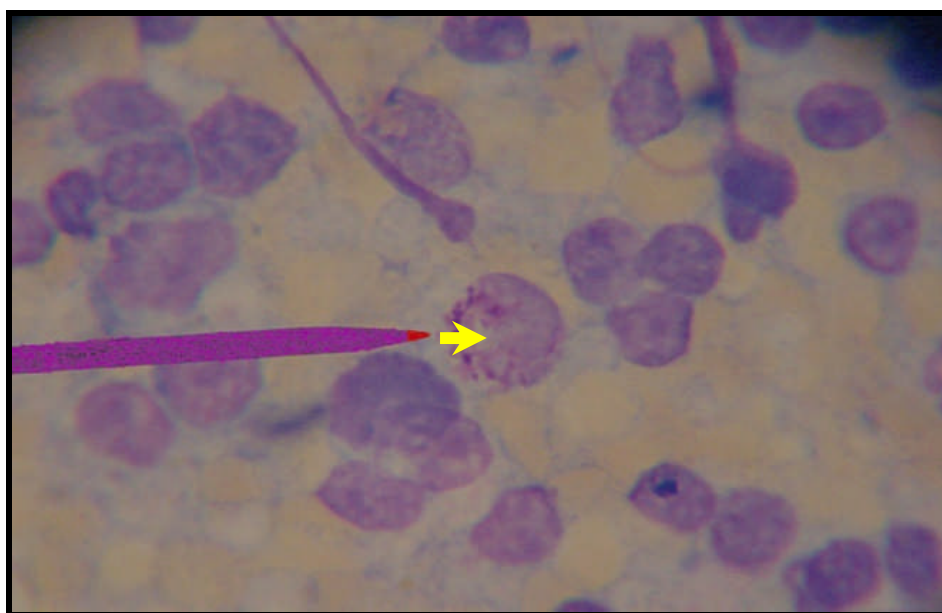


Figure 6: Liver impression smear of rat eight weeks post inoculation, showing presence of *T. gondii*, Giemsa stain (X 100).

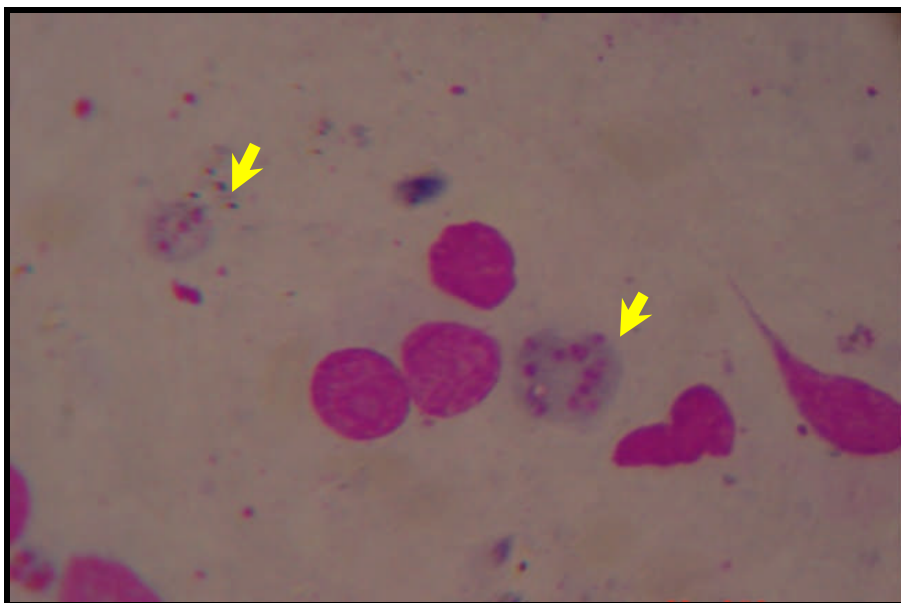


Figure 7: Heart impression smear of rat eight weeks post inoculation, showing presence of *T. gondii* , Giemsa stain (X 100).

