

Non Enzymatic Method to Isolation *Toxoplasma Gondii* from Placental Tissue and Effect on Lipid Peroxidation in Pregnant Women in Al-Ramadi City

¹Ridhab Ajeel Jasim , ²Thaer Abdulqader Salih, ³Al-kubaisi S. M. A.

¹ College of science / University of Anbar.

ridhab90@uoanbar.edu.iq

²College of Education for pure science / University of Anbar.

sc.thaerparasite@uoanbar.edu.iq

³College of Veterinary Medicine / University of Fallujah .

doctor_salah73@uofallujah.edu.iq

ABSTRACT

Background: Toxoplasmosis is known to be one of the most common infections worldwide in humans and warm-blooded animals .One-third of the world's population is infected with toxoplasmosis. **The current study aimed** to isolate the toxoplasma gondii parasite from aborted and un aborted placentas for women with toxoplasmosis within AL Ramadi city by non-enzymatic methods to take care of the vitality of the rapidly multiplying phase of the parasite found within the placental tissue. Additionally , the present study aims to detection the effect of parasitic on Lipid Peroxidation by measuring malondialdehyde (MDA) levels within the pellet of solution extracted from the placenta after mashing it to examine the parasite **The results** of the microscopic examination of the pellet and suspension that extracted from placental solution showed the presence of bradyzoite in most of the diagnosed samples additionally to the emergence of the rapidly multiplying phase tachyzoite of others. The results of Lipid Peroxidation measurement showed increase in MAD levels in infected samples compared to control samples but its non-significant .

Keywords: *Toxoplasma gondii*, MDA, Lipid Peroxidation Placenta .

INTRODUCTION

Toxoplasma gondii belongs to coccidia, habit the domestic cat and other fields as its definitive host and wide range of birds and mammals as intermediate hosts (1,2). *T. gondii* specific IgM antibody production, and low numbers of concanavalin A binding sites have been located on the surface of tachyzoites. Glycoproteins that bind specific IgG and IgM have been identified on the surface of *T. gondii*, and it appears that *T. gondii* is capable of both N. and O. glycosylation (2).

Abortion is the term refers to the ending of pregnancy by loss of a fetus before it can be outside the uterus after 9 months. An abortion which occurs spontaneously is called miscarriage or may be caused purposely and is called induced abortion (3). Infectious toxoplasmosis is a specific type of toxoplasmosis in which a fetus is infected by the placenta is associated with fetal death and abortion, and in an infant, it is associated with a defect in neurons, neurocognitive deficits, and chorioretinitis Early pregnancy loss, which occurs in the

first trimester, is the most common type. The nonspecific symptoms of vaginal bleeding and uterine cramping associated with pregnancy loss can occur in normal, ectopic, and molar pregnancies, which can be a source of frustration for patients and clinical confusion for care providers (4,5).

Malondialdehyde (MDA) in cells of the human body and tissue is an indicator of the oxidative stress arising from the lipid peroxidation (6). The Lipid peroxide is the derivative enzyme of feeble unsaturated fatty acid produced from the result of deposition of a set of complex component (7).

Materials and Method

Samples

Samples were collected from the maternity wards at Ramadi Teaching Hospital for childbirth and children with (8) uninfected samples as a control group, (8) samples with aborted *Toxoplasma gondii* and (8) samples with unaborted. *Toxoplasma gondii* and kept the samples in sterile containers containing normal saline (NaCl) as well as antibiotics to prevent contamination such as streptomycin and Penicillin

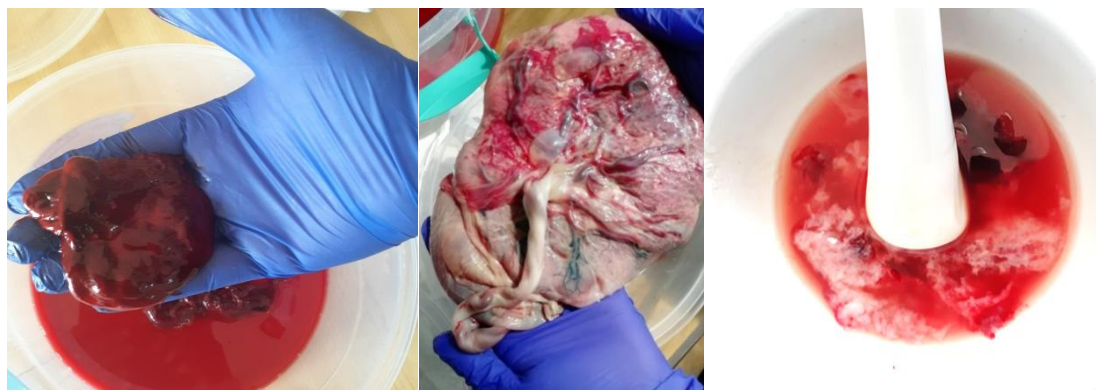
Methods

1/ Detect IgG and IgM antibodies by using ELISA technique:

