Medical Journal of Ahl al-Bayt University, Vol.4, No.1, Aug.2025

Detection of *Toxoplasma gondii* antibody by Latex technique among some of Universities students

Abeer M. Alsheikly[1], Marwa L. Jabur[2], Ali J. Turki[3] , Shahad M. Alsheikly[4]

abeer.mahmood@uomustansiriyah.edu.iq[1], marwahjebur93@uomustansiriyah.edu.iq[2], Alijamal1996@uomustansiriyah.edu.iq[3], Shahad.m.shaker@uotechnology.edu.iq[4]

[1,2,3] **University of Al Mustansiriyah, College of Pharmacy, Dept. of clinical laboratory sciences**

[4] **University of Technology, College of Chemical Engineering**

ABSTRACT

Toxoplasma gondii exhibits a global distribution and is among the most common infectious agents in Iraq. The current study aimed to assess the seroprevalence of toxoplasmosis across various universities students in different regions in Baghdad. Venous blood samples were taken from 200 male and female students, both pre-marital and married in females only. Serum was obtained from everyone for the identification of specific anti-*T. gondii* antibody by LAT test. The overall percentage of seropositive anti-*T.gondii* antibodies by LAT 87 is 43.5%. According to the age, the higher seropositive found in age ranging between (19-23) 45 (22.5%). High Significant differences (P≤0.01) were observed in the results across different regions based on age groups. Females exhibited a substantial increase in the percentage of seropositivity for toxoplasmosis compared to male students, as determined by LAT test. The prevalence of *T. gondii* infection in pre-marital females was substantially higher at 20 (10%) compared to married females at 5 (2.5%), as determined by latex testing.

Keywords: Toxoplasma gondii; Serological test; Latex agglutination tes

INTRODUCTION

A small North African rodent, Ctenodactylus gundi, is the source of toxoplasmosis (Smith *et al.,* 2021). The protozoan Apicomplexa causes the disease, which is common in humans and other animal species and has been documented in numerous countries with varying climates (Jiménez-Martín *et al.,* 2020). Felidae serve as both intermediate and definitive hosts, while all mammals, including humans and birds, are intermediate hosts. Meat from sheep and goats is a significant source of toxoplasmosis infection (Esubalew *et al.,* 2020) . Humans can contract *T. gondii* by eating raw infected meat from intermediate hosts, such as

sheep, goats, and cattle, or by consuming contaminated food or water that contains sporulated oocysts. Additionally, the virus is spread through the placenta (Gisbert Algaba *et al.,* 2020) . Although the disease is usually benign, it can cause serious morbidity and mortality in immunocompromised people, particularly those who have acquired immunodeficiency syndrome, or AIDS (Almeria and Dubey, 2020), as well as in developing fetuses (Mata *et al.,* 2021). During the acute stage of infection, millions of oocysts are formed in the intestinal mucosa of cats, the last host, and may be expelled in the feces in a single day. Cats have been regarded as a health concern for both humans and wildlife because they are recognized to be a source of the disease (Odeniran *et al.,* 2020). Breeders of sheep and goats suffer large financial losses because of the parasite, which is a leading cause of abortion (Dubey *et al.,* 2020). One of the major causes of abortion in humans is toxoplasmosis (Kalantari *et al.,* 2021).

Although parasite infection is common worldwide, seroprevalence varies significantly by nation and population group (Ducrocq et al., 2021) . In the absence of immunosuppression. T. gondii antibodies in mammal serum have been found using a variety of serologic techniques such as ELISA, or enzyme-linked immunosorbent assay, and miniVIDAS, or Vitek Immunodiagnostic Assay System. The most often used diagnostic tests are the latex agglutination test (LAT), modified agglutination test (MAT), indirect fluorescent antibody test (IFAT), Sabin-Feldman (SF) dye test, complement fixation test (CFT), and indirect haemagglutination test (IHAT) and the SF test which is the primary method for detecting toxoplasmosis in both humans and animals. Toxoplasma gondii infection is recognized by IgG,IgM,IgA and IgE antibodies in patients with acute and chronic toxoplasmosis depending on the strain and the stage of the parasite , therefore essential to estimate the time of infection as precisely as possible to properly manage the risk to the fetus of a maternal infection, A positive IgM antibodies result in a single serum specimen may reflect an acute infection .