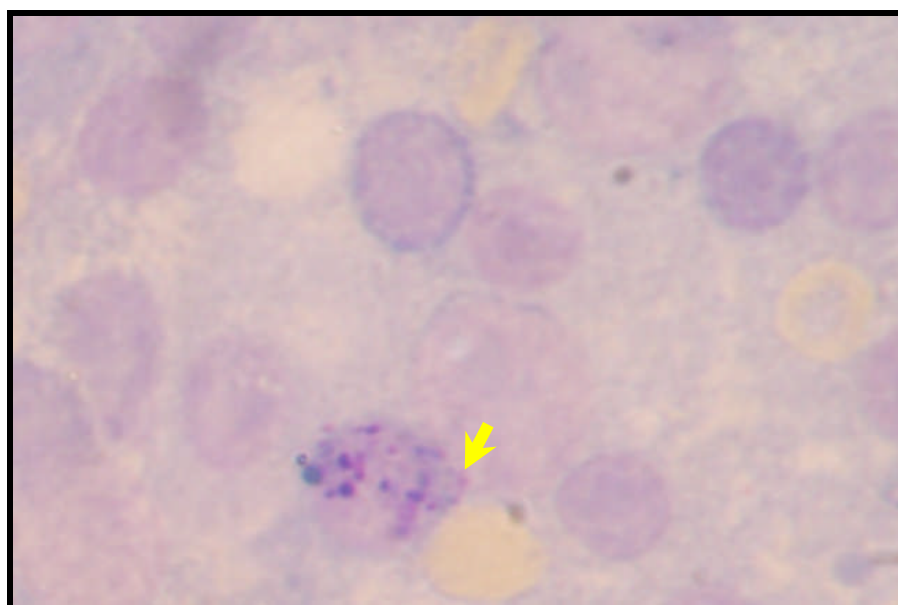


Figure 8: Spleen impression smear of rat eight weeks post inoculation, showing presence of *T. gondii*, Giemsa stain (X 100).

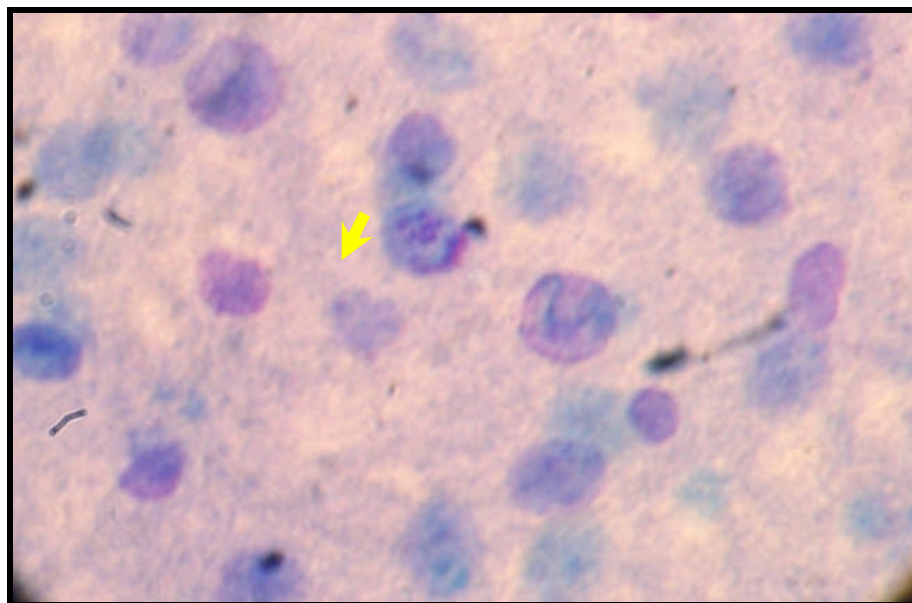


Figure 9: Kidney impression smear of rat eight weeks post inoculation, showing presence of *T. gondii*, Giemsa stain (X 100).

Discussion

The detection of *T. gondii* in meat is fundamental to assess its importance as a source of infection ⁽²⁴⁾. Also human acquire the infection through contact with infected animal carcasses, when there is skin abrasions, cuts ⁽²⁵⁾, or through touching mucous membrane of the mouth and eyes while handling raw meat ⁽²⁶⁾.

In the present study, the attempt for the parasite isolation from diaphragmatic muscle of slaughtered sheep and goats, this was due to the easily obtaining samples, and the muscle tissues more infected than brain in sheep and goats ⁽²⁷⁾. The direct microscopic examination of tissue suspension sediments of diaphragmatic muscle revealed the presence of the parasite (Figure 1, 2). Results showed in Table 1 12 samples (48 %) of the total 25 samples were positive, of these 7 samples (58.33 %) were from sheep and 5 samples (41.67 %) were from goats. Similarly ⁽¹⁴⁾ reported 41

positive cases out of the total 100 diaphragmatic muscle, of these 22 were from sheep and 19 from goats, again by direct microscopic examination of sediments. Also ⁽²⁸⁾ reported that sheep are one of the animals which have been identified as a possible source of infection for people.