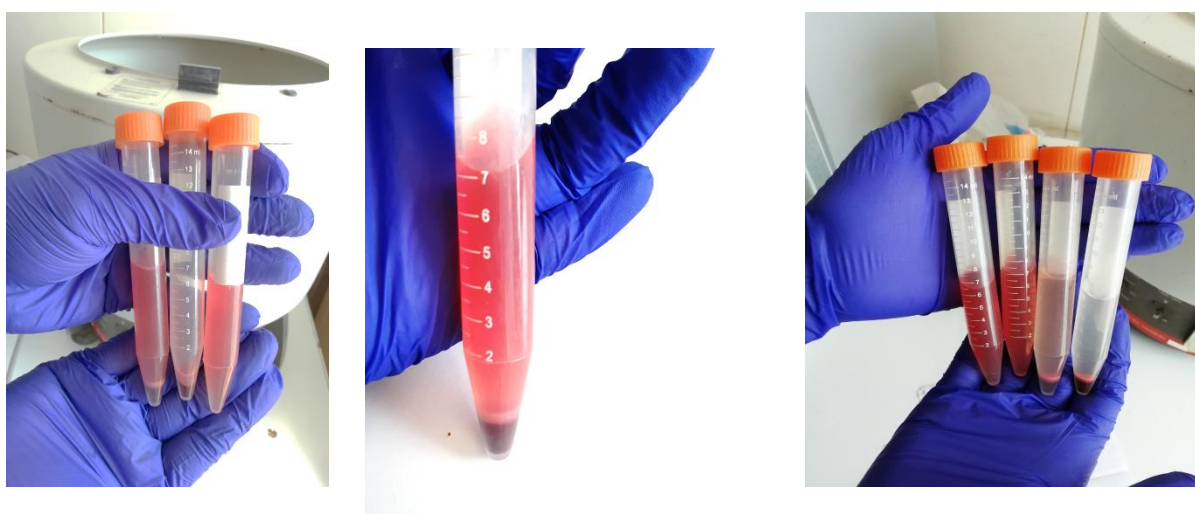
In the present study, the ELISA test was utilized to detect and identify IgM and IgG antibodies. Several tests were utilized by the German company (Human). The principle of testing depends on the interaction between the antibodies found in the patient's serum and the antigens which covered well of kit. This test was conducted to confirm samples already infected with *Toxoplasma gondii*.

2/ Isolation *Toxoplasma gondii* From placenta

The samples were then transferred to the Laboratories of the College of Science sat in Anbar University. The samples were Crushed by using ceramic mortar and were mixed with a quantity of normal saline and then filtered the result with sterile gauze to get rid of large pieces, then expelled the centrifuge (3000 rpm , 10 min, 37°C) saves the first stuck tube to examine it, then suspended the deposit with the solution of normal saline and repeated the process three times and then added to the output 1000 units of penicillin and 100g of streptomycin to prevent contamination. A drop of the product was placed on a glass slide and dyed with Giemsa stain to be examined under a microscope and to confirm the presence of parasitic phases. (8)



A/ Abortion and non-abortion placentas and its hemolysis



B/ centrifugation of sample

2/ Determination of MDA in biological samples

For TBARS determinations 5.00 g of mixed sample in a 50 mL centrifuge tube was weighed. Subsequently 0.5 mL of EDTA was added and stirred, then 2.5 mL of 0.8% BHT, and stirred again. Before homogenization was added 4 mL of 5% TCA was added and homogenized with Ultra-Turrax T18 basic (IKA, Staufen, Germany) at 10,000 rpm for 1 min. After homogenization, the sample was left for 10 min at room temperature and then centrifuged at 4 °C and 3500 rpm for 5 min (Jouan BR4 centrifuge (Jouan Technology for Life, Winchester, VA, USA). Next, the top hexane layer was removed with a pipette and the sample was filtered using filter paper (Whatman No. 4, GE Healthcare, Freiburg, Germany). The filtered sample was transferred to 10 mL volumetric flask and completed with 10% TCA. From the volumetric flask was transferred 4 mL was transferred into the tube and 1 mL of TBA was added, and closed with aluminum foil. A blank sample was prepared in a separate tube using 4 mL of 10% TCA and 1 mL of TBA. We incubated the tubes in a water bath at 70 °C for 90 min. After that time, the tubes were cooled in an ice bath and after reaching a laboratory temperature the

rest of the samples was measured opposite to the blank sample. The calibration curve was set on the UV-VIS spectrophotometer (Helios γ , Thermo spectronic, Cambridge, UK) and TBARS values were measured at [532](#) nm. The results were quantified as malondialdehyde equivalents (mg/MDA/kg sample).

