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The relationship between the infection of *Toxoplasma gondii* and breast cancer among women

A Thesis

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BY

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بسم الله الرحمن الرحيم

﴿وَتِلْكَ ٱلْأَمْثَـٰلُ نَضْرِبُهَا لِلنَّاسِ ۖ وَمَا يَعْقِلُهَآ إِلَّا ٱلْعَـٰلِمُونَ﴾

صدق الله العلي العظيم

سورة العنكبوت آية٤٣

Dedication

To the Remnant of God on earth,

To the promised light and the awaited hope,

To the present yet unseen Imam —the Master of the Age and the Time —

May Allah hasten his reappearance and make us among his sincere helpers and loyal supporters.

I humbly dedicate this modest scientific endeavor as a token of loyalty and devotion to the path of knowledge, the very path cherished by Ahlulbayt (peace be upon them).

I sincerely pray that Allah grants me the honor of applied, purposeful knowledge in the era when his blessed hands will raise the truth-righteous banner.

With all reverence and gratitude.

Elaf Alzaidy

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Summary:

Certain parasites have been proposed as potential carcinogenic agents due to their significant impact on human health. *T. gondii* is one of the most widely distributed intracellular parasites, infecting more than 30% of the global population. Humans serve as one of over 300 intermediate hosts for this parasite, which is capable of infecting nearly all nucleated cells in endothermic animals.

Breast cancer represents the most prevalent malignant tumor among women worldwide. Although genetic alterations influenced by environmental and hormonal factors are primarily implicated in its pathogenesis, the possible contribution of parasitic infections, such as *T. gondii*, in inducing or accelerating these cellular changes warrants thorough investigation.

This study was conducted between December 2023 and May 2025 to explore the association between *T. gondii* infection and breast tumors in women using two complementary diagnostic techniques: histopathology and immunohistochemistry. A total of 61 breast tissue samples were collected from a private laboratory under specialist supervision. Among these, 46 samples were diagnosed with breast cancer, five with benign breast tumors, and ten were free from malignant or benign lesions.

The results demonstrated that IHC was more sensitive and reliable than HP in detecting *T. gondii*. Infection with *T. gondii* caused severe tissue damage, including necrosis and structural destruction. The prevalence of *T. gondii* infection was 47.83% in breast cancer tissues and 20% in benign tumors, whereas no infection (0.00%) was detected in tissues free of tumors. Statistical analysis revealed a significant association between *T. gondii* infection and breast cancer ($\chi^2 = 7.877$, $p = 0.005$), while no significant relationship was observed with benign tumors ($\chi^2 = 2.143$, $p = 0.143$).

These findings highlight the potential role of *T. gondii* infection in breast cancer pathogenesis, although factors such as cancer type, parasite strain, and host immune response likely influence this relationship. Further studies are recommended to elucidate the underlying mechanisms linking *T. gondii* infection to breast carcinogenesis.

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List of Abbreviations

Abbreviations	Meaning
Ab	Antibody
AIDS	Acquired Immunodeficiency Syndrome
AP	Alkaline Phosphataes
BAK	Bcl-2 homologous antagonist/killer
BAX	Bcl-2-associated X protein
BRCA1	Breast Cancer Gene 1
BSA	Bovine Serum Albumin
°C	Celsius
Ca^{2+}	Calcium Ion
$CD8^{+}$ T cells	Cluster of Differentiation 8 T cells
DAB	Diaminobenzidine
DHF	Dihydrofolate Reductase
DHP	Dihydropteroate Synthetase
CNS	Central Nervous System
DNA	Deoxyribonucleic Acid
DPX	Dibutylphthalate Polystyrene Xylene
ECM	Extracellular Matrix
ELISA	Enzyme-Linked Immunosorbent Assay
GRAs	Dense Granule Proteins
H&E	Hematoxylin and eosin stain
HIV	Human Immunodeficiency Virus
HMGB1	High Mobility Group Box 1
HOTAIR	HOX Transcript Antisense Intergenic RNA
HP	Histopathology
HRP	Horseradish Peroxidase
IDC	Invasive Ductal Carcinoma
IFN-γ	Interferon-Gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-12	Interleukin-12
IL-23	Interleukin-23
LAT	Latex Agglutination Test
MCF-7	Michigan Cancer Foundation-7
MICs	Microneme Proteins
MJ	Moving Junction

MMPs	Metalloproteinases
MRI	Magnetic Resonance Imaging
MYC	Myelocytomatosis Oncogene
NFkB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
p38-MAPK	p38 Mitogen-Activated Protein Kinase
p53	Tumor suppressor gene
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PD-1	Programmed Cell Death Protein-1
PD-L1	Programmed Death-Ligand 1
PI3K	Phosphoinositide 3-Kinase
PKC	Protein Kinase C
PPT	Parts Per Thousand
PV	Parasitophorous Vacuole
PVM	Parasitophorous Vacuole Membrane
Pyr-Sulf	Pyrimethamine And Sulfadiazine
RH-Δompdc	*Toxoplasma gondii* RH strain with deletion of the orotidine 5'-monophosphate decarboxylase (ompdc) gene
RNA	Ribonucleic Acid
RONs	Rhoptry Neck Proteins
ROPs	Rhoptry Proteins
RT	Room Temperature
SAGs	Surface Antigens
SFDT	Sabin Feldman Dye Test
spp	Species pluralis
STAT3	Signal Transducer and Activator of Transcription 3
4T1 Cells	Murine mammary carcinoma cell line
TBS	Tris-Buffered Saline
Th17	T helper 17 cells
TME	Tumor Microenvironment
T. gondii	*Toxoplasma gondii*
µm	Micrometer
µL	Microliter
WHO	World Health Organization

Chapter One

Introduction

1. Introduction:

Parasites encompass diverse organisms that significantly spread human diseases, including protozoa, helminths, and arthropods (Mahmud *et al.*, 2017). The apicomplexa phylum includes many intracellular protozoan parasites, such as *Eimeria, Sarcocystis, Isospora*, and *Toxoplasma* (Corvi *et al.*, 2012). *Toxoplasma gondii* (*T. gondii*) is an opportunistic coccidian parasite with an obligate intracellular parasitism ability, affecting over 30% of the global population; this parasite lives inside host cells to persist (Chen *et al.*, 2023). *T. gondii* parasitizes nucleated cells of various tissues of over 350 species of endothermic vertebrates, including humans (Innes, 2010; Salim *et al.*, 2022; Hotea & Dărăbuș, 2023). The infection in immunocompetent individuals is typically described as mild and asymptomatic; however, it is severe and symptomatic in immunocompromised individuals and congenitally infected newborns (Kalantari *et al.,* 2017; Castro & Dubey, 2019; Ghenciu *et al*., 2024).

Cancer is a life-threatening and ongoing challenge to global health, according to statistics from the World Health Organization (WHO), which estimates approximately 20 million new cancer cases and 9.7 million cancer-related deaths annually (WHO, 2024). Cancer is the second leading of mortality, trailing cardiovascular diseases (WHO, 2024). Cancer is mainly defined by the uncontrolled development of cells that can arise in all tissue types across all age groups; hereditary factors, environmental pollutants, and lifestyles are not the sole contributor to carcinogenesis; some pathogenic factors, including viruses, bacteria, fungi, and parasites, play a key role in cancer onset (Weinberg, 1996; Devi, 2004).

Breast cancer stands as the most prevalent malignancy and the primary contributor to cancer-related mortality among women worldwide (Chen *et al*., 2023). Breast cancer develops in women after puberty, affecting

populations in both economically developed and developing countries (WHO, 2024). Nevertheless, the rate increases in older age, approximately 2.3 million cases have been diagnosed with breast cancer, and 670,000 deaths among women worldwide (WHO, 2024). It is a complex malignancy, and both hereditary and environmental factors play a crucial role in its onset (Nikbaksh *et al*., 2022).

Several investigations reveal that toxoplasmosis is more prevalent in cancer patients compared to healthy individuals, including breast cancer patients (Haghbin *et al.*, 2023). Women diagnosed with breast cancer exhibit a higher seroprevalence rate of toxoplasmosis compared to women without malignancy (Kalantari *et al.*, 2017).

The infection with *T. gondii* can inhibit the development of many types of malignant tumors, such as ovarian tumors (Baird *et al*., 2013), pancreatic tumors (Sanders *et al*., 2015), and breast tumors (Xu *et al*., 2021; Ye *et al*., 2024). Also, *T. gondii* is one of the active agents that prevents cancer by inducing cellular immunity (Bolhassani & Zahedifard, 2012; Chen *et al*., 2022). *T. gondii* exhibits anti-cancer activities by the excretion of dense granule antigens (GRAs), rhoptry proteins (ROPs), and cancer-associated mucin-type O-glycans, which prevent tumor progression by activating the antigen-presenting system (Ye *et al*., 2023).

Conversely, specific publications suggest that *T. gondii* contributes to the development of breast malignancy (Salim *et al*., 2022). It has been shown that *T. gondii* plays a significant role in the etiology or existence of breast cancer (Mostafa *et al*., 2018).

Given the conflicting promotive and inhibitory evidence regarding the role of *T. gondii* in breast tumors, a critical question remains: Is there direct histological evidence demonstrating the parasite's impact on malignant breast tumor tissue compared to benign and normal tissue?

1:2 Aims of the study:

This study aimed to investigate the role of *T. gondii* in breast tissue in women through the following objectives:

1- Determine the prevalence of *T. gondii* in women with or without breast tumors.

2- To employ immunohistochemistry for the detection of *T. gondii* infection in breast tissue samples.

3- To conduct histopathological examinations to describe tissue alterations and assess the potential influence of *T. gondii* infection on the development of breast tumors.

Chapter Two

Literature Review

2. Literature Review

2:1 The history of *Toxoplasma gondii*:

Toxoplasmosis is one of the most widespread zoonotic diseases worldwideit ; infects the nucleated cells of 300 members of the endothermic animal species (Dubey, 2022). In 1908, Charles Nicolle and Louis Herbert Manceaux at the Pasteur Institute in Tunisia made a significant discovery while studying leishmaniasis ;they unintentionally found a protozoan organism within the tissues of the rodent species *Ctenodactylus gundi* (Innes, 2010).

Initially, *Leishmania spp*. were the primary suspect, followed by piroplasma based on its morphology and the assumption that the rat acts as the host; scientists named the new organism *Toxoplasma gondii*; "Toxo" refers to the arc, "plasma" denotes form, and "gondii" signifies the host rat species (Dubey, 2022).

Simultaneously, the same parasite was recognized in a rabbit in Brazil in 1908 by parasitologist Alfonso Splendore, who also classified it as *Leishmania spp.*; however, he did not refer to it (Innes, 2010; Al-hajj & Kekillioğlu, 2023).

Toxoplasmosis was first described in a domestic animal, a dog, in 1910; however, in 1923, a newborn with microphthalmia and hydrocephalus was reported to have the first suspected case of congenital toxoplasmosis (Wolf *et al*., 1939).

In addition, throughout the 1920s, researchers Albert Sabin and Henry Feldman proposed an innovative serological staining technique to detect toxoplasmosis in humans; this method quickly gained popularity and became a widely adopted diagnostic tool in the medical community, significantly advancing the understanding and detection of this parasitic disease (Reiter-Owona *et al*., 1999).

The first confirmed case of congenital toxoplasmosis was reported in 1939, which is significant in understanding this infectious disease (Wolf *et al.,* 1939).

During the 1940s, autopsy slides revealed fascinating cysts indicative of a latent, asymptomatic infection (Dubey, 2022). In addition, research on anti-toxoplasmosis drugs began in the 1940s, with studies demonstrating the efficacy of sulfonamides in treating murine toxoplasmosis; in the 1950s, the synergistic effect of combining pyr-sulf for treating toxoplasmosis in mouse models was observed (Innes, 2010; Dunay *et al.,* 2018).

The classic clinical manifestations related to congenital toxoplasmosis in humans were identified in 1952; these manifestations encompass cerebral calcifications, chorioretinitis, hydrocephalus or microcephaly, and psychomotor disorders (Hotea & Dărăbuș, 2023).

In the 1960s, the oocysts of *T. gondii* were identified, and it was recognized that oocysts can be transmitted into the environment through cat feces (Dubey, 2022). Following the description of its life cycle in 1960, researchers observed that the bradyzoites within the tissue cysts prove a notable tolerance to gastric acids and enzymes; this remarkable resilience is crucial in effectively transmitting the infection (Dubey, 2022).

Moreover, the distinct coccidian characteristics of *T. gondii* were identified during research conducted in the 1960s (Hotea & Dărăbuș, 2023). In 1968, *T. gondii* was recognized as a significant complication in patients suffering from malignancies (Hotea & Dărăbuș, 2023).

During the 1970s, intermediate and definitive hosts were identified, with felids recognized as the exclusive hosts responsible for excreting oocysts (Frenkel *et al.,* 1970; Dubey, 2022).

Subsequently, toxoplasmosis emerged as one of the most prominent opportunistic infections affecting individuals with HIV-related immunosuppression during the 1980s, and the researchers found the first CNS toxoplasmosis case in patients with AIDS was identified (Luft *et al.,* 1983).

2:2 Taxonomic classification:

The classical classification of *T. gondii* is as follows (de Oliveira, 2016; Hotea & Dărăbuș, 2023):

Kingdom: Protista

Subkingdom: Protozoa

Phylum: Apicomplexa

Class: Sporozoasida

Subclass: Coccidiasina

Order: Eucoccidiorida

Suborder: Emeriorina

Family: Sarcocystidae

Genus: *Toxoplasma*

Species: *Toxoplasma gondii*

2:3 The morphology of *Toxoplasma gondii:*

Like other apicomplexa, *T. gondii* is a unicellular eukaryotic organism; it possesses an apical complex, an apicoplast (a type of plastid found in apicomplexans), and a glideosome (Frénal *et al*., 2017). It also exhibits a quite varied lifestyle, including phototrophy, intracellular parasitism, and predatory behavior (Delgado *et al*., 2022).

The parasite undergoes three distinct infective stages during its life cycle; two of these stages, the tachyzoites and tissue cysts that sustain bradyzoites, appear in infected humans, birds, and mammalsthe ; third stage, the oocyst, which contains sporozoites, exclusively occurs in the definitive host, the felids (Dubey, 2022; Al-hajj & Kekillioğlu, 2023).