The number of *T. gondii* tissue cysts in meat from food animals is very low, it is estimated that as few as one tissue cyst may be present in 100 g of meat. So without using a concentration method, it's not practical to detect this low level of *T. gondii* infection. Therefore digestion of meat samples in trypsin or pepsin is used to concentrate *T. gondii* in meat ⁽²⁹⁾, and for this purpose acid-pepsin solution was used in the present study for isolation of the parasites from diaphragmatic muscle by digestion process because, until recently acid-pepsin digestion was a generally accepted method to recover *T. gondii*

from tissues ⁽³⁰⁾, also the components of the solution were available and prepared easily, and when used for experimental infection in laboratory animals, it requires less washing time compared to trypsin as it cause trypsin toxicosis ⁽²⁷⁾.

Sediments of tissue suspension from the diaphragm samples which were the digested materials, were bio assayed in laboratory animals. Albino rats were used, because animal inoculation is usually considered the most sensitive method for *T. gondii* isolation from tissue or body fluids ^(31,32). However, several studies have demonstrated that susceptibility to *Toxoplasma*, and the course of infection may be affected by several factors such as the route of infection and the infecting dose ^(33,34,32), also this procedure is not practical for mass scale samples ⁽³⁵⁾. Intraperitoneal route was used for isolation of the parasite *T. gondii*, which is considered as the best method for parasite isolation from uncontaminated samples and it's a sensitive method ⁽¹⁵⁾. The sediment samples with large number of parasites are used for this purpose in the study, although ⁽¹²⁾ reported that the minimum infecting dose was three tachyzoites or bradyzoites, inoculation of 10 - 30 and 100 parasites resulted in an increase in the infection rate.

There was no mortality in the experimentally infected rats, which means none of the *T. gondii* infected rats died of toxoplasmosis, but this dose not mean lack of infection, and this result might be indicated two things, either the rat is one of the hosts that has some resistant degree to the clinical *T. gondii* infection ⁽²¹⁾. Also reported by ⁽³⁸⁾ that, like human rats usually develop subclinical infection, or

might be related to the strain of the parasite, when most of the present strains are with low virulence that dose not result in the death of the host and may result in sub acute infection. The presence of the parasite in the peritoneal fluid and seropositive cases by LAT will indicate that the present strain cause sub-clinical infection in rats.

Results showed the presence of tachyzoite stage of the parasite in the peritoneal fluid of inoculated rats 10 days post inoculation, after they were smeared and stained with Geimsa stain,(Figure 3). The tachyzoite stage of the parasite were seen in 5 rats 35.71 %, out of 14 infected rats, they were of different sexes(2 males and 3 females), these indicate the susceptibility of both sexes to the infection. The serological results by LAT in (6) weeks post inoculation revealed the presence of antibodies against *T. gondii* in 7 cases 50 %, this agree with ^(36,37) who observed seropositive cases among inoculated laboratory animals four weeks post inoculation by LAT. While ⁽¹⁴⁾ not obtained any seropositive cases out of the total 250 inoculated mice.

Examination of stained impression smears eight weeks post inoculation also showed the presence of the parasite in liver, heart, spleen, kidney, mesenteric lymph node, and brain (fig:4,5,6,7,8,9). Similar to our results ⁽³⁹⁾ observed the tissue cysts in the brain, heart, kidney, mesenteric lymph node, liver and spleen of experimentally infected rats. Different organs were involved because the parasite disseminated through blood circulation, ⁽⁴⁰⁾ reported that the distribution of the parasite through blood mainly occurs during a restricted period of time

and after that, the tachyzoites established in different tissues and organs of the host and continue to invade all cell types except the red blood cells. In the liver, tachyzoites may be found within liver or kuffer cell in cyst containing a large number of organisms either singly or in pairs scattered in both the necrotic and viable tissues. In spleen, tachyzoites are seen inside the macrophage and in lymph nodes tachyzoites may be found in endothelial cells of veins, but may be within the cytoplasm of monocytic cells or free in the tissue, usually, both lymphatic and reticuloendothelial hyperplasia occurs. In heart myocardium is invaded and the parasite present in large or small groups within the cytoplasm of cardiac muscle cells, and in the brain tachyzoites may be found scattered singly or in pairs through the parenchyma or in aggregation containing 50 organisms tissue cysts seen near the blood vessels in the cerebral tissue⁽²³⁾

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