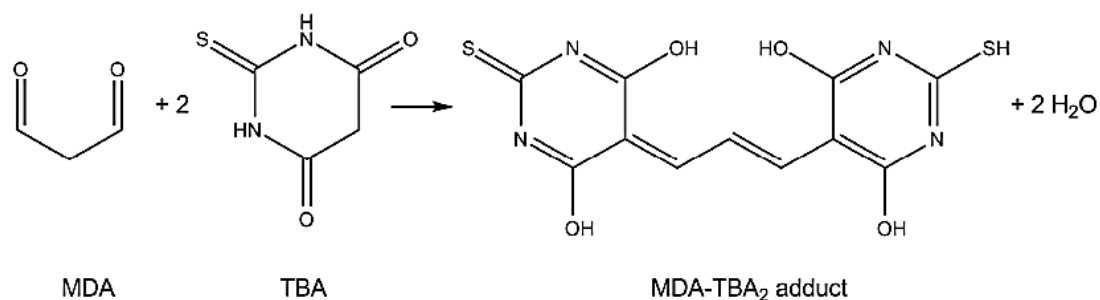


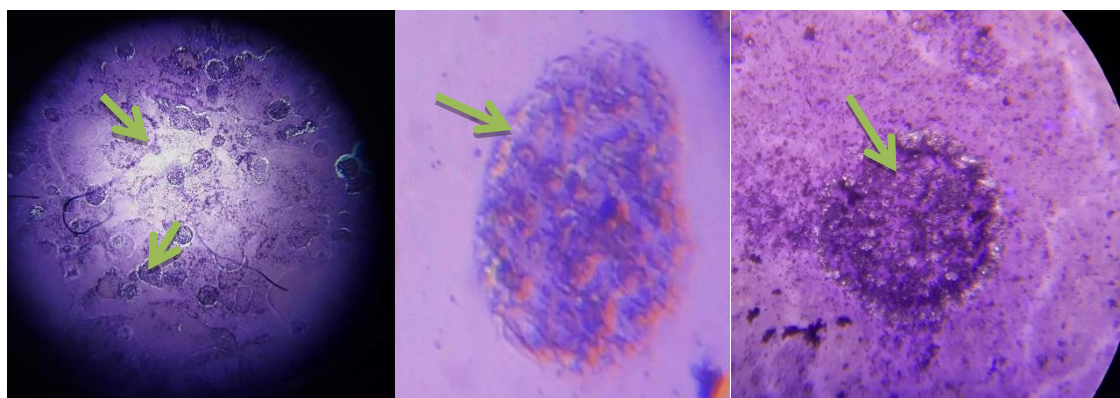
Figure 1. Reaction of malondialdehyde (MDA) with 2 molecules of 2-thiobarbituric acid (TBA).

Statistical Analysis

Data are expressed as mean \pm standard deviation. Statistical analysis of the data (descriptive statistics and linear regression) was performed with Statistica 8.0 software (Stat Soft Ltd., Bedford, UK). Paired t-test was used to analyze differences between methods and samples. Differences were considered significant for values of two-tail $p < 0.05$.

Results

1/ The results of the parasite's isolation from the placenta showed the presence of different phases of the parasite in most of the samples that were examined microscopically, confirming the actual incidence of the parasite of placentas that were digested in a non-enzymatic way to maintain the vitality of the parasite's reproductive phases .