Aims of this study

To determine the seroprevalence of anti-Toxoplasma antibodies among male and female students in different ages from different regions in Baghdad province and then examine these samples by Latex test for calculating the percentage of toxoplasmosis infection. and examine the connection between Toxoplasma infection and some parameters (age, sex, and marital status) examined in this investigation.

Historical Background

Toxoplasmosis is a significant disease caused by the microscopic parasite *Toxoplasma gondii*. This disease was first identified in 1908 by Nicolle and Manceaux in North Africa, as well as by Splendore in Brazil. The species name derives from the North African rodent Ctenodactylus gondii, from which the parasite was initially isolated (Ajioka and Morrissette, 2009) . The genus name is rooted in the Greek word toxon, meaning "bow," which refers to the organism's crescent shape (Michael and John, 2000) . Toxoplasmosis was first reported in humans by Junku in 1923, who discovered parasitic cysts in the retina of a child suffering from congenital hydrocephalus (Remington and Desmonts, 1990) .

In Iraq, the parasite was first identified by Machattie (1938) through smears taken from the spleens and lungs of two street dogs in Baghdad.

Stages of *Toxoplasma gondii*

There are three stages of *T. gondii*: -

1. Oocysts, which give rise to sporozoites.

2. Tachyzoite or trophozoite, the actively proliferating form.

3. Cystozoite or bradyzoite, the resting form.

The Life Cycle of Toxoplasmosis:

The life cycle of *Toxoplasma gondii* consists of stages occurring in both feline and non-feline hosts, which are associated with sexual and asexual development, respectively.

Transmission of T. gondii:

There are multiple pathways for parasite transmission:

1- Transplacental transmission (Maternal transmission).

2- Oral Transmission.

3- Blood Transfusion.

Diagnosis of Toxoplasmosis:

The diagnostic laboratory procedures for toxoplasmosis include the isolation of the organism and the detection of antibodies to Toxoplasma in the patient's serum. Furthermore, a cutaneous response akin to the tuberculin reaction may be elicited in positive patients using an antigen derived from Toxoplasma (Sharma *et al*. 2023). The clinical pathology of the disorder provides minimal diagnostic assistance; nevertheless, during the acute phase, an elevation in cells and protein in the cerebrospinal fluid may be observed, although an increase in protein without a corresponding rise in cells is occasionally noted in seemingly resolved instances. No specific blood changes or eosinophilia have been documented.

1- Direct Microscopic Examination:

Tissues obtained by biopsy or autopsy can undergo direct microscopic inspection by being fixed, sectioned, and stained, or by creating impression smears treated with polychrome stains. Suspected fluid may undergo centrifugation, and the sediment can be analyzed in stained smears. Cerebrospinal and ventricular fluid must be analyzed promptly following aspiration. Toxoplasma gondii can be extracted from the placenta, umbilical cord, or newborn blood through inoculation into mice or cell culture.