The tachyzoite and bradyzoites stages had a crescent-shaped cell, a thin anterior (apical) pole, and a more rounded posterior (basal) pole measuring 1–2 × 5–8.5 μm (Dubey, 2022); *T. gondii* shows the typical eukaryotic organelles: nucleus, endoplasmic reticulum, Golgi complex, ribosomes, mitochondria, and centrosome (Robert-Gangneux & Dardé, 2012). The same basic formation is shared by the three infectious stages (tachyzoite, bradyzoite, and sporozoite), a distinct apical complex (consisting of 2 sets of secretory organelles: rhoptries and micronemes, as well as a conoid); also, a marked specialization in the anterior portion, where the apical complex is located, is utilized to initiate the process of infecting host cells (Attias *et al*., 2020; Dubey, 2022).

The morphology of *T. gondii* stages differs slightly; tachyzoites and merozoites have a central nucleus and few micronemes, whereas bradyzoites and sporozoites have a basal nucleus and many micronemes; lipid bodies are irregularly present in tachyzoites, absent in bradyzoites, and abundant in sporozoites (Delgado *et al.*, 2022).

Since *T. gondii* lacks specialized motility organelles such as cilia, flagella, and pseudopodia, its movement relies on a unique mechanism known as gliding motility; this form of locomotion, rather than amoeboid movement, enables the parasite to invade, traverse, and exit host cells efficiently; gliding motility in *T. gondii* is characterized by circuitous gliding, straight twirling, and helical rotation, and is considered essential for its survival and pathogenicity (Håkansson et al., 1999; Soldati & Meissner, 2004; Dubey, 2022).

The gliding is accomplished via the interaction between glide structures, the apical complex, the actomyosin system, and other associated proteins (Meissner *et al.*, 2002; Brossier *et al.*, 2003).

2:3:1 Life cycle stages:

2:3:1:1 Tachyzoites:

Tachyzoite (Greek: táchos = speed) is used instead of trophozoite, depending on its division rate (Smith & Evans, 2009). Tachyzoite is considered the simplest form of the *T. gondii* life cycle when investigated in vivo and in vitro; when grown in animal models or culture, a vast number of them can be obtained, measuring 2 × 7 μm (Al-hajj & Kekillioğlu, 2023).

Beneath the plasma membrane at the apical portion of the parasite, two apical bodies comprise the cytoskeleton and the secretory organelles, apicoplast, mitochondrion, and the typical organelles shared by eukaryotic cells, the pellicle encloses all of them, a membranous structure (Fig. 2-1) (Dubey, 2022).

The rapid reproduction of the tachyzoite stage caused the acute infection, which is expected at the onset of the parasite infection (Al-hajj & Kekillioğlu, 2023). However, it is undoubtedly less risky than other stages; it is essential to note that it cannot endure the harsh environment of gastric secretions (Roberts & Janovy, 2009).

In immunocompromised patients, the reactivation of the chronic disease through the conversion of the bradyzoites into tachyzoites might result in a lethal infection (Zhou *et al*., 2011). In addition, tachyzoites can be transmitted through several vital routes, including blood transfusions, organ transplants, and maternal-fetal transmission via the placenta (Lazar *et al.*, 2021).

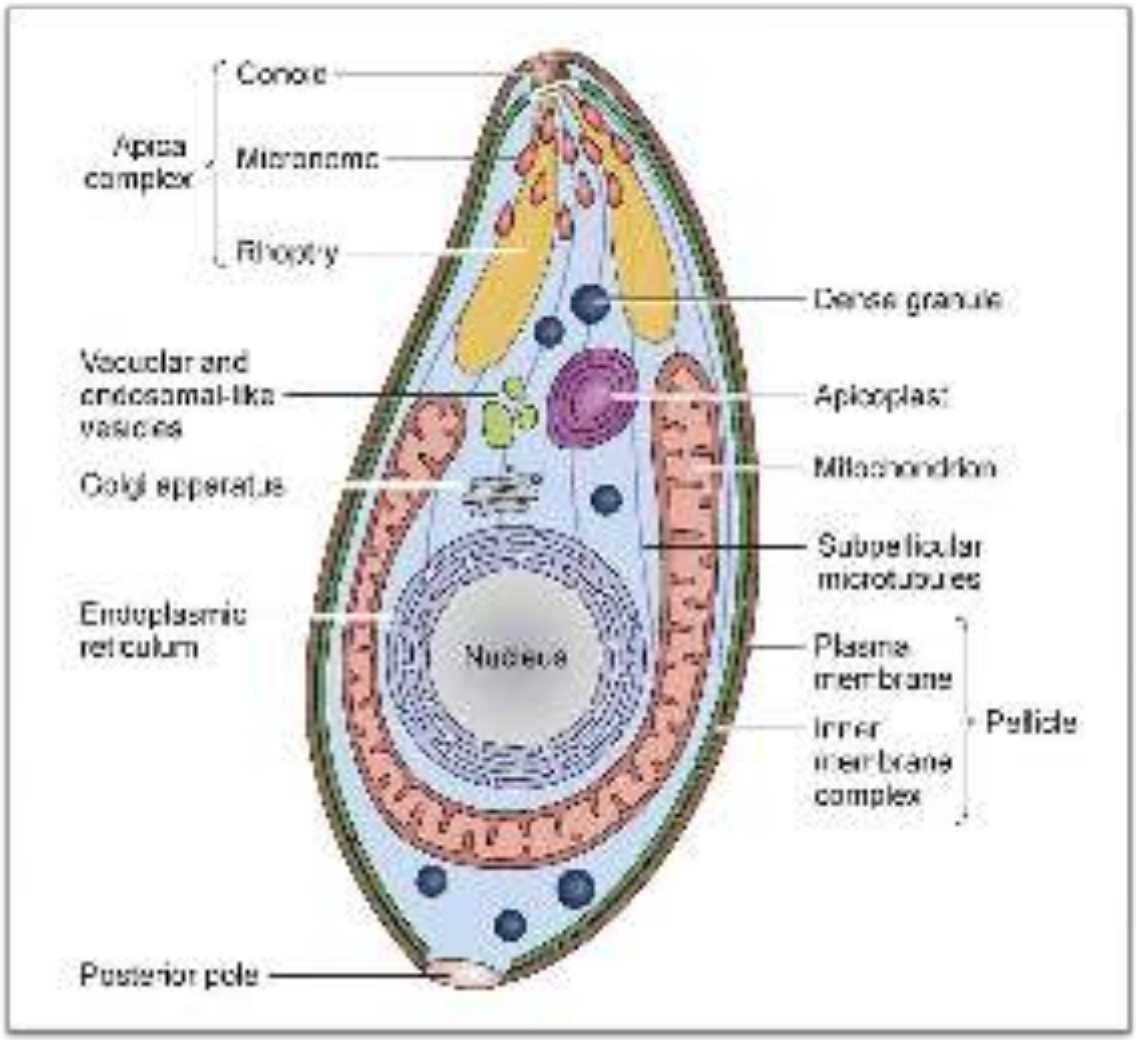

Figure 2-1: Tachyzoite stage (Sanchez & Besteiro, 2021).

2:3:1:2 Bradyzoites:

The transition to the bradyzoite stage, which is encystment within a tissue cyst and is characterized by a reduced replication rate, typically occurs following several multiplication cycles during the tachyzoite stage (Dubey, 2022). The number of bradyzoites within the tissue cysts varies according to size and harbors 2-1000 bradyzoites (Dubey, 2022).

Frenkel described the tissue stage as bradyzoite (Greek: Brady = slow), previously referred to as cystozoites tissue; cysts were renamed in 1988 to distinguish them from oocyst stage; bradyzoites are crucial in the *T. gondii* life cycle, as they are transmitted by ingesting infected meat (Fig. 2-2) (Frenkel, 1973; Gazzinelli *et al.*, 2014; Al-hajj & Kekillioğlu, 2023).

Once a tachyzoite is converted into the bradyzoite stage, the tissue cysts develop and persist inside the host cells through the endodyogeny-mediated division of the bradyzoite (Dubey, 2022); the encystment provides a unique ability to infect new hosts without undergoing the sexual stage (a privilege to felids only) (Sullivan & Jeffers, 2012).

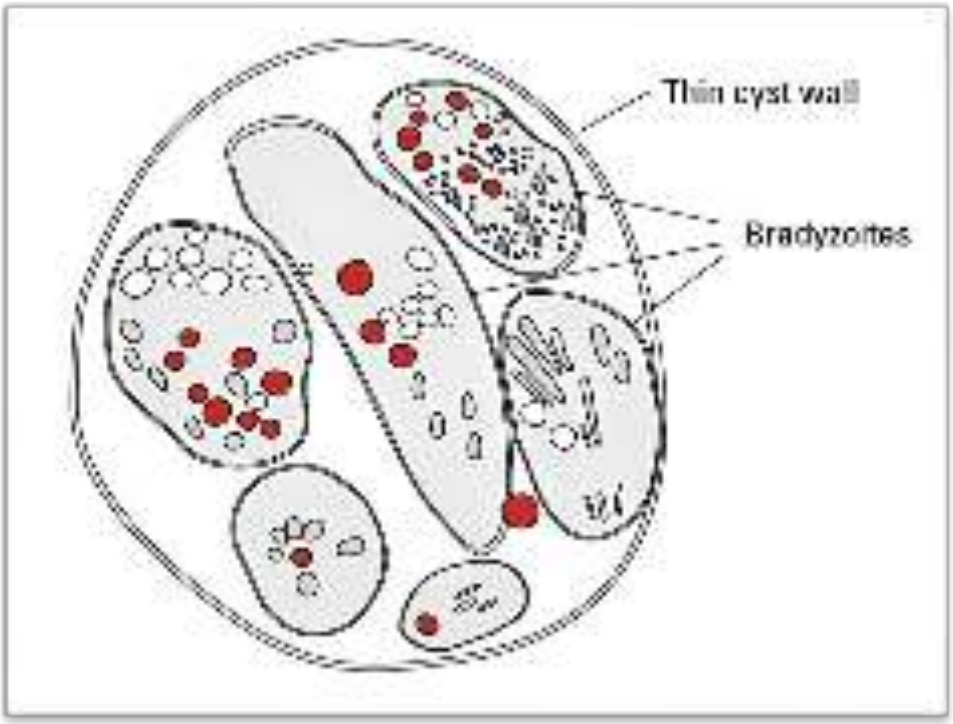

Figure 2-2: Young tissue cyst (Hill & Dubey, 2014).

2:3:1:3 Oocyst stage:

The oocyst stage is formed exclusively within the intestinal epithelial cells of the definitive host during sexual reproduction (Tomasina & Francia, 2020). It is characterized by an almost spherical shape and is estimated to be 10 × 12 μm in size (Dubey, 2022).

During zygote formation, microgametes use their flagella to move toward and penetrate mature macrogametes, facilitating fertilization (Dubey, 2022). Subsequently, a protective oocyst wall, comprising five layers, develops around the zygote; following the rupture of the infected epithelial

cells, oocysts are released into the intestinal lumen, from which they are excreted into the external environment (Fig. 2-3) (Hotea & Dărăbuș, 2023).

The oocysts of *T. gondii* demonstrate a remarkable tolerance to salinity, surviving in environments with salinity levels of up to 15 parts per thousand (ppt) (Al-hajj & Kekillioğlu, 2023). Additionally, they maintain viability across a broad temperature range, from −20 to +37 °C, and exhibit high disinfectant resistance (Al-hajj & Kekillioğlu, 2023). Notably, the only significant reduction in oocyst viability occurs through desiccation and exposure to elevated temperatures exceeding 45 °C; temperatures above 60 °C, are particularly lethal to the oocysts, leading to rapid destruction (Dubey, 2022).

The resilience of oocysts in contaminated water and soil represents a significant challenge for removing or eliminating *T. gondii;* this characteristic significantly contributes to the persistence of *T. gondii* in contaminated environments (Al-hajj & Kekillioğlu, 2023).

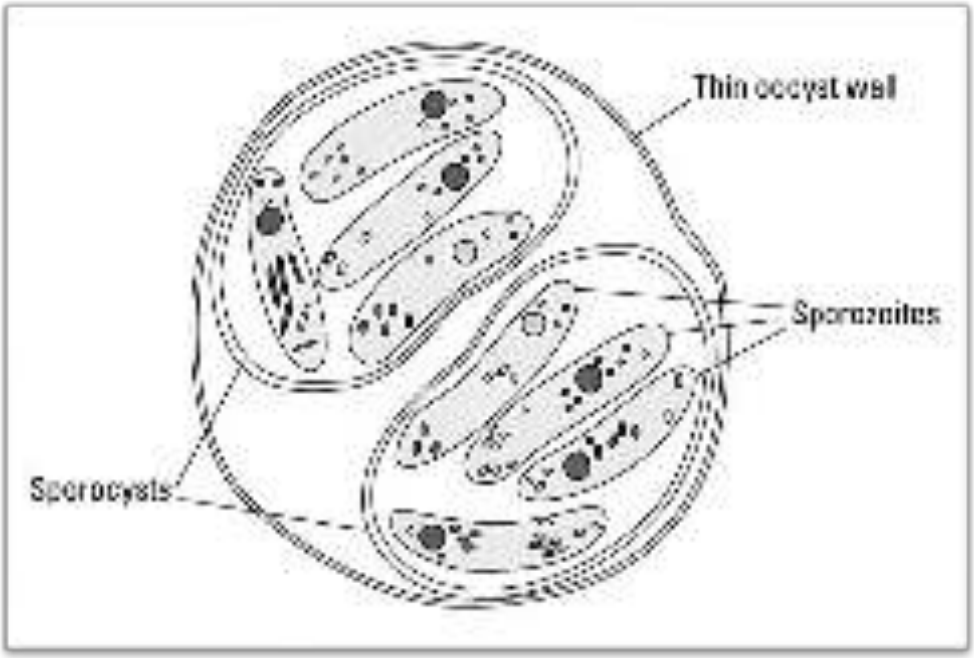

Figure 2-3: Oocyst stage (Hill & Dubey, 2014).

2:4 The life cycle of *Toxoplasma gondii*:

The life cycle of *T. gondii* is uniquely fascinating, encompassing both an asexual reproduction cycle that occurs in the intermediate hosts and a sexual reproduction cycle that arises within the definitive hosts (Al-Malki, 2021; Hotea & Dărăbuș, 2023). Felines are prominent endothermic organisms that can serve as intermediate and definitive hosts, and the global distribution of *T. gondii* is related to the ability of all stages to infect both intermediate and definitive hosts (Fig. 2-4) (Delgado *et al.*, 2022).