A

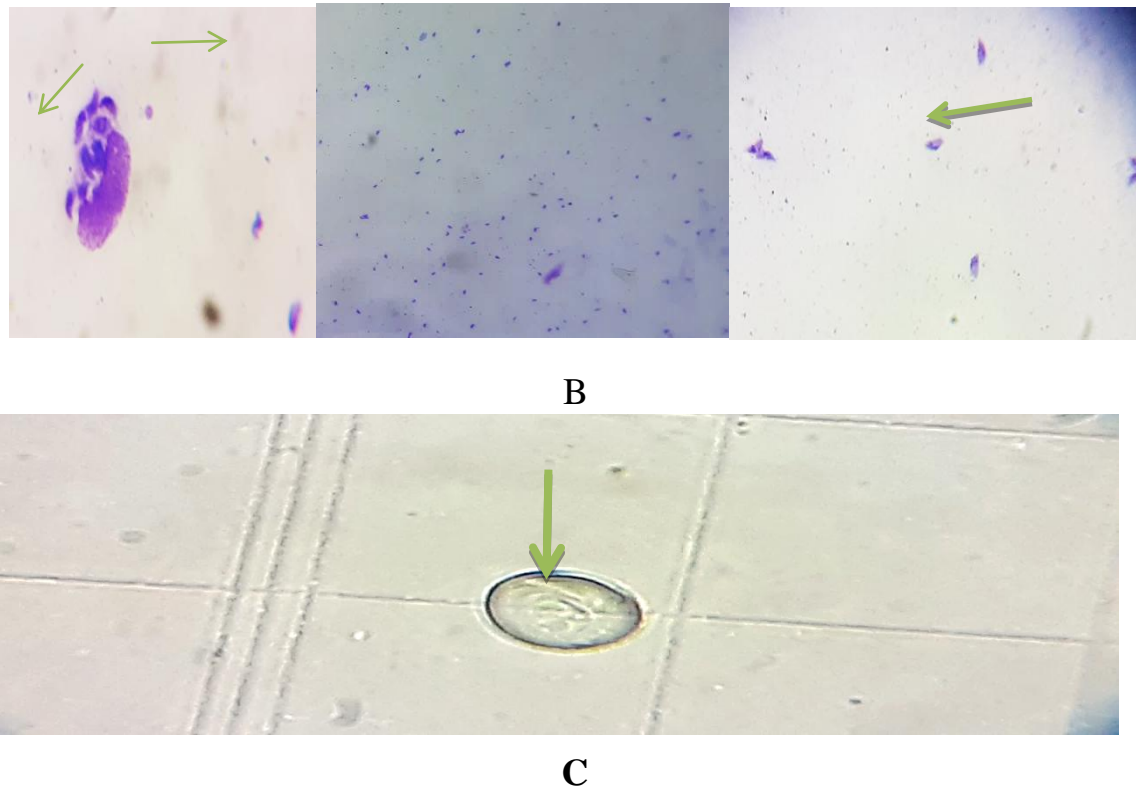


figure 1: The results of the microscopic examination of the tissue isolation of the placenta show the different parasitic phases after isolating it with centrifuge and staining it with Giemsa stain .A/ bradyzoite . B/trachyzoite . C/ Oocyte

2/ To measure the level of lipid peroxidation in the isolation output of the Crushed placenta, the samples were divided into two groups (without abortion, with an abortion) and the results of the examination showed a middle rise in the level of lipid peroxidation between the two groups, but it was non-significant differences.

Sauce of variation (SOV)	Degrees of Freedom (DF)	Significant Level 5%	Chi square
with control	1	N.S	0.74

n.s = nan-signifi

Sauce of variation (SOV)	Degrees of Freedom (DF)	Significant Level 5%	Chi square
without control	1	N.S	0.42

n.s = nan-significant

Discussion

In this study, the method of researchers Sharma and Dubey was used in the isolation process in a year (1988) (10) and Remington and Desmotsin 1974 (9), which were transformed by the dispensation of digestion. The tissue of the placenta tissue using acid pepsin solution or trypsin solution. He used the researcher. Jacobs and others in the year (1960) (11), the method of tissue digestion with acid bisin to isolate the *Toxoplasmosis gondii* parasite is from the placental tissue, and this researcher has not been able to isolate the parasite, and he has indicated that this is due to That the acid pepsin solution causes the rapid killing of the rapidly multiplying phase found in the tissue The placenta has therefore been dispensed with the tissue digestion of placental tissue in the current study for the purpose of isolating the parasite which may be one of the main reasons that jacobs researcher did not enable Others isolated the parasite as it used acid pepsin solution in the process of tissue digestion and therefore With the possibility of containing placental tissue in a small number of tissue bags compared to the rapidly multiplying phase Led to the inability to isolate the parasite.

The toxoplasma parasite is known to cross the placenta. It is an important cause of abortion (12). This disease is now recognized pathophysiologically as an endometrial dysfunction that probably is related to the accumulation of lipid peroxides produced in the placenta (13 ; 14). The placenta also has opposite physiological activities, including the production of antioxidants that decompose the placental lipid peroxides (15). Generally, a healthy pregnancy is maintained by the quantitative balance between such oxidants and antioxidants (13; 14; 15), and the disintegration of this equilibrium might lead to endometrial dysfunction.