2- Serological Diagnosis:

The predominant technique employed to identify toxoplasmosis is serologic testing for Toxoplasma-specific immunoglobulin G (IgG) antibodies. Toxoplasma immunoglobulin M

(IgM) antibody titters increase shortly after infection but generally decrease rapidly and become undetectable within weeks or months. IgG titters increase within one to two months post-infection and persist elevated for a lifetime. The presence of toxoplasma IgG antibodies signifies an existing T gondii infection, whereas IgM antibodies indicate a recent infection. Serum anti-Toxoplasma antibody titters can be assessed using many approaches, including the following:

A- Dye Test (D.T.):

It is referred to as Sabin-Feldman. The dye test was developed in 1948 by Sabin and Feldman (Jeske *et al.,* 2024). DT is the definitive serological assay for Toxoplasma antibodies in humans. Live Toxoplasma tachyzoites are treated with a complement-like accessory factor and test serum at 37°C for one hour prior to the addition of methylene blue. A specific antibody enhances membrane permeability in the parasite, allowing cytoplasmic leakage, resulting in the tachyzoite's inability to absorb the dye, therefore appearing colorless. Tachyzoites that are not exposed to a specific antibody (i.e., a negative serum sample) absorb the dye and exhibit a blue coloration. DT is both specific and sensitive in humans but may be unreliable in other species.

B- Indirect Hemagglutination Assay (IHAT):

Jacobs and Lunde described an indirect hemagglutination test (IHAT) utilizing sheep red cells as an antigen carrier (Ehrens *et al.,* 2020). The reagent consists of a suspension of stabilized erythrocytes coated with a pure antigen derived from Toxoplasma gondii, incubated in mouse peritoneal exudate. These erythrocytes interact with certain antibodies found in human or animal serum, thereby creating a uniform network on the plate (positive reaction). In the absence of specific antibodies, erythrocytes aggregate to form a distinct button at the bottom of the plate, indicating a negative reaction. This method is applicable to both humans and animals, proving efficient for testing large quantities of sera; however, it is not suitable for detecting congenital and neonatal infections, and it is subject to variability in red blood cell quality and antigen differences.

C- Complement Fixation Test (CFT):

The complement fixation test (CFT) is the fundamental technique for diagnosing toxoplasmosis (Nova *et al.,* 2023) . Antigens were extracted from the peritoneal exudates of infected mice and guinea pigs. False positives may occasionally occur in malaria, TB, salmonellosis, and trypanosomiasis. This test is infrequently utilized currently due to technological challenges and insufficient sensitivity.

D- Latex Agglutination Test (LAT):

The toxoplasmosis latex reagent is a dispersion of polystyrene particles sensitized with Toxoplasma gondii antigens (Angarita-Corzo *et al.,*2025). When serum or plasma from an infected individual is combined with latex particles, a characteristic agglutination pattern is seen due to the development of antigen-antibody complexes. In the absence of infection, no agglutination will occur. A positive result shows the presence of antibodies.

E- Indirect Fluorescent Antibody Test (IFAT):

The IFAT is a straightforward and often utilized technique. Whole, killed Toxoplasma tachyzoites are incubated with diluted test serum, followed by the addition of the appropriate fluorescent antispecies serum, and the outcome is subsequently examined using a fluorescence microscope (Voyiatzaki *et al.,*2024). Commercially accessible fluorescent-labelled antibodies exist for various animal species; the procedure is very cost-effective, and kits can be purchased. Nonetheless, the technique necessitates a fluorescent microscope, and the results are interpreted visually, which may lead to individual variability. Locating certain species-specific conjugates may prove challenging, and there exists a potential risk of cross-reactivity with rheumatoid factor.

F- Enzyme-Linked Immunosorbent Assay (ELISA):

Ban Waeman and Schurs conducted research in Holland in 1971, whereas Engavell Perlamann originally created this test in Sweden in 1972. The test is highly sensitive and utilizes an enzyme coupled with either the antibody or the antigen to measure the respective antigens and antibodies (Voyiatzaki *et al.,*2024). This is a straightforward, quick, and precise method for detecting IgM antibodies in persons with acute acquired toxoplasmosis and for assessing whether pregnant women were infected during gestation or before to conception. IgM generally appears within the first week of infection, rises rapidly, and thereafter decreases at varied rates, eventually disappearing after a few months. IgM antibodies may persist for years beyond the acute infection, and the reliability of commercially available assays varies significantly. The IgM-ELISA identifies recently acquired acute Toxoplasmosis and congenital infection. Positive ELISA-IgG reactivity signifies a prior infection, regardless of its regency or duration. Positive IgA reactivity serves as a reliable indicator of recent active infection and can confirm primary, congenital (maternal, fetal, and neonatal), and reactivating infections. Immunosuppressed individuals may exhibit diminished IgA responses.