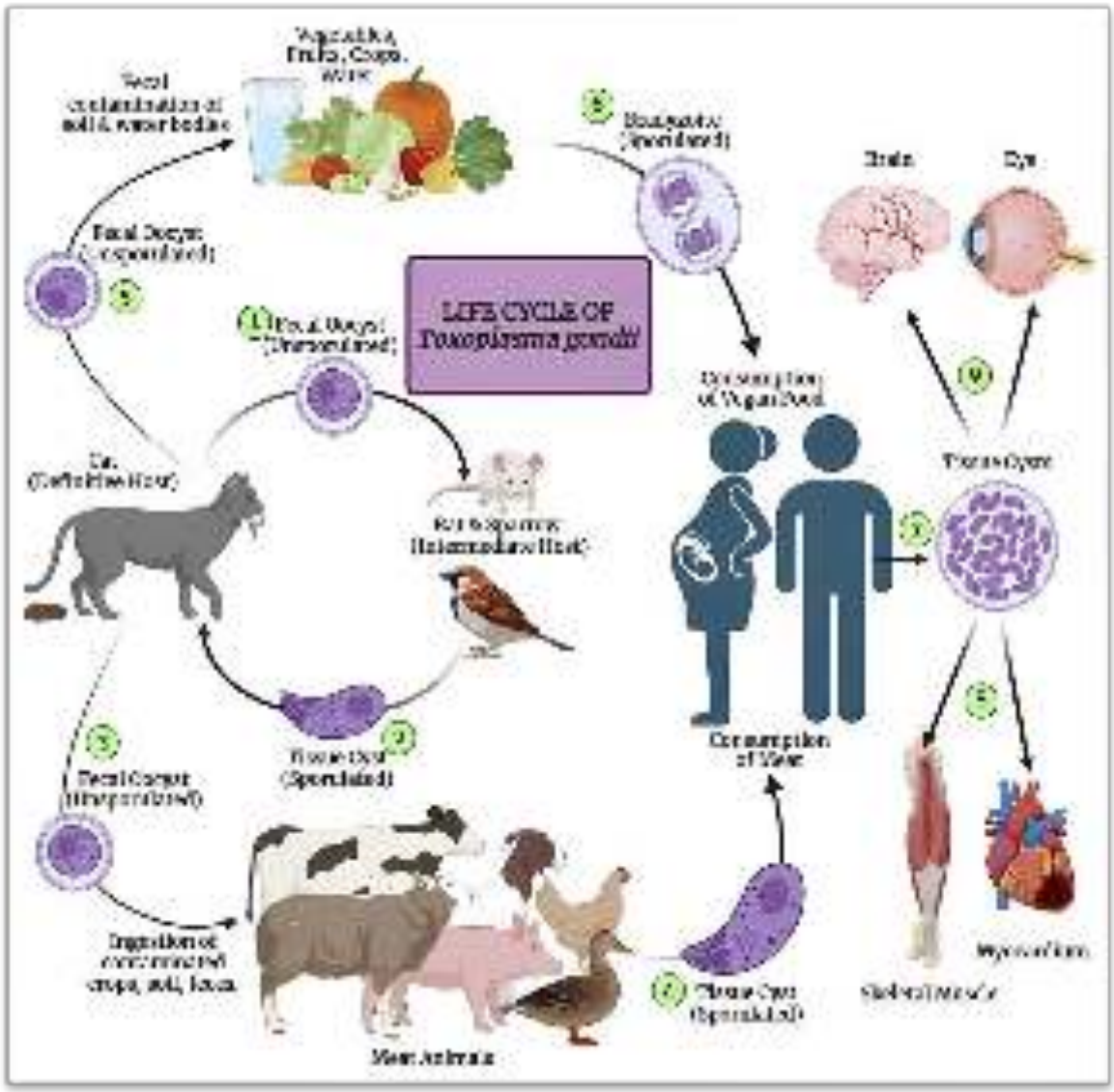

Figure 2-4: The life cycle of *T. gondii* (Naeem *et al.*, 2023).

2:4:1 The life cycle in the definitive hosts:

The three stages of *T. gondii* infection can potentially infect definitive hosts, as these hosts typically consume organisms that contain tissue cysts, they are frequently infected by tissue cysts that harbor bradyzoites (Dubey, 2022; Naeem *et al.*, 2023).

After the digestive processes break down the tissue cyst wall with proteolytic enzymes, the infection enters the gastrointestinal tract and invades the enterocytes (Delgado *et al.*, 2022). The infection can then be transformed into tachyzoites, which spread throughout the host's body, or merozoites, which are retained in the host's gastrointestinal tract (Delgado *et al.*, 2022).

Subsequently, several asexual rounds of expansion in the enterocytes and merozoites initiate the sexual reproduction cycle (Dubey, 1998; Tomasina & Francia, 2020). The asexual expansion occurs through schizogony, endodyogeny, and endopolygeny (Dubey, 1998; Tomasina & Francia, 2020). A new generation of merozoites develops once a merozoite moves from one cell to invade another (Dubey, 1998; Hotea & Dărăbuș, 2023). It is worth noting that schizonts are classified into five different types of schizonts (from A to E) based on morphology, each with a unique generation of merozoites (Dubey, 1998; Hotea & Dărăbuș, 2023).

Following multiple generations, the merozoites derived from the D or E-type forms will play an essential role in developing either microgametes or macrogametes; microgametes undergo division to produce biflagellates microgametes, which are released and directed toward the macrogametes for penetration; following the fertilization, a protective wall develops around the fertilized macrogametes, ultimately forming an oocysts, this cycle is conducted within 3-10 days depending on the nature of the infective stage within the intraepithelial (Fig. 2-5) (Dubey, 2022; Hotea & Dărăbuș, 2023).

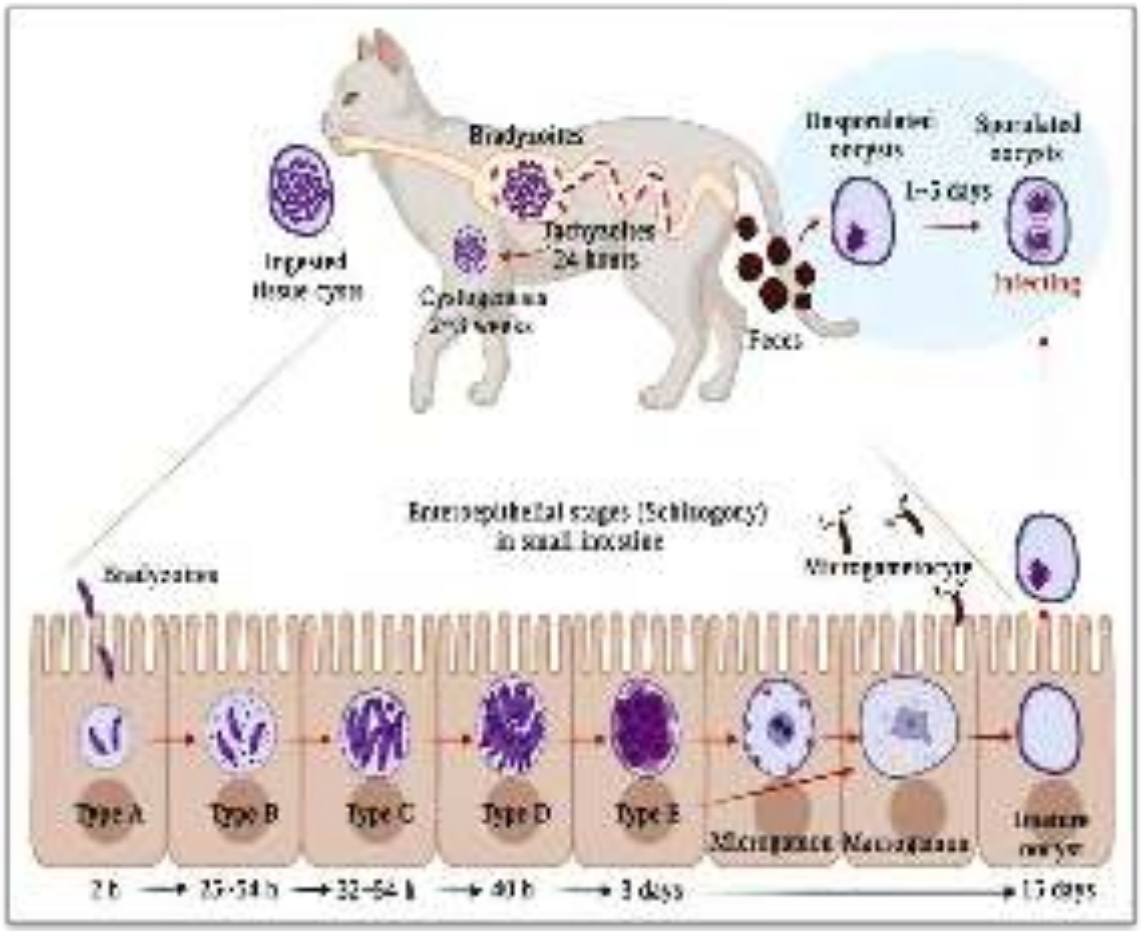

Figure 2-5: Intraepithelial sexual cycle of *T. gondii* (Hsieh & Yang, 2022).

Felids can produce 107 to 109 oocysts during the 4 to 13 days following their initial infection with *T. gondii*, unsporulated oocysts are excreted in the feces of infected felids, typically occurring 2-10 days after ingesting bradyzoites; on the other hand, this shedding process may commence after 18 days post-ingestion of oocysts of *T. gondii* (Delgado *et al.*, 2022).

Oocysts undergo sporulation in the environment, allowing them to become infectious to new hosts; this transformation involves three nuclear divisions: one meiosis followed by two mitoses and the development of sporocyst walls (Dubey, 2022; Delgado *et al.*, 2022).

2:4:2 The life cycle in the intermediate hosts:

Intermediate hosts can be infected by ingesting sporulated oocysts, tissue cysts, and tachyzoites; however, it is essential to note that tachyzoites are restricted in epidemiology (Dubey, 2022).

Upon the infection of the intermediate host, the sporozoites and bradyzoites differentiate into tachyzoites; these tachyzoites replicate within a parasitophorous vacuole (PV) through a process known as endodyogeny, leading to rapid asexual proliferation, and this proliferation enables the dissemination of the tachyzoites throughout the host's tissues via the circulatory system when the tachyzoite attacks the macrophages and establishes the PV (Delgado *et al*., 2022).

The invasion strategy, followed by intracellular asexual replication and eventual egress from the host cell, is the lytic cycle in the life cycle of *T. gondii* (Fig. 2-6) (Sanchez & Besteiro, 2021).

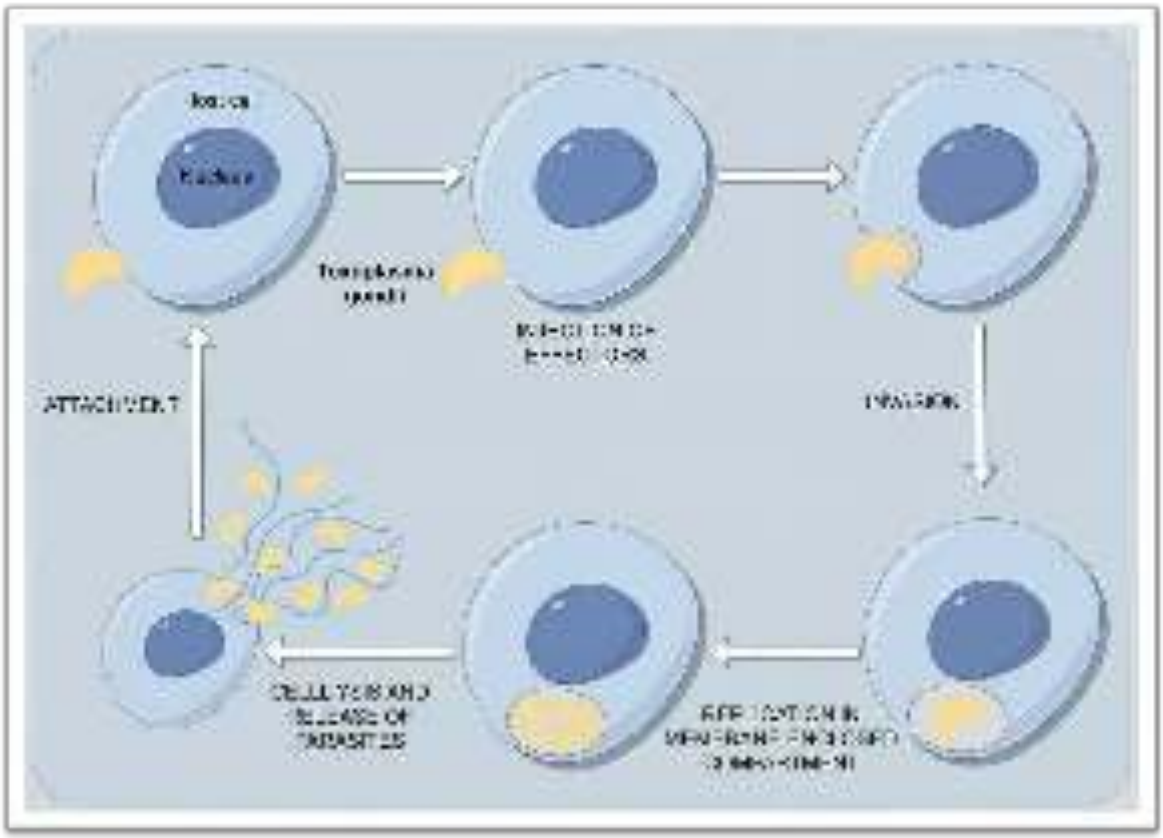

Figure 2-6: The lytic cycle of *T. gondii* (Cao *et al.*, 2024).

Toxoplasma gondii is capable of invading various cell types across different host organisms; this invasion occurs through a remarkably rapid process, often completed in under one minute (Dubey, 2022; Delgado *et al.*, 2022; Cao *et al.*, 2024).