Another study shows an increase of MDA which refers to the increment of lipid peroxidation, due to the decreasing activity of the defense system protecting tissues from the free radical (16).

The Free radicals have been implicated in the pathogenesis of a variety of diseases. Thus, the large amount of lipid accumulates produced in the placenta in many diseases, involving toxoplasma infestation and must be prevented through the production of antioxidants (16), But in the study MDA values were simple differences and non- significant because MDA was measured in the solution caused by placenta crushing and not directly in the blood because the parasite directly affects the blood, moreover, the cause may be the end of the parasite's effect on placental tissue outside the organism's.

References

- 1/ Alvarez C, De-La-Torre A, Vargas M, Herrera C, Uribe-Huertas LD, Lora F, Gómez-Marín JE. Striking divergence in *Toxoplasma* ROP16 nucleotide sequences from human and meat samples. *The Journal of infectious diseases*. 2014 Dec 23;211(12):2006-13.
- 2/ Gomez-Marín JE. Evidence based guideline for toxoplasmosis during pregnancy. *Infectio*. 2017 Mar 24;21(2).
- 3/ Sepidarkish M, Almasi-Hashiani A, Maroufizadeh S, Vesali S, Pirjani R, Samani RO. Association between previous spontaneous abortion and pre-eclampsia during a subsequent pregnancy. *International Journal of Gynecology & Obstetrics*. 2017 Jan;136(1):83-6.

- 4/ Flegr J, Prandota J, Sovičková M, Israeli ZH. Toxoplasmosis—a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. *PloS one*. 2014 Mar 24;9(3):e90203.
- 5/ Clement EG, Horvath S, McAllister A, Koelper NC, Sammel MD, Schreiber CA. The Language of First-Trimester Nonviable Pregnancy: Patient-Reported Preferences and Clarity. *Obstetrics & Gynecology*. 2019 Jan 1;133(1):149-54.
- 6/ Ito F, Sono Y, Ito T. Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis, and chronic inflammation. *Antioxidants*. 2019 Mar;8(3):72.
- 7/ Lopresti AL, Maker GL, Hood SD, Drummond PD. A review of peripheral biomarkers in major depression: the potential of inflammatory and oxidative stress biomarkers. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2014 Jan 3;48:102-11.
- 8/HO AL-KHAFFAF, Farah; A ABDULLAH, Basima. Isolation of *Toxoplasma gondii* Parasite from Placental Tissue of Aborted Women in Nenavah Governorate. *Rafidain Journal of Science*, 2005, 16.10: 93-99.
- 9/ DESMONTS, GEORGES; COUVREUR, JACQUES. Toxoplasmosis in pregnancy and its transmission to the fetus. *Bulletin of the New York Academy of Medicine*, 1974, 50.2: 146.
- 10/ DUBEY, Jitender Prakask, et al. *Toxoplasmosis of animals and man*. CRC Press, Inc., 1988.
- 11/ JACOBS, Leon; REMINGTON, Jack S.; MELTON, Marjorie L. The resistance of the encysted form of *Toxoplasma gondii*. *The Journal of parasitology*, 1960, 46.1: 11-21.
- 12/ OLARIU, Tudor Rares, et al. Severe congenital toxoplasmosis in the United States: clinical and serologic findings in untreated infants. *The Pediatric infectious disease journal*, 2011, 30.12: 1056-1061.
- 13/ HUBEL, Carl A., et al. Lipid peroxidation in pregnancy: new perspectives on preeclampsia. *American journal of obstetrics and gynecology*, 1989, 161.4: 1025-1034.
- 14/ WANG, Yuping; WALSH, Scott W.; KAY, Helen H. Placental lipid peroxides and thromboxane are increased and prostacyclin is decreased in women with preeclampsia. *American journal of obstetrics and gynecology*, 1992, 167.4: 946-949.
- 15/WALSH, SCOTT W.; WANG, YUPING. Trophoblast and placental villous core production of lipid peroxides, thromboxane, and prostacyclin in preeclampsia. *The Journal of Clinical Endocrinology & Metabolism*, 1995, 80.6: 1888-1893.
- 16/ Karaman U, Celik T, Kiran TR, Colak C, Daldal NU. Malondialdehyde, glutathione, and nitric oxide levels in *Toxoplasma gondii* seropositive patients. *The Korean journal of parasitology*. 2008 Dec;46(4):293.