G- VITEK Immunodiagnostic Assay System (miniVIDAS):

The miniVIDAS is referred to as a "multiparametric" instrument. The phrase "multiparametric" describes the capabilities of the VIDAS. The user can execute compatible tests using identical protocols inside the same area. Salmonella, Listeria, and E. coli 0157 can be processed concurrently in the same section. The test principle integrates a two-step enzyme immunoassay sandwich technique with a concluding fluorescence detection method (ELFA), specifically utilizing mini VIDAS. It is a straightforward, quick, and precise method for quantifying anti-toxoplasma IgM or IgG in individuals with acute or chronic toxoplasmosis. The VIDAS apparatus executes all procedures autonomously. This technique exhibits great specificity for the identification of IgM and IgG (Fiedler *et al.,* 1999).

Materials and Methods

Subject selection and blood sample collection:

Blood samples were collected from 200 students (males and females) from different Universities in different regions in Baghdad province with age ranging from 19 to 28 years. Five ml of venous blood were collected from each person by using a disposable syringe. The blood sample was placed in a plain tube and let stand for 20 minutes at room temperature to clot. Serum was separated from clot by centrifugation at 3000 rpm for 10 min of (5ml), Then the obtained serum was divided into three portions in different appendrof tubes to avoied repeated freezing and thawing, and stored at -20ºC until being analyzed for detection of total Toxoplasma antibodies by Latex Agglutination Test (LAT). There was ethical approval from all students in this paper.

*(The kit used in this study from BIOKIT, S.A. used for detection of total Antibodies).

Results

Table (1): The percentage distribution of LAT Sero +ve and sero –ve of students from different ages groups.

Test / Age Groups(year)	Latex test					
	Latex+ve		Latex-ve		Total	
	NO	%	NO	%	NO	%
19-23	45	22.5	64	32	109	54.5
23-25	27	13.5	29	14.5	56	28
25-28	15	7.5	20	10	35	17.5
Total	87	43.5	113	56.5	200	100
P-value	--	0.0001 **	--	0.0001 **	--	0.0001 **
** (P≤0.01).						

Table (2): The distribution of LAT sero +ve in different ages group according to the sex

Test / Sex	LAT sero +ve							
	19-23		23-25		25-28		Total	
	NO	%	NO	%	NO	%	NO	%
Male	9	4.5	7	3.5	10	5	26	13
Female	25	12.5	19	9.5	17	8.5	61	30.5
Total	34	17	26	13	27	13.5	87	43.5
P-value	--	0.0096 **	--	0.275 NS	--	0.459 NS	--	0.0006 **
** (P≤0.01).								

Table (3): The distribution of LAT sero +ve in different ages group according to the female group

Test / Female groups	LAT sero +ve							
	19-23		23-25		25-28		Total	
	NO	%	NO	%	NO	%	NO	%
Premarital	20	10	11	5.5	14	7	45	22.5
Married	5	2.5	6	3	5	2.5	16	8.
Total	25	12.5	17	8.5	19	9.5	61	30.5
P-value	--	0.0382 *	--	0.563 NS	--	0.392 NS	--	0.0027 **
* (P≤0.05), ** (P≤0.01).								