2:4:3 The *Toxoplasma gondii* mechanism for cell invasion:

A critical stage in the life cycle of *T. gondii* is the invasion of the host cell, which is followed by rapid asexual reproduction (Sanchez & Besteiro, 2021). These processes facilitate the development within definitive and intermediate hosts through the developmental stages of merozoites and tachyzoites (Sanchez & Besteiro, 2021).

The initial invasion step involves gliding motility, followed by tachyzoite's attachment to a host cell (Delgado *et al.*, 2022; Cao *et al.*, 2024). This attachment relies on the presence of actin and myosin proteins, microneme proteins (MICs), and the level of surface antigen (SAGs) expression (Delgado *et al.*, 2022; Cao *et al.*, 2024).

Following the attachment step, *T. gondii* penetrates the host cell while it drags a part of the host membrane to the center of the host cell; this process forms a parasitophorous vacuole, which serves as an intracellular niche; within this specialized environment, *T. gondii* can replicate asexually (Fig. 2-7) (Kochanowsky & Koshy, 2018; Cao *et al.*, 2024).

Toxoplasma gondii employs a distinctive mechanism for host cell invasion called active penetration, during this process, the parasite establishes the moving junction (MJ) between it and the host cell membranes; this formation acts as a tight junction contact point and is facilitated by the secretion of specific proteins, including rhoptry neck proteins (RONs), micronemes, and dense granules; during the invasion

process, the protrusion of the conoid and the secretion of microneme and rhoptry contents together form a moving junction, these two events depend on calcium ions (Ca^{2+}) (Kochanowsky & Koshy, 2018; Sanchez & Besteiro, 2021; Dubey, 2022; Cao *et al.*, 2024).

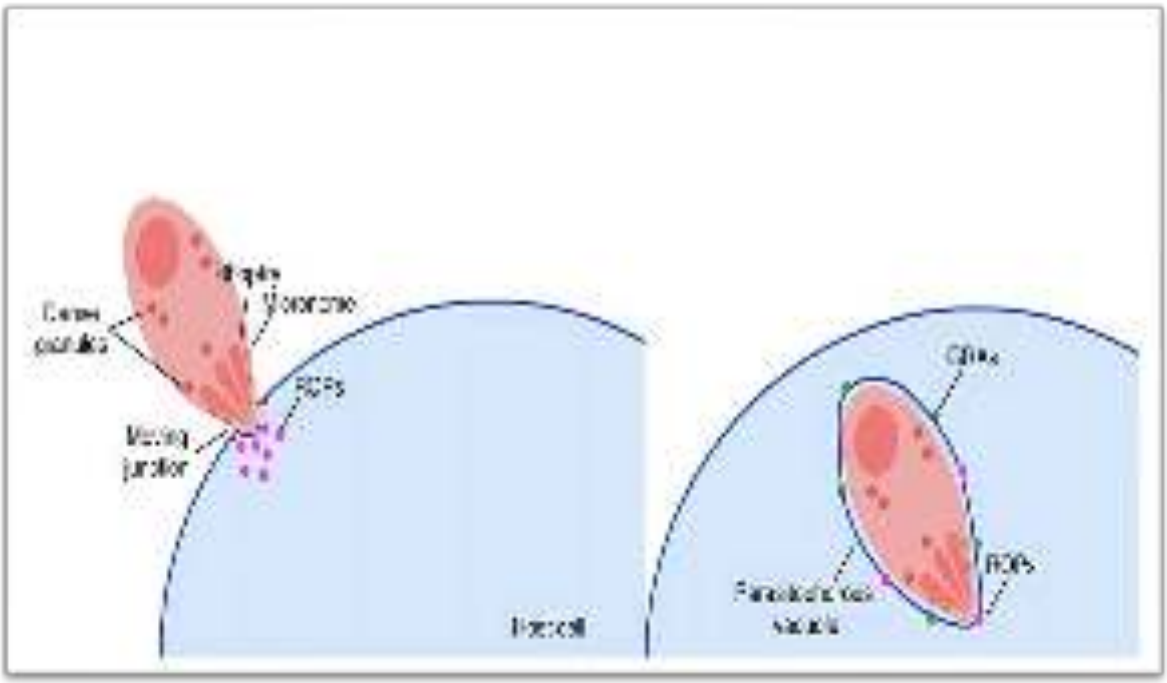

Figure 2-7: *Toxoplasma gondii* mechanism for cell invasion (Kochanowsky & Koshy, 2018).

During penetration, the tachyzoite develops a transient structure resembling a rose, known as the parasitophorous vacuole (PV); the proteins secreted by the Rhoptry and the dense granule antigens (GRAs) collectively form a strong parasitophorous vacuole membrane (PVM) that effectively envelops *T. gondii*; additionally, *T. gondii* secretes rhomboid proteases to precisely cleave the junction region, ensuring successful invasion of the host cell, as a result, the host cell's plasma membrane forms an isolated bubble of cytoplasm that extends toward the center of the host cell, effectively integrating with the host (Fig. 2-6); finally, it adjusts the components of the PVM, this mechanism is regarded as a protective strategy aimed at evading lysosomal activity, thereby enhancing the survival of the parasite (Beyer *et*

al., 2002; Clough & Frickel, 2017; Sanchez & Besteiro, 2021; Cao *et al.*, 2024).

The host cell undergoes manipulation by the parasite, characterized by interactions with microtubules; furthermore, the parasite employs essential organelles, such as mitochondria, the endoplasmic reticulum, and the Golgi apparatus (Sehgal *et al*., 2005; Coppens *et al.*, 2006; Dubey, 2022).

The tachyzoite's ability to evade the immune response is illustrated through mechanisms such as paracellular crossing, active penetration, and the use of Trojan horses (Soldati & Meissner, 2004; Mendez & Koshy, 2017). These strategies enable the parasite to move within immune cells (Soldati & Meissner, 2004; Mendez & Koshy, 2017). Consequently, *T. gondii* induces significant modifications in macrophages through altering their signaling pathways (Lima & Lodoen, 2019).

2:4:4 Endodyogeny:

Endodyogeny is a unique form of asexual reproduction process utilized by tachyzoites in the parasitophorous vacuole; in this process, two daughter cells are formed within a mother cell, which gradually shrinks as the daughter cells expand; ultimately, a small residual body is constructed due to this distinctive reproduction method (Hu *et al*., 2002; Delgado *et al*., 2022).

In this specific form of replication, the budding of daughter cells and DNA replication are synchronized, with specific organelles synthesized from scratch (such as rhoptries and micronemes); on the other hand, the other organelles including the apicoplast and Golgi apparatus, are duplicated and spread into emerging daughter buds, which are effectively controlled by the centrosomes (Fig. 2-8) (Sanchez & Besteiro, 2021; Dubey, 2022).

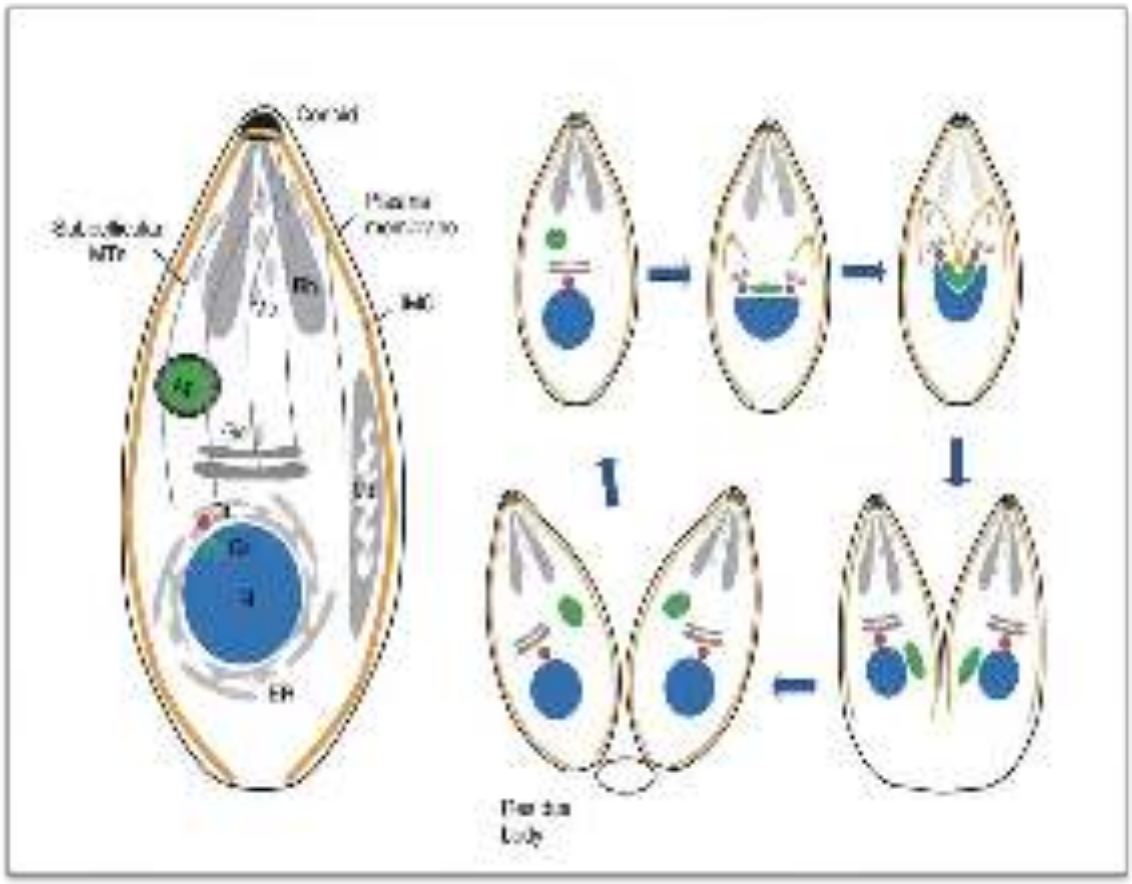

Figure 2-8: Endodyogeny (Jacot *et al.*, 2013).

2:5 The epidemiology and transmission:

The latent infection of *T. gondii* typically does not induce disease in individuals with a competent immune system; however, it has the potential to lead to various neuropsychiatric conditions in individuals with compromised immune systems (Delgado *et al.*, 2022).

Several factors can influence the prevalence of toxoplasmosis, including cultural considerations, climatic conditions, and the presence of domestic cats; the prevalence of *T. gondii* flourishes in warm climates, while it diminishes in colder regions (Al-Malki, 2021).

The transmission of *T. gondii* stages is mainly attributed to several critical pathways, including carnivorism, fecal-oral paths, and transplacental transmission, and less common modes, such as venereal and through

breastfeeding or the consumption of unpasteurized milk (Lazar *et al.*, 2021; Dubey, 2022). Furthermore, blood transfusions and organ transplants from an infected individual are possible ways of transmitting tachyzoites (Fig. 2-9) (Lazar *et al.*, 2021).

Cats acquire toxoplasmosis by consuming infected prey, such as rodents or birds, in this process, they serve as definitive hosts, facilitating the sexual cycle and the production of oocysts (Al-Malki, 2021). Humans get *T. gondii* via the primary modes of transmission: contaminated water and food, ingesting oocysts or tissue cysts, and congenital transmission (Al-Malki, 2021; Dubey, 2022).

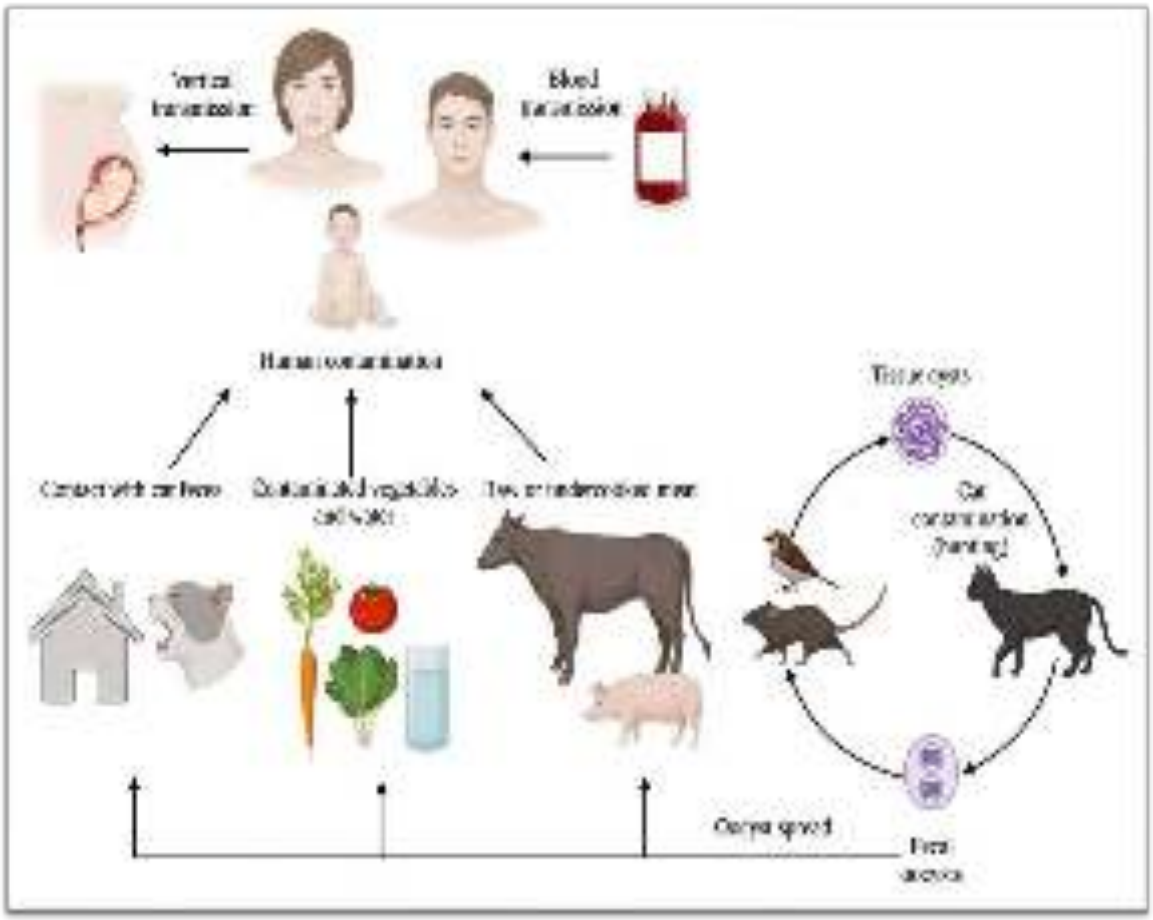

Figure 2-9: The main routes of *T. gondii* transmission (Hsieh & Yang, 2022).

2:6 The pathogenicity of *Toxoplasma gondii*:

Toxoplasmosis is mainly acquired through consuming tissue cysts in the infected meat or ingesting oocysts from food and water contaminated with cat feces; therefore, the bradyzoites and sporozoites invade the intestinal epithelial cells, where they undergo multiplication within the intestine (Attias *et al.*, 2020).

Toxoplasma gondii has the potential to disseminate locally to the mesenteric lymph nodes and distant organs through lymphatic and bloodstream invasion, a host may undergo mortality due to necrosis of the intestine and mesenteric lymph nodes before significant damage occurs in other organs (Dubey, 2022).

Toxoplasmosis manifestations vary significantly among immunocompetent and immunocompromised individuals (Frenkel, 1985; Daher *et al*., 2021).

2:6:1 Toxoplasmosis in immunocompetent individuals:

2:6:1:1 Acute Toxoplasmosis:

Acute toxoplasmosis typically occurs after a short incubation period, during which tachyzoites are disseminated and replicated within the host; over 80% of immunocompetent individuals remain asymptomatic, when symptoms occur, they may present as asthenia, myalgia, and flu-like manifestations, including fever and mononucleosis (Frenkel, 1988; Daher *et al*., 2021; Sanchez & Besteiro, 2021).

Variations in susceptibility may be attributed to several factors, including age, host species, the strain of *T. gondii*, the prevalence of *T. gondii*, and the method of *T. gondii* treatment (Dubey, 2022).

Upon the host's immune system response, tachyzoites disseminate to the brain and skeletal muscles, developing into bradyzoites in tissue cysts;

this process initiates the chronic form of the disease (Daher *et al.*, 2021).

2:6:1:2 Congenital toxoplasmosis:

The primary congenital infection occurs when the parasite is transmitted from the placenta to the fetus; the severity of congenital infection is inversely correlated with the gestational trimester (Sanchez & Besteiro, 2021; Dubey, 2022).

Also, not every infected mother would pass the infection to her fetus, mainly if exposure occurred before gestation, this is especially the case unless the mother is immunocompromised (Gomella *et al.*, 2004).

During the first trimester, infection of the fetus can pose significant risks, including the possibility of abortion or stillbirth; however, congenital toxoplasmosis in newborns can manifest with a wide range of outcomes, from severe cases that may result in death shortly after birth to asymptomatic presentations, this condition can also lead to complications such as premature birth, liver enlargement, and variations in head size, including microcephaly and macrocephaly; additionally, affected infants may experience learning difficulties, intellectual disability, hepatosplenomegaly, umbilical bleeding, jaundice, and retinitis (Fig. 2-10) (Tesini, 2020; Daher *et al*., 2021; Nagalo *et al*., 2022).

2:6:1:3 Ocular toxoplasmosis:

Ocular toxoplasmosis is a progressive condition distinguished by necrotizing retinitis, which has the potential to result in serious complications that may threaten vision and even entire blindness; during the initial congenital and acquired toxoplasmosis, tachyzoites can disseminate to the eyes, which can encyst and reactivate within the eye (Sanchez & Besteiro, 2021; Dubey, 2022; Ghenciu *et al*., 2024).

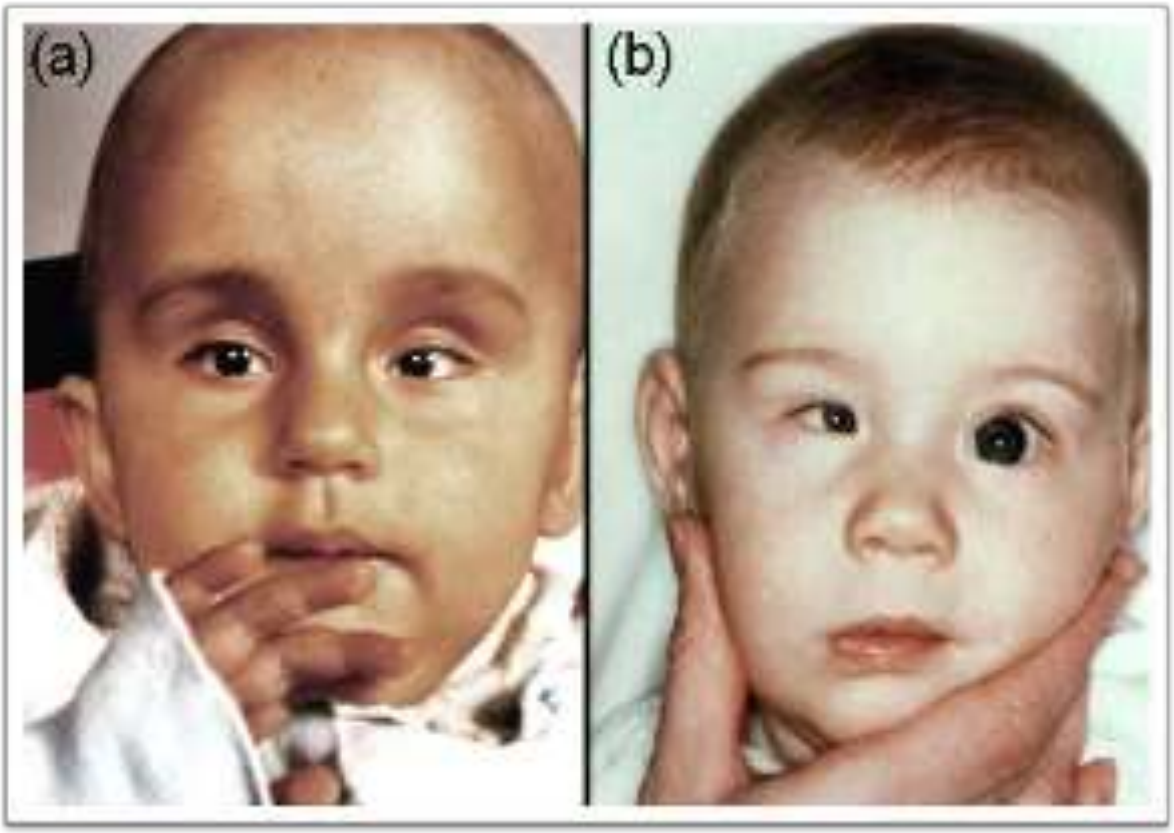

Figure 2-10: (a) Child with congenital toxoplasmosis. Hydrocephalus with bulging forehead. (b) Microphthalmia of the left eye (CABI International, 2022).

The anatomical site of the lesions influences the clinical manifestations of ocular toxoplasmosis, which is typically characterized by retinochoroiditis (Fig. 2-11) (Daher *et al.*, 2021).

2:6:1:4 Chronic toxoplasmosis:

Toxoplasma gondii is a neurotropic pathogen with a strong affinity for the central nervous system, it reaches the brain parenchyma from the bloodstream by either hijacking leukocytes or entering as free parasites (Daher *et al.*, 2021). Particularly due to its effects on the central nervous system, this may significantly influence behavioral changes and psychiatric disorders (Sanchez & Besteiro, 2021).

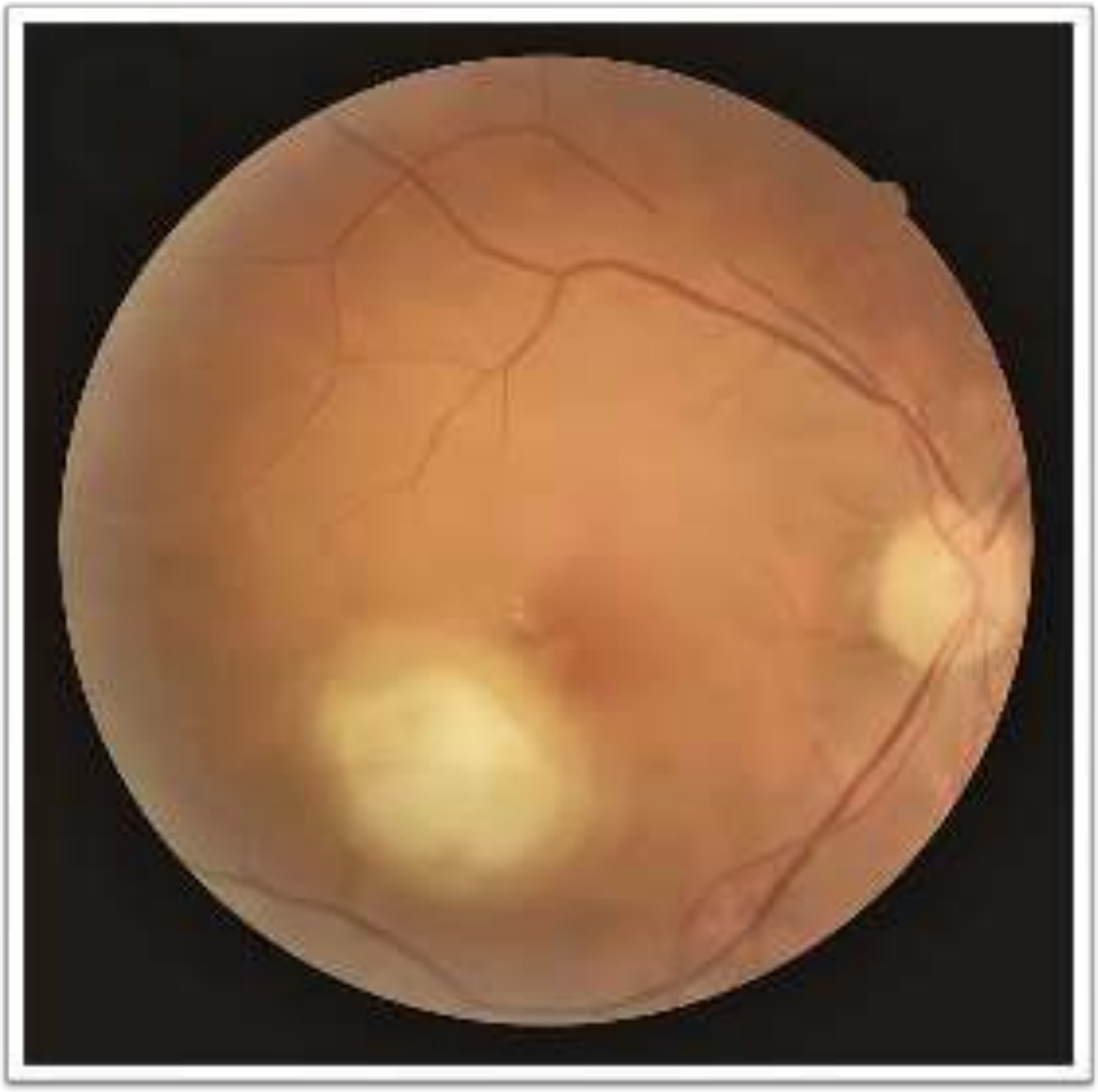

Figure 2-11: Ocular toxoplasmosis (retinochoroiditis) (Smith *et al.*, 2021).

The parasite remains dormant within the host's tissues and can become active opportunistically in the presence of immunodeficiency, thereby progressing to its active stage (Montoya & Liesenfeld, 2004; Dubey, 2022). This infection is characterized by fatal encephalitis, presenting with clinical manifestations such as headache, cognitive difficulties, memory impairment, and hemiparesis (Luft & Remington, 1992).

2:6:2 Toxoplasmosis in immunocompromised individuals:

Although there are both preventive and therapeutic strategies available, the potential for reactivation of chronic toxoplasmosis persists, presenting considerable health implications, especially in immunocompromised individuals; various factors can impair the cellular immune response; conditions such as HIV infection, as well as the immunosuppressive therapies utilized in hematopoietic stem cell transplantation, solid organ transplants, and cancer chemotherapy, can elevate the risk of reactivating chronic toxoplasmosis (Daher *et al.*, 2021; Sanchez & Besteiro, 2021).

2:7 The genotype of *Toxoplasma gondii*:

Understanding the genotypes of *T. gondii* is crucial in influencing the course and intensity of infection (Dubey, 2022). Globally, *T. gondii* exhibits over 200 distinct genotypes, among which genotypes I, II, and III are particularly dominant (Allamy, 2023).

The distribution of strain types reflects varying prevalence across different areas, the strain I is the least abundant and is primarily found in Europe and America; in contrast, strain II is more prevalent in Europe and North America (Hotea & Dărăbuș, 2023). Finally, strain III has a relatively lower infection rate and has been observed in various regions worldwide (Delgado *et al.*, 2022).

Notably, patients with HIV have been found to carry Type I and Type II strains; in contrast, the strain III has only been isolated from animal hosts and is less common than the others (Al-Malki, 2021; Hotea & Dărăbuș, 2023).

One of the most effective approaches for differentiating *T. gondii* genotypes is PCR-RFLP, a technique first employed by Sibley and colleagues, this technique demonstrates high accuracy in distinguishing

between various strains of the parasite (Allamy, 2023).

2:8 The laboratory diagnosis

The diagnosis of *T. gondii* can be effectively achieved through biological, histological, and serological techniques or by a combination of these techniques (Dubey, 2022).

2:8:1 The serological diagnosis:

The detection of *T. gondii* antibodies is essential for accurate diagnosis. Several serological methods are employed to identify humoral antibodies, which can be present in various specimens, including whole blood, serum, plasma, body fluids, meat juice, and milk (Dubey, 2022).

Serological tests assess various immune response components, including antibodies, antigens, cytokines, interleukins, and additional factors (Arnold & Chung, 2018).

Upon the first *T. gondii* infection, IgM is the first immunoglobulin produced in response to infection, it serves as a short-term response that can last for weeks or years; IgG appears about two weeks after infection and can remain in the body much longer (Innes, 2010; Allamy, 2023).

There are many serologic techniques, such as Sabin-Feldman Dye Test (SFDT), Enzyme-linked immunosorbent assay (ELISA), Latex Agglutination Test (LAT), Western Blotting, and Avidity Tests (Robert-Gangneux & Dardé, 2012; Dubey, 2022).