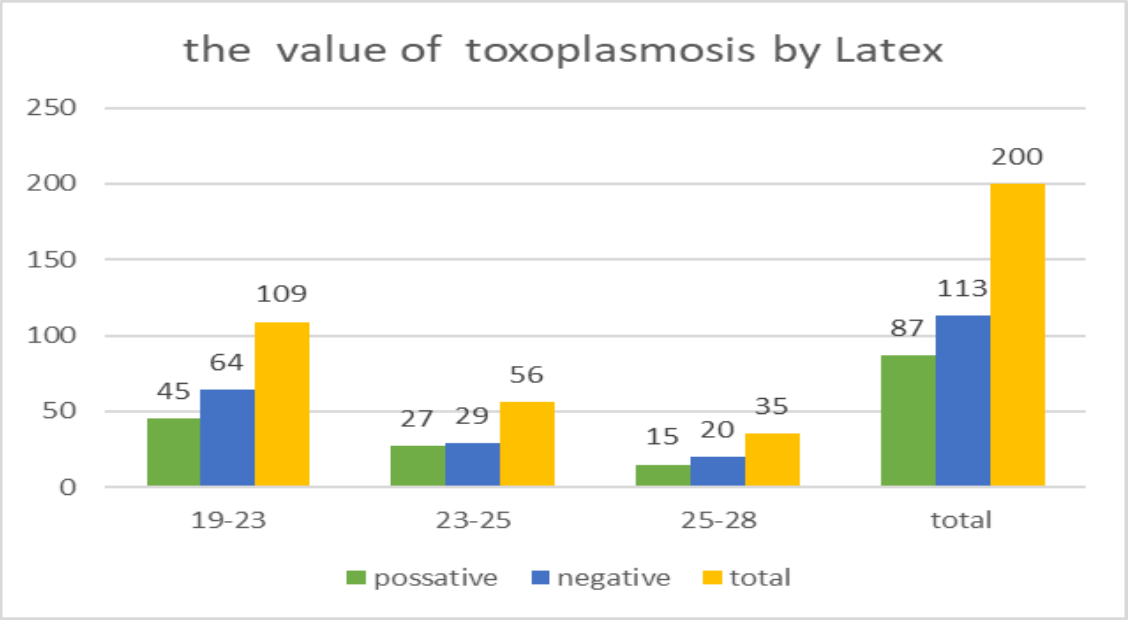
The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of different groups/ factors in study parameters. The Chi-square test was used to significantly compare between percentage (0.05 and 0.01 probability in this study.

Note:

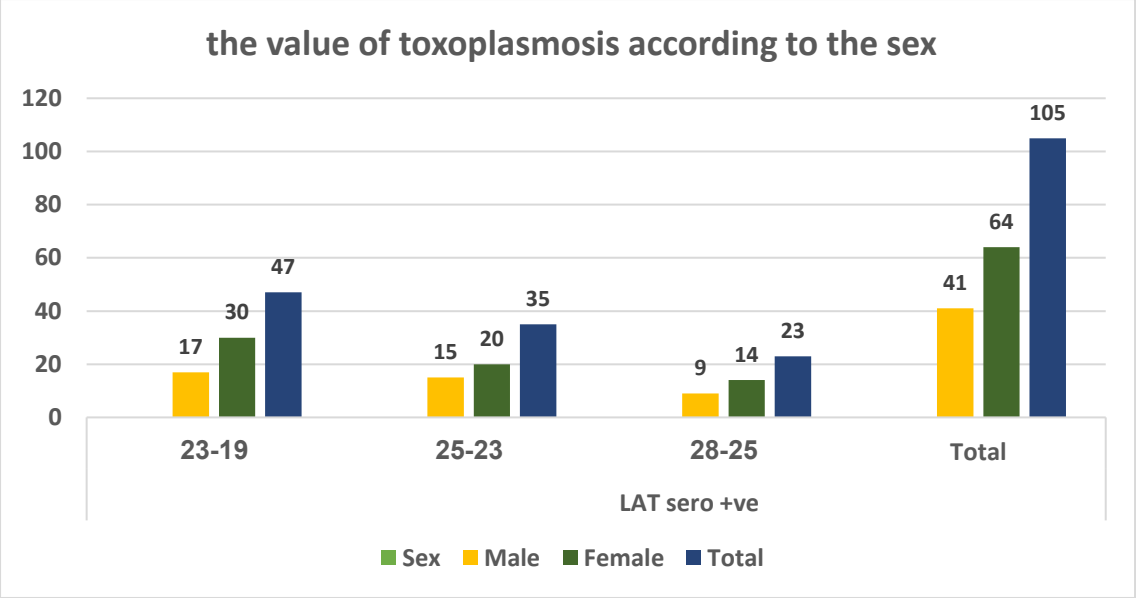
* Means significant (P≤0.05).