2:8:2 The Molecular diagnosis:

Molecular techniques, particularly polymerase chain reactions (PCR), play a crucial role in investigating DNA and RNA; these techniques amplify specific segments of parasite DNA, generating millions of copies that can be detected and utilized for diagnosing pathogens and classifying organisms;

(PCR) encompasses various types, including real-time PCR and restriction fragment length polymorphism PCR (RFLP-PCR) (Al-Malki, 2021; Allamy, 2023).

These techniques are instrumental in accurately detecting both acute and chronic infections; additionally, they play a crucial role in identifying the genotype of *T. gondii* (Dubey, 2022).

2:8:3 The Histological diagnosis

2:8:3:1 Histopathology technique:

Histopathology thoroughly examines prepared tissue samples under a microscope, allowing for a detailed examination of cellular structures and disease processes (Slaoui & Fiette, 2011). Hematoxylin and Eosin are essential histopathological stains that enhance our ability to visualize cellular components within tissue samples (Allamy, 2023).

Hematoxylin is a naturally occurring substance utilized in laboratories globally, the first extraction in 1502 from the logwood tree *Haematoxylum campechianum*, a discovery made by the Spaniards; in the 1800s, amateur microscopists utilized hematoxylin as a staining agent, this stain effectively colors the nuclei blue or the gradients of blue; in subsequent advancements, researchers established various techniques to illustrate multiple cellular structures, nevertheless, hematoxylin continues to be regarded as the most widely utilized nuclear stain in histology (Titford, 2005).

On the other hand, eosin, developed in 1874 by the director of a German chemical company, is a vibrant red dye that stains the cytoplasm of cells; together, these dyes play an essential role in histology, enhancing our understanding of tissue structure and various pathologies (Allamy, 2023).

The primary steps of the histopathology technique are tissue samples

collection, fixation, tissue processing, embedding, sectioning, staining, mounting, and microscopic examination (Fig. 2-12) (Bancroft & Gamble, 2008; Slaoui & Fiette, 2011).

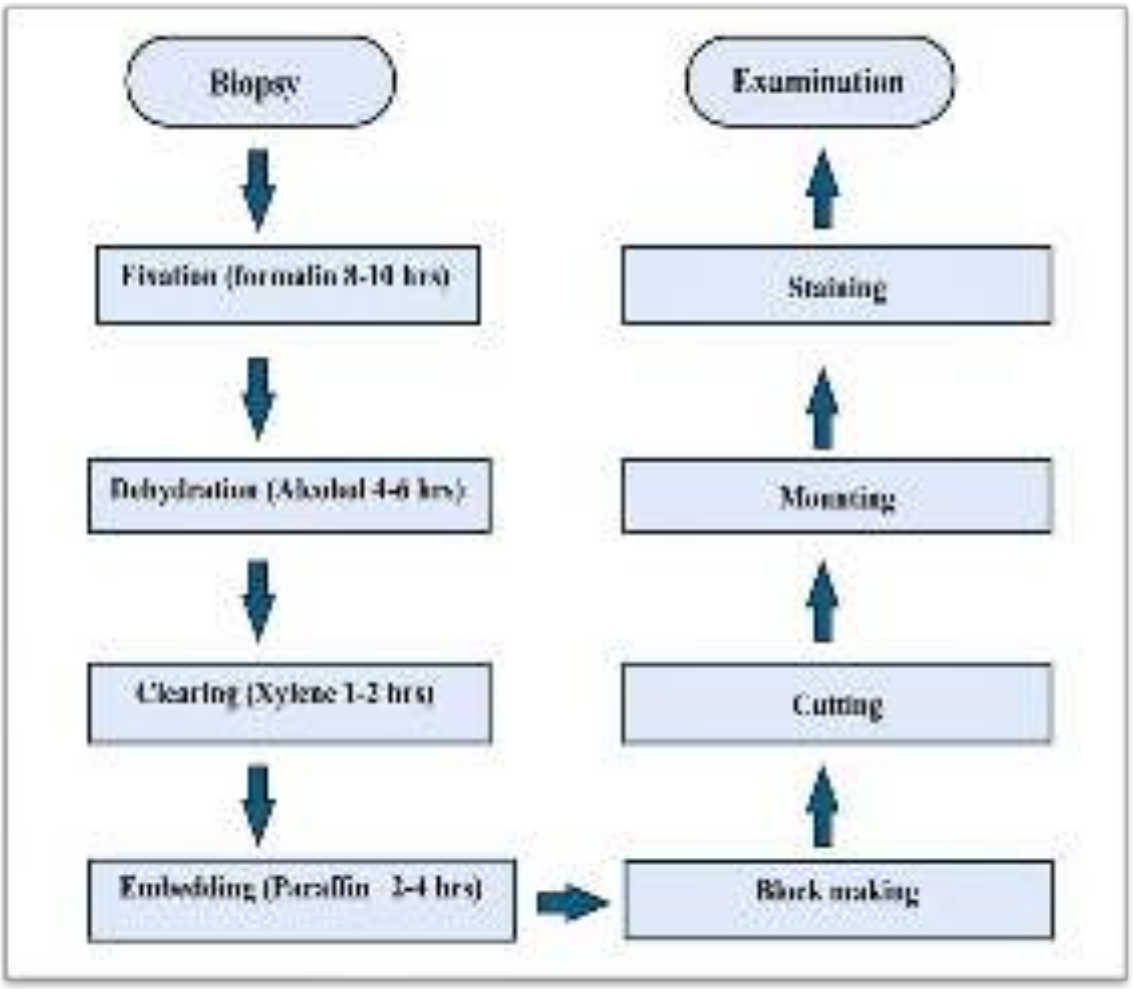

Figure 2-12: The primary steps of Histopathology technique. Based on data from Slaoui & Fiette (2011).

2:8:3:2 Immunohistochemistry technique:

Immunohistochemistry (IHC) is an essential technique for researchers engaged in routine diagnostics and clinical studies, particularly in investigating biomarkers; this technique enables the confirmation of target molecule expressions within the context of the surrounding microenviron-

ment, enriching our knowledge of their roles and influence (Kim *et al.*, 2016). IHC visualizes a particular molecule's distribution and quantity within tissue samples through specific interactions (Kim *et al.*, 2016).

The foundational principles of the immunohistochemistry technique have been recognized since the 1930s; however, the first study utilizing IHC techniques was not published until 1941 by Coons and his colleagues (Coons *et al.*, 1941).

2:8:3:3 The principle of immunohistochemistry:

Immunohistochemistry is a fascinating method that integrates anatomical, immunological, and biochemical techniques to provide a unique approach for detecting antigens or haptens within the cells of a tissue section; it utilizes the principle of specific antibody binding to antigens present in biological tissues; various methods can be employed to visualize antibody-antigen interactions, commonly employed enzymes, such as Horseradish Peroxidase (HRP) or Alkaline Phosphatase (AP), facilitate color-producing reactions that improve the detection strategy (Fig. 2-13) (Boster Biological Technology; Dubey, 2022; Kohale *et al*., 2023).

2:8:3:4 The applications of immunohistochemistry:

Immunohistochemistry is utilized for various purposes, such as in disease diagnosis, where physicians use it to distinguish whether a tumor is benign or malignant, determine its stage, and recognize the origin of metastasis; it is also used in drug development to assess drug efficacy; IHC can be used independently or in conjunction with other analytical techniques to study normal tissue, pathological processes, and many fields of biological investigation (Boster Biological Technology).

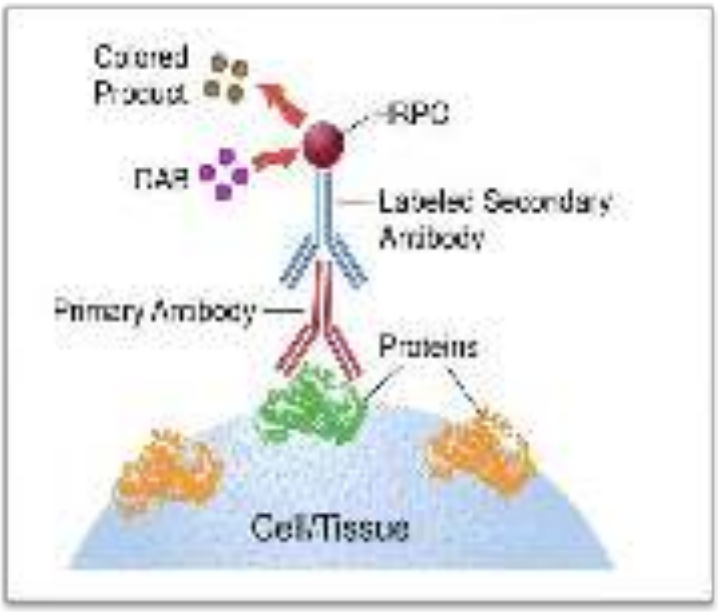

Figure 2-13: Immunohistochemistry principle (Leinco Technologies, Inc.)

2:8:3:5 The main steps of the immunohistochemistry protocol:

Sample collection and preparation are crucial in IHC, as the quality of the tissue sample significantly influences the visibility and localization of antigens; confirming high-quality tissue samples enhances the technique's overall effectiveness (Boster Biological Technology). The samples must be fixed promptly, as antigens can denature, disappear, or diffuse; timely fixation is crucial to preserve the integrity of the samples (Boster Biological Technology). The primary materials: Paraffin-embedded tissue samples on slides, xylene, ethanol (100%, 95%, 70%), distilled water, antigen retrieval buffer (e.g., citrate buffer pH 6.0 or Tris-EDTA pH 9.0), blocking serum (e.g., 5% Phosphate-Buffered Saline (PBS) or normal serum), primary antibody (diluted in blocking buffer), secondary antibody (Horseradish Peroxidase (HRP)- or Alkaline Phosphatase (AP)-conjugated), chromogen (e.g., Diaminobenzidine (DAB) for HRP), Hematoxylin (for counterstaining), and mounting medium and coverslips to examination under the microscope (Fig. 2-14) (Boster Biological Technology; Leinco Technologies, Inc.).

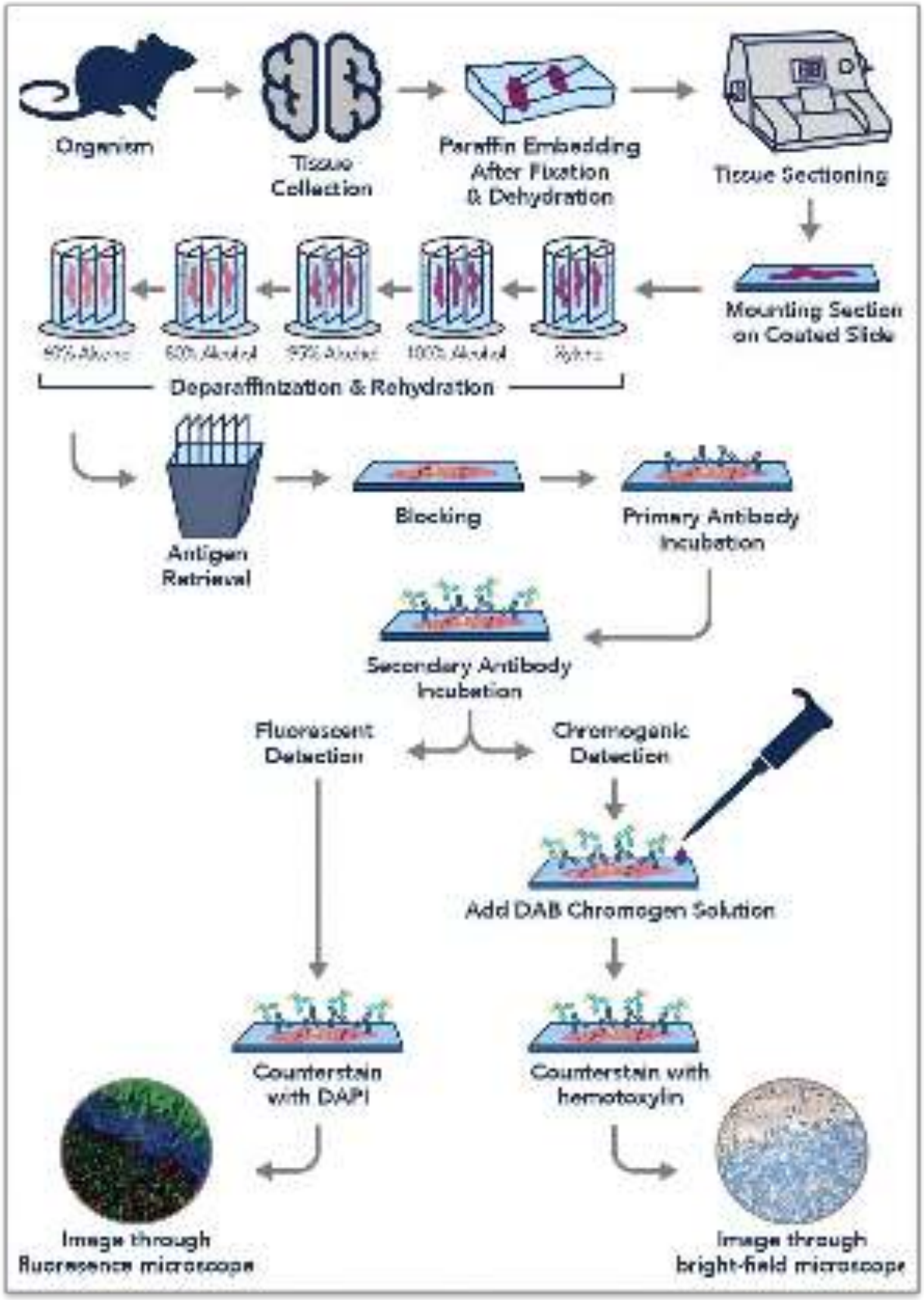

Figure 2-14: The main steps of Immunohistochemistry technique (Proteintech Group, 2023).

2:9 The treatment:

Medication choices for toxoplasmosis are notably restricted. Dihydrofolate reductase (DHFR) and dihydropteroate synthetase (DHPS) are components of the folate pathway, which is crucial for DNA synthesis and considered the a key target of most medication regimens (Konstantinovic *et al.*, 2019).

Frequently, two antimicrobial combinations are used to treat toxoplasmosis; the combination therapy of pyrimethamine and sulfadiazine (Pyr-sulf) is currently accepted as the most effective strategy for treating human toxoplasmosis (Dunay *et al*., 2018). The Pyr-Sulf combination demonstrates strong efficacy in managing acute infections. While several associated side effects exist, incorporating folinic acid and yeast has proven effective in mitigating many; also, the atovaquone drug has been confirmed to treat tissue cysts (Lapinskas & Ben-Harari, 2019; Daher *et al.*, 2021; Dubey, 2022).

Acquiring infection just before or during pregnancy leads to congenital toxoplasmosis, and Spiramycin is a proven and effective treatment for this infection; it is crucial to avoid pyrimethamine, as it is known to induce fetal malformations during the early months of gestation (Dunay *et al.*, 2018; Daher *et al*., 2021).

Spiramycin is not used for postnatal toxoplasmosis; newborns with congenital infections are treated with pyrimethamine-sulfadiazine, along with options like atovaquone, azithromycin, and dapsone (Singh, 2016).

The classical treatment regimen for ocular toxoplasmosis consists of pyrimethamine-sulfadiazine plus corticosteroids; the Trimethoprim-sulfamethoxazole plus oral prednisolone regimen is another alternative

adequate treatment option; furthermore, pyrimethamine-clindamycin plus folinic acid and prednisone and pyrimethamine-azithromycin plus folinic acid and prednisone regimens (Park & Nam, 2013; Kalogeropoulos *et al*., 2022).

Immunocompromised patients with toxoplasmosis can be treated with the golden approach, pyr-sulf, however, good options such as Pyrimethamine-clindamycin, folinic acid, and atovaquone-sulfadiazine can be used (Dunay *et al.*, 2018). The imiquimod drug has demonstrated significant potential in treating acute and chronic infections in murine models (Daher *et al.*, 2021).

2:10 The prevention:

Prevention measures are essential to reduce the risk of *T. gondii* infection, adhere to the following health recommendations:

1) After dealing with meat, hands and utensils must be thoroughly washed with soap and water to kill the parasite stages (Castro & Dubey, 2019).
2) Make it a priority to avoid the consumption of raw or undercooked meat (Kalogeropoulos *et al.*, 2022; Hotea & Dărăbuș, 2023).
3) To effectively eliminate *T. gondii* stages in meat, ensure it reaches temperatures above 66 °C or is frozen solid at -12 °C; these strategies ensure safety and provide peace of mind when enjoying meals (Dubey, 2022).
4) Washing fruits and vegetables thoroughly is essential to safeguarding our health and preventing the risk of infection (Robert-Gangneux & Dardé, 2012).
5) Guaranteeing the supply of pristine, well-filtered water (Kalogeropoulos *et al.,* 2022; Hotea & Dărăbuș, 2023).

6) It is essential to wash hands with soap and water after returning indoors or after any contact with soil, particularly garden soil, to minimize the risk of the infection (Robert-Gangneux & Dardé, 2012; Dubey, 2022).
7) Domestic cats should be prefed with a dry, canned, or cooked diet, and their litter boxes must be emptied daily; gloves and masks should be worn to avoid potential hazards; to ensure health and safety reasons, pregnant women should abstain from performing this task (Dunay *et al.,* 2018; Castro & Dubey, 2019).

2:11 Breast cancer:

2:11:1: The onset of carcinogenesis:

Carcinogenesis is a multifaceted and evolving dynamic process comprising three distinct stages: initiation, progression, and metastasis (Wang *et al.*, 2017). Cancer is characterized by the unregulated proliferation of cells, which can lead to significant alterations in the adjacent normal tissues, ultimately affecting their essential functions (Fig. 2-15) (Hanahan & Weinberg, 2011; Brown *et al*., 2023).

Cancer cells typically arise from normal cells because of DNA damage, the body possesses mechanisms to repair such damage; however, this DNA repair process in cancerous cells is often compromised; consequently, these abnormal cells disobey the standard regulatory signals instructing cells to cease division and evade apoptosis (Gataa, 2022; Brown *et al.*, 2023; Alipanahi *et al*., 2025).

Tumors are surrounded by a specialized environment known as tumor microenvironment (TME), which consists of cellular and noncellular components: the stromal cells, fibroblasts and myofibroblasts, neuroendocrine cells, immune and inflammatory cells, adipose cells, the

vascular networks of blood and lymphatic systems, and extracellular matrix (ECM) (Bussard *et al.*, 2016; Wang *et al.*, 2017; Arneth, 2019).

The TME plays a significant role in each stage of carcinogenesis, in a healthy state, the microenvironment plays a crucial role in defending against tumorigenesis and invasion; conversely, it can inadvertently contribute to these adverse processes when it is not in optimal condition (Wang *et al*., 2017; Arneth, 2019). The tumors can adjust the microenvironment by influencing the balance of immune cells and stromal components, thereby establishing conditions supporting cancer progression (Joyce & Pollard, 2009).

Cancer cell's various mechanisms to evade apoptosis illustrate their resistance to numerous pro-apoptotic signals, consequently, cancer cell proliferation occurs independently of the regulatory influence exerted by the surrounding tissue and adjacent cells (Brown *et al*., 2023).

According to Coussens and Werb (2002), normal tissues acquire the exacerbation of damage as a consequence of the chronic inflammation that emerges from the cancer; furthermore, essential enzymes that play a key role in the degradation of the extracellular matrix, such as matrix metalloproteinases (MMPs), are produced by tumors; this degradation promotes tissue refurbishing and improves the invasive potential of the cancer (Coussens & Werb, 2002; Quail & Joyce, 2013).

Eventually, in the advanced stages of cancer, malignant cells penetrate the surrounding normal tissues and can metastasize to distant areas, leading to significant tissue damage throughout the body (Hanahan & Weinberg, 2011).

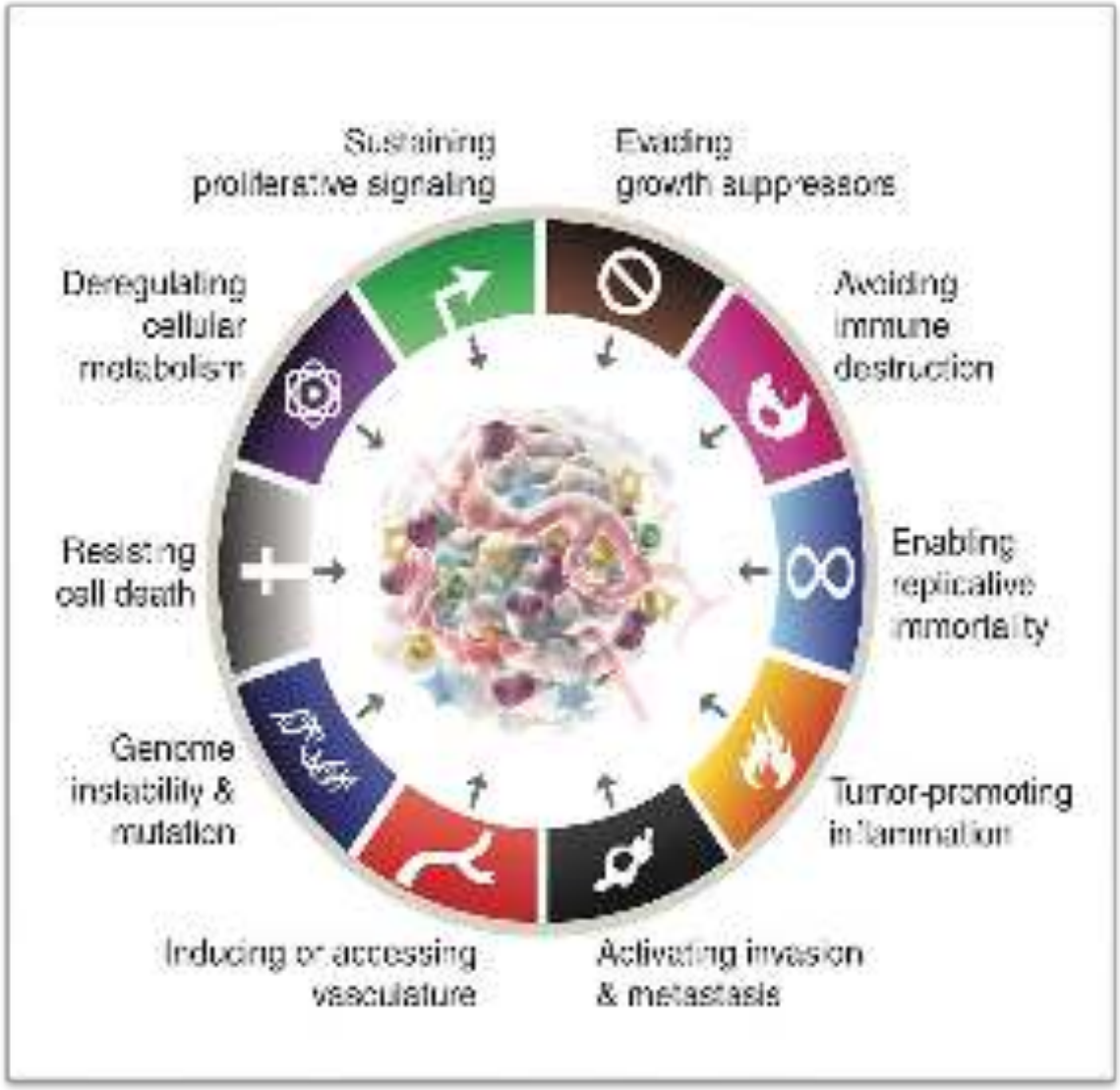

Figure 2-15: The hallmarks of cancer (Hanahan, 2022).

The concept of "breast cancer" refers to the disease that arises when the breast's epithelial cells multiply beyond their typical range with the potential to invade and metastasize (Cuthrell & Tzenios, 2023). Worldwide, breast cancer is the most frequently confirmed malignancy in women, along with being the main trigger of malignant tumor-related mortality (Houghton & Hankinson, 2021).

Industrialized countries possess the highest incidence of this malignancy, though throughout the world, the incidence of this malignancy

is rising; in contrast, populations in Southeast Asia and Africa exhibit the lowest incidence (Smolarz *et al.,* 2022).

It is estimated that between 5% and 10% of breast cancer cases are hereditary, whereas the remaining 90% to 95% are classified as sporadic; this distinction reveals that sporadic cases occur randomly and are not influenced by genetic predispositions (Gataa, 2022).

Various risk factors contribute to the incidence of breast tumors; the most significant risk factor is age, where elderly females exhibit higher incidence rates; substantial risk factors can be categorized into two types: reproductive and non-reproductive, with economic expansion affecting both (Shahidi *et al.*, 2020).

Reproductive factors include menstruation occurring at an early/late age, oral contraceptive use, no pregnancy, the primi gravida after reaching thirty years, lack of breastfeeding, postmenopausal conditions, and hormone replacement treatment use (Wilkinson & Gathani, 2022).

Non-reproductive factors include genetic predisposition, western-style diet, overindulgence in fats, excessive red meat consumption, obesity or gaining weight at menopausal age, adolescence with rapid development and adulthood with high growth, getting older (risk increases after age 35), previous appearance of breast, endometrial, and ovarian cancer, atypical hyperplasia occurs consequence of the development of benign changes in the breasts, an oncogenic viral infection, nicotinism, alcohol consumption, ionizing radiation exposure, and lack of physical exercise (Smolarz *et al.,* 2022; Wilkinson & Gathani, 2022).

Histological studies suggest that a wide range of malignancies can be categorized into one of six main types (Fig. 2-16) (Houghton & Hankinson, 2021; Smolarz *et al.*, 2022; Cuthrell & Tzenios, 2023).

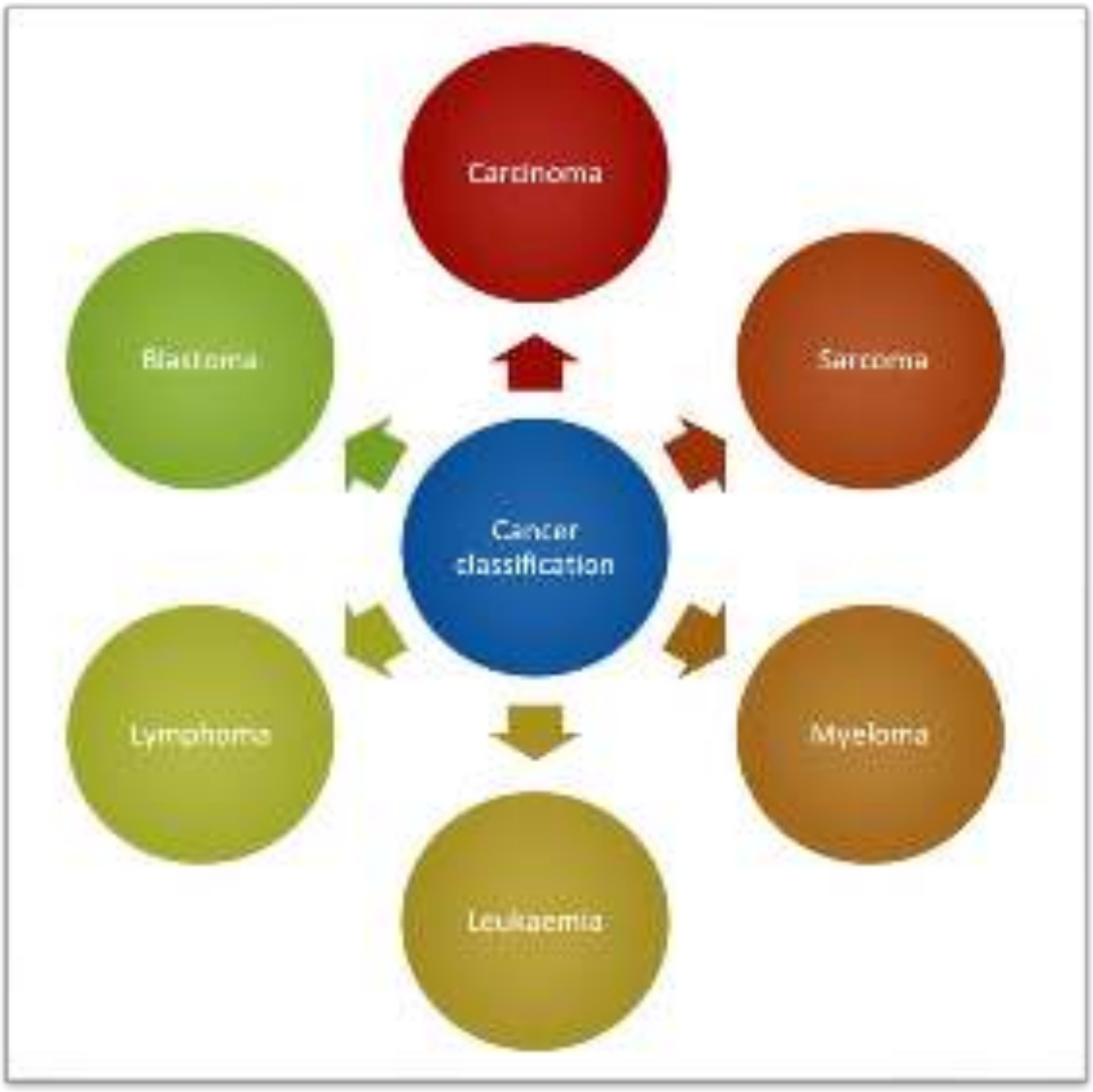

Figure 2-16: Classification of cancer (Cuthrell & Tzenios, 2023).