** means highly significant (P≤0.01).

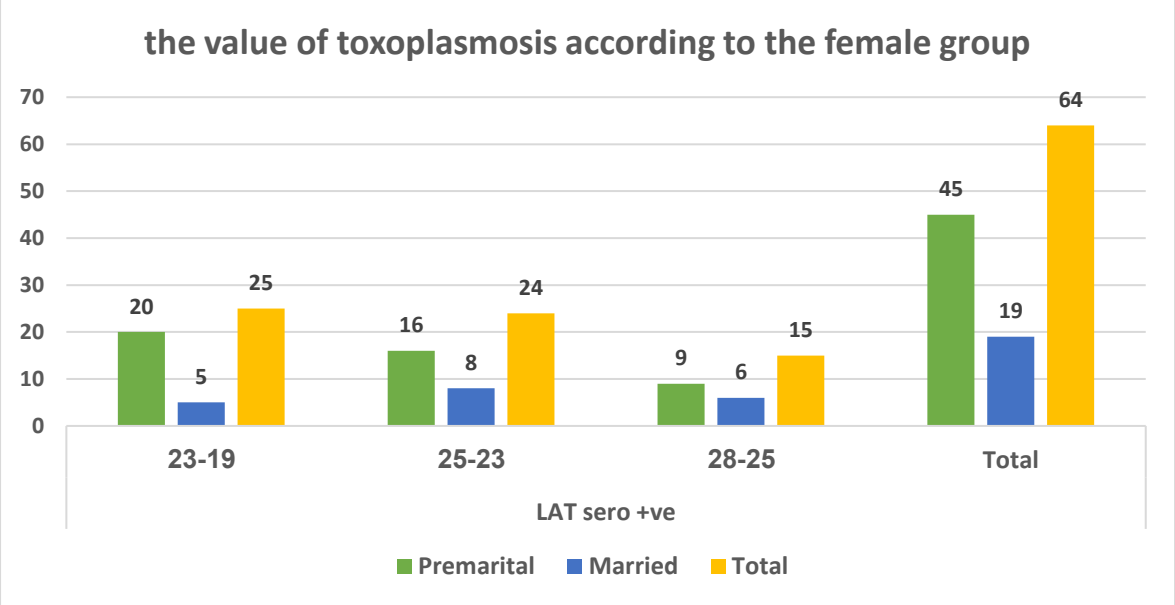
NS means not significant.



The value distribution of toxoplasmic steudents according to the age groups from the students Universities in Baghdad province by latex agglutination test (LAT).



The value distribution of toxoplasmosis of LAT sero +ve in each ages according to the sex.



The value distribution of LAT sero +ve in each ages according to the female groups.

Discussions:

The total percentages of latex seropositive is 87(43.5%) and there were highly significant differences (P≤0.01) according to the ages group. The infection in younger age groups may be more common due to lifestyle, handling animals, eating patterns, etc Table (1).

The latex seropositive in female more than in male and there were highly significant differences (P≤0.01) according to the sex in first age group and not significant in other ages group. Toxoplasmosis prevalence is often reported as higher in females than males, but the reasons for this are complex and not fully understood. Several factors, including behavioral

differences, immune responses, and potential sexual transmission, Also, handling fresh meat, soil and animals may be a cause of infection Table (2).

The latex seropositive in Premarital female more than in Married female and there were significant differences ($P \leq 0.05$) according to the females' group in first age group and not significant in other ages group and the total percentages was highly significant ($P \leq 0.01$) . Toxoplasmosis prevalence may appear higher in unmarried females due to a combination of factors including age, lifestyle, and potential exposure to risk factors. While it's not a direct consequence of being unmarried, certain risk factors associated with Toxoplasma infection may be more common in this group. These factors include increased likelihood of close contact with cats, consumption of undercooked meat, and engaging in outdoor activities like gardening, which can lead to soil contamination Table (3)

References

1. Ajioka , J . W . and N . S . Morrissette (2009) . A century of Toxoplasma research . Int . J . Parasitol., 39:859-860 .
2. Almeria, S., & Dubey, J. P. (2020). Foodborne transmission of Toxoplasma gondii infection in the last decade. An overview. Research in Veterinary Science. doi:10.1016/j.rvsc.2020.10.019 .
3. Angarita-Corzo K, Henao-Montoya LM, Franco-G M, Riva HG, Correa-Valencia NM. Occurrence of Toxoplasma gondii in Three Tamarin Species (Saguinus geoffroyi, Saguinus oedipus, and Leontocebus fuscicollis) Ex Situ in Colombia. J Med Primatol. 2025 Apr;54(2):e70005. doi: 10.1111/jmp.70005. PMID: 39962995 .
4. Dubey, J. P., Murata, F. H. A., Cerqueira-Cézar, C. K., Kwok, O. C. H., & Su, C. (2020). Economic and public health importance of Toxoplasma gondii infections in sheep: the last decade. Veterinary Parasitology, 109195.
5. Ducrocq, J., Ndao, M., Yansouni, C. P., Proulx, J., Mondor, M., Hamel, D. Talbot, D. (2021). Epidemiology associated with the exposure to Toxoplasma gondii in Nunavik's Inuit population using the 2017 Qanuilirpitaa cross-sectional health survey. Zoonoses and Public Health, 68(7), 803–814. doi:10.1111/zph.12870 .
6. Ehrens A, Lunde CS, Jacobs RT, Struever D, Koschel M, Frohberger SJ, Lenz F, Fendler M, Turner JD, Ward SA, Taylor MJ, Freund YR, Stefanakis R, Easom E, Li X, Plattner JJ, Hoerauf A, Hübner MP. In vivo efficacy of the boron-pleuromutilin AN11251 against Wolbachia of the rodent filarial nematode Litomosoides sigmodontis. PLoS Negl Trop Dis. 2020 Jan 27;14(1):e0007957. doi: 10.1371/journal.pntd.0007957. PMID: 31986143; PMCID: PMC7004383.
7. Esubalew, S., Seyoum, Z., Temesgen, W., Dagnachew, S., Kussa, M., Ayele, A., … Chanie, M. (2020). Seroepidemiology of Toxoplasma gondii in small ruminants in Northwest Ethiopia. Veterinary Parasitology: Regional Studies and Reports, 100456.
8. Fiedler K, Hülsse C, Straube W, Briese V. Toxoplasmose-Durchseuchungsstudie in Mecklenburg-Vorpommern [Toxoplasmosis-antibody seroprevalence in

Mecklenburg-Western Pomerania]. Zentralbl Gynakol. 1999;121(5):239-43. German. PMID: 10408076 .

9. Gisbert Algaba, I., Verhaegen, B., Murat, J. B., Coucke, W., Mercier, A., Cox, E., De Craeye, S. (2020). Molecular Study of Toxoplasma gondii Isolates Originating from Humans and Organic Pigs in Belgium. Foodborne Pathogens and Disease. doi:10.1089/fpd.2019.2675
10. Jeske R, Becker N, Kroeller L, Mentzer AJ, Brenner N, Guy E, Waterboer T, Butt J. Advancing *Toxoplasma gondii* multiplex serology. Microbiol Spectr. 2024 Apr 2;12(4):e0361823. doi: 10.1128/spectrum.03618-23. Epub 2024 Feb 22. PMID: 38385741; PMCID: PMC10986549.
11. Jiménez-Martín, D., García-Bocanegra, I., Almería, S., Castro-Scholten, S., Dubey, J. P., Amaro-López, M. A., & Cano-Terriza, D. (2020). Epidemiological surveillance of Toxoplasma gondii in small ruminants in southern Spain. Preventive Veterinary Medicine, 105137.
12. Kalantari, N., Gorgani-Firouzjaee, T., Moulana, Z., Chehrazi, M., & Ghaffari, S. (2021). Toxoplasma gondii infection and spontaneous abortion: A systematic review and meta-analysis. Microbial Pathogenesis, 158, 105070.
13. Machattie , C . (1938) . Notes on two cases of naturally occurring toxoplasmosis in Baghdad Tran . Roy .Top. Hyg ., 23:373-376.
14. Matta, S. K., Rinkenberger, N., Dunay, I. R., & Sibley, L. D. (2021). Toxoplasma gondii infection and its implications within the central nervous system. Nature Reviews Microbiology, 19(7), 467–480.
15. Michael, W. B. and C. B. John (2000). Lytic cycle of Toxoplasma gondii.M.M.B.R.,64(3):607-623.
16. Nicolle , C. and L. Manceaux, (1908). Sur une infection a` corps ou organismes voisins du gondi. C. R. Seances Acad. Sci., 147:763-766 .
17. Novoa MB, Aguirre N, Ormaechea N, Palmero S, Valentini B, Vanzini V, Molineri AI. Evaluation of frequency of antibodies against Neospora caninum, Toxoplasma gondii and Brucella melitensis, risk factors and spatial distribution of infection in goat and sheep flocks from Argentina. Vet Parasitol Reg Stud Reports. 2023 Nov;46:100939. doi: 10.1016/j.vprsr.2023.100939. Epub 2023 Oct 13. PMID: 37935540 .
18. Odeniran, P. O., Omolabi, K. F., & Ademola, I. O. (2020). Risk factors associated with seropositivity for Toxoplasma gondii in population-based studies among immunocompromised patients (pregnant women, HIV patients and children) in West African countries, Cameroon and Gabon: a meta-analysis. Acta Tropica, 105544.
19. Remington, J.S .and G. Desmonts (1990). Toxoplasmosis. Bull. N.Y. Acad.Med.,50:211-227.
20. Sharma K, Srivastava S, Kundu A, Tak V. Detection of Antibodies against Toxoplasma from Human Serum Sample using ELISA. Infect Disord Drug Targets. 2023;23(2):e130922208785. doi: 10.2174/1871526522666220913152447. PMID: 36100992.

21. Smith, N. C., Goulart, C., Hayward, J. A., Kupz, A., Miller, C. M., & van Dooren, G. G. (2021). Control of human toxoplasmosis. International Journal for Parasitology, 51(2-3), 95–121 .
22. SPSS (2019). Statistical Packages of Social Sciences-SPSS/ IBM Statistics 26 step by step. 16th Edition.
23. Voyiatzaki C, Zare Chormizi AD, Tsoumani ME, Efstathiou A, Konstantinidis K, Chaniotis D, Chrysos G, Argyraki A, Papastamopoulos V, Kotsianopoulou M. Seroprevalence of *Toxoplasma gondii* among HIV Positive Patients under Surveillance in Greek Infectious Disease Units: A Screening Study with Comparative Evaluation of Serological Methods. Pathogens. 2024 Apr 30;13(5):375. doi: 10.3390/pathogens13050375. PMID: 38787227; PMCID: PMC11123859 .