Breast cancer encompasses a variety of types, as it can develop in different areas of the breast, including the lobules, ducts, and surrounding tissue (Fig. 2-17) (Cuthrell & Tzenios, 2023).

Each region of breast tissue has the potential to be a site for cancer development, breast cancer types can be classified based on the affected cell types within malignant tumors (Cuthrell & Tzenios, 2023). Although approximately 20 primary breast cancer categories exist, most are categorized within two predominant histological classes (Fig. 2-18) (Feng *et al*., 2018).

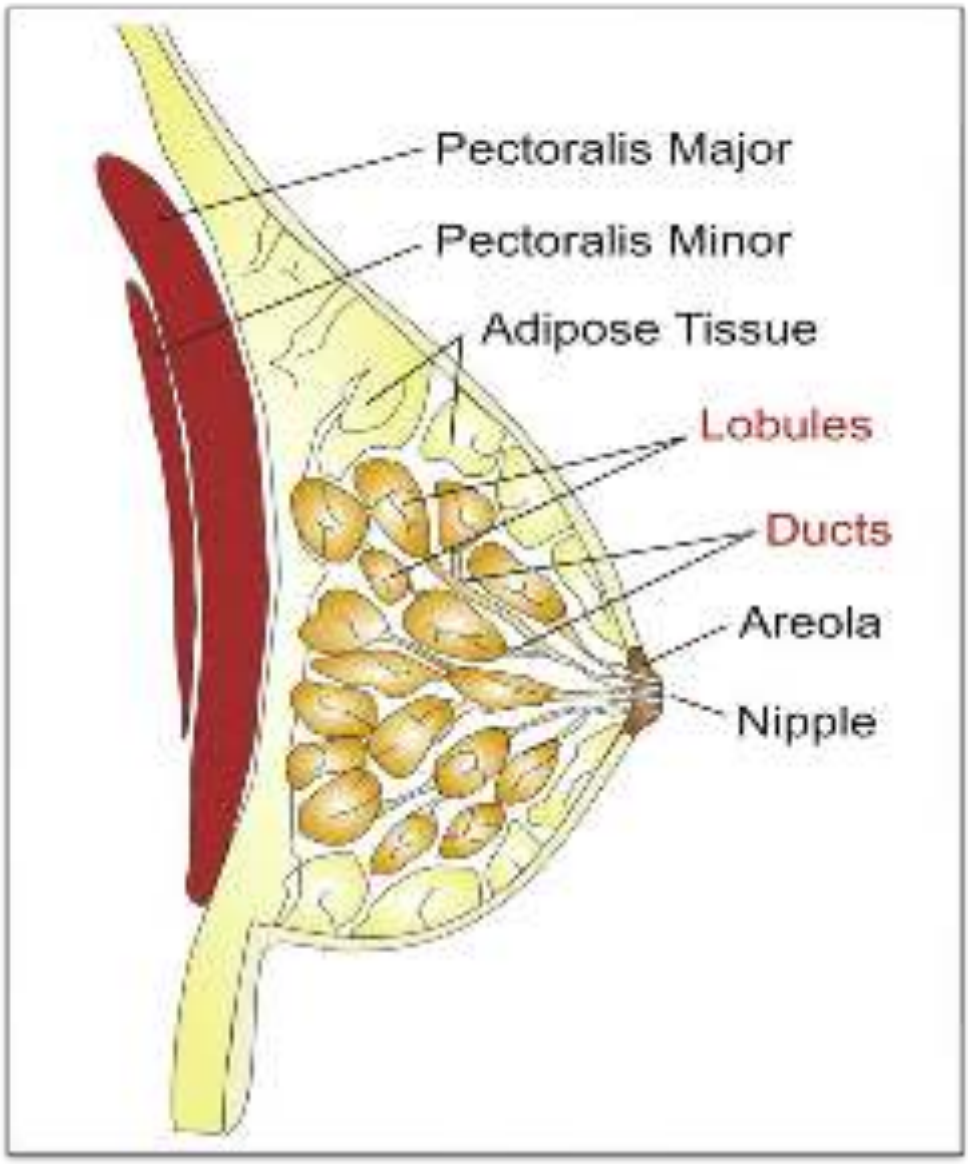

Figure 2-17: The anatomical origin of breast cancer (Feng *et al.,* 2018).

Non-invasive breast cancer: This carcinoma possesses an extremely good chance of developing into an invasive tumor, although it is unable to expand to other regions of the body on its own; also, it is one of the most prevalent forms of breast cancer is ductal carcinoma in situ, which is both non-invasive and pre-invasive (Shahidi *et al*., 2020).

Invasive breast cancer: as invasive, infiltrating breast cancers, these tumors expand to the surrounding breast stroma, they have the potential to migrate throughout the body and reach other organs and lymph nodes (Shahidi *et al*., 2020).

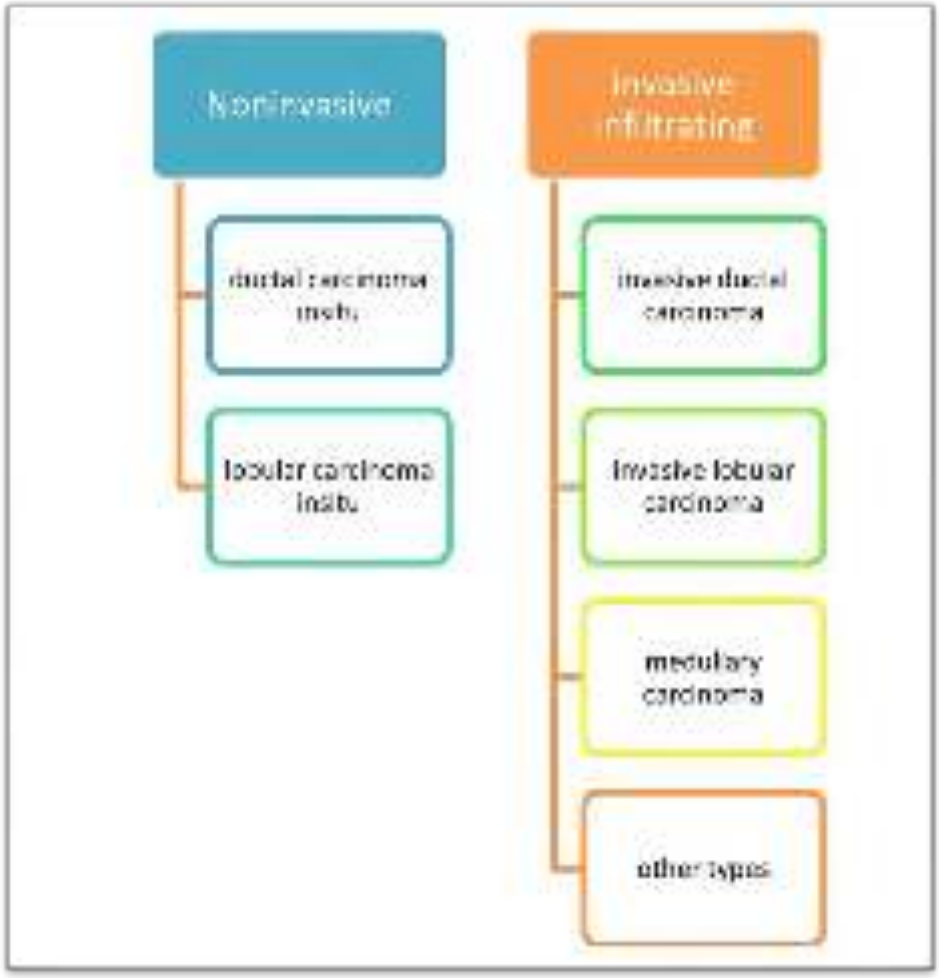

Figure 2-18: WHO classification of breast cancer (Cuthrell & Tzenios, 2023).

2:11:2: The diagnosis of breast cancer:

The main diagnostic tools are magnetic resonance imaging (MRI), mammography, and pathological validation, which are the techniques utilized in this malignancy's current investigation, of those techniques, histopathological images are the most effective in increasing diagnosis accuracy (Feng *et al.*, 2018; Cuthrell & Tzenios, 2023).

2:11:3: The treatment of breast cancer:

Various therapeutic approaches can be used to treat breast cancer, each determined based on the molecular subtype (Smolarz *et al*., 2022). These include chemotherapy, immunotherapy, radiation therapy, surgery, hormone therapy for hormone-positive diseases, and anti-HER2 therapy for HER2-positive diseases (Smolarz *et al.,* 2022; Cuthrell & Tzenios, 2023).

2:12: The relationship between *Toxoplasma gondii* and breast cancer:

The exact relationship between *T. gondii* and breast cancer has not been fully understood until now; however, it has been reported that *T. gondii* plays a key role in maturing the dendritic cells which activate the $CD8^+$ T cells that able to eliminate the malignant cells (Baird *et al*., 2013).

Also, the attenuated *T. gondii* has demonstrated great promise as a creative immunotherapy for breast cancer treatment; further investigations are needed to exploit its full potential and clarify the specific mechanisms involved (Ye *et al*., 2023).

Moreover, the experimental investigation on animals has revealed that *T. gondii* possesses an effective ability to inhibit the development and metastasis of breast tumors (Xu *et al*., 2021; Song *et al*., 2024). Although the whole mechanism is unclear, it has been demonstrated that *T. gondii* can reduce the threat of breast tumors by regulating the signaling pathway of breast cancer, thus suppressing malignant cell development and metastasis (Ye *et al.*, 2024).

Toxoplasma gondii can have a significant role in the development of breast cancer, probably by elevating the expression of PD-1 (Programmed Cell Death Protein-1) and PD-L1 (Programmed Cell Death Ligand-1) genes, the PD-1 and PDL-1 signaling pathways significantly facilitate tumor growth by allowing cancer cells to evade the immune system's recognition (Al-Muskakeh *et al*., 2022; Parvez *et al*., 2023; Ali & Khudair Khalaf, 2024).

Furthermore, it has been revealed that the seroprevalence of human toxoplasmosis is significantly correlated with breast cancer development (Mostafa *et al.*, 2018).

Chapter Three

Materials & Methods

3. Materials and Methods:

3:1 Materials:

3:1:1: Equipment and Instruments:

Equipment and Instruments are summarized in Table (3-1).

Table 3-1: Equipment and Instruments

No.	Equipment/Instruments	Company	Origin
1	Pipettes	Darwell	China
2	Surgical gloves	Conex	Malaysia
3	Plastic sample cup	Qingdao Jindian	China
4	Disposable Face Mask	Albatross	USA
5	Safety Glasses	Y1	China
6	Water Bath	FALC BI	Italy
7	Positively charged glass slides (Electro-Statically Charged)	SANTA CRUZ BIOTECHNOLOGY GY	USA
8	Hot plate	K&K	Korea
9	Immunohistochemistry Pens	Gene tech	China
10	Automated tissue processor	Histo-line	Italy
11	Semiautomated tissue processor	Histo-line	Italy
12	Tissue embedding system	HESTION	China
13	Incubator	ESCO	China

3:1:2: The Chemicals and reagents:

The Chemicals and reagents are summarized in Table (3-2).

Table 3-2: Chemicals and reagents.

No.	Materials and Solutions
1	Different Ethanol Changes (70%, 90%, 95%, 100%)
2	Harris's Hematoxylin
3	Mayer Hematoxylin
4	Acid Alcohol Solution
5	Ammonia Water Solution 0.2%
6	Formaldehyde Solution 37%
7	Tris-Buffered Saline
8	Formalin Solution 10%
9	Antibody Dilution
10	Anti-Antibody Dilution
11	Antigen Retrieval Solution
12	Peroxidase Block Solution
13	DAB^{+} Substrate-Chromogen Solution
14	Horseradish Peroxidase

3:1:3: Immunohistochemistry:

3:1:3:1 The primary Antibody, anti-*Toxoplasma gondii*:

Table 3-3: An overview of the primary antibody utilized in the immunohistochemistry technique and the Immunohistochemistry technique kit.

The name	Cat. No.	Company	Country
Polyclonal Rabbit Antibody IgG	MBS373041	Mybiosource	USA
Envision FLEX	K8000	Dako	Denmark

3:1:3:2 kit component:

Table 3-4: The components of the immunohistochemistry kit.

Material & buffer	Cat. No.
EnVision FLEX Antibody Diluent.	K8006
EnVision FLEX Wash Buffer 20X as a Tris-buffered saline (TBS) bath.	SM831
EnVision™ FLEX Target Retrieval Solution, High pH as Antigen retrieval solution.	DM828
EnVision FLEX Peroxidase-Blocking Reagent.	SM801
EnVision FLEX /goat anti-rabbit IgG-HRP as secondary antibody labeled to horseradish peroxidase.	SM802
EnVision FLEX Substrate Buffer.	SM803
EnVision FLEX DAB^{+} Chromogen.	SM827

3:2 Methods:

3:2:1 Population of the study & samples collection:

Sixty-one tissue samples were obtained throughout the study. Forty-six are found to be diagnosed with malignant tumors, five are diagnosed with benign breast tumors and ten women are free of malignant or benign breast tumors.

The breast tissue sections were collected randomly from a private laboratory in Basrah province / South of Iraq with the help of a specialist doctor (Dr. Sawsan Alharoon) from December 2023 to January 2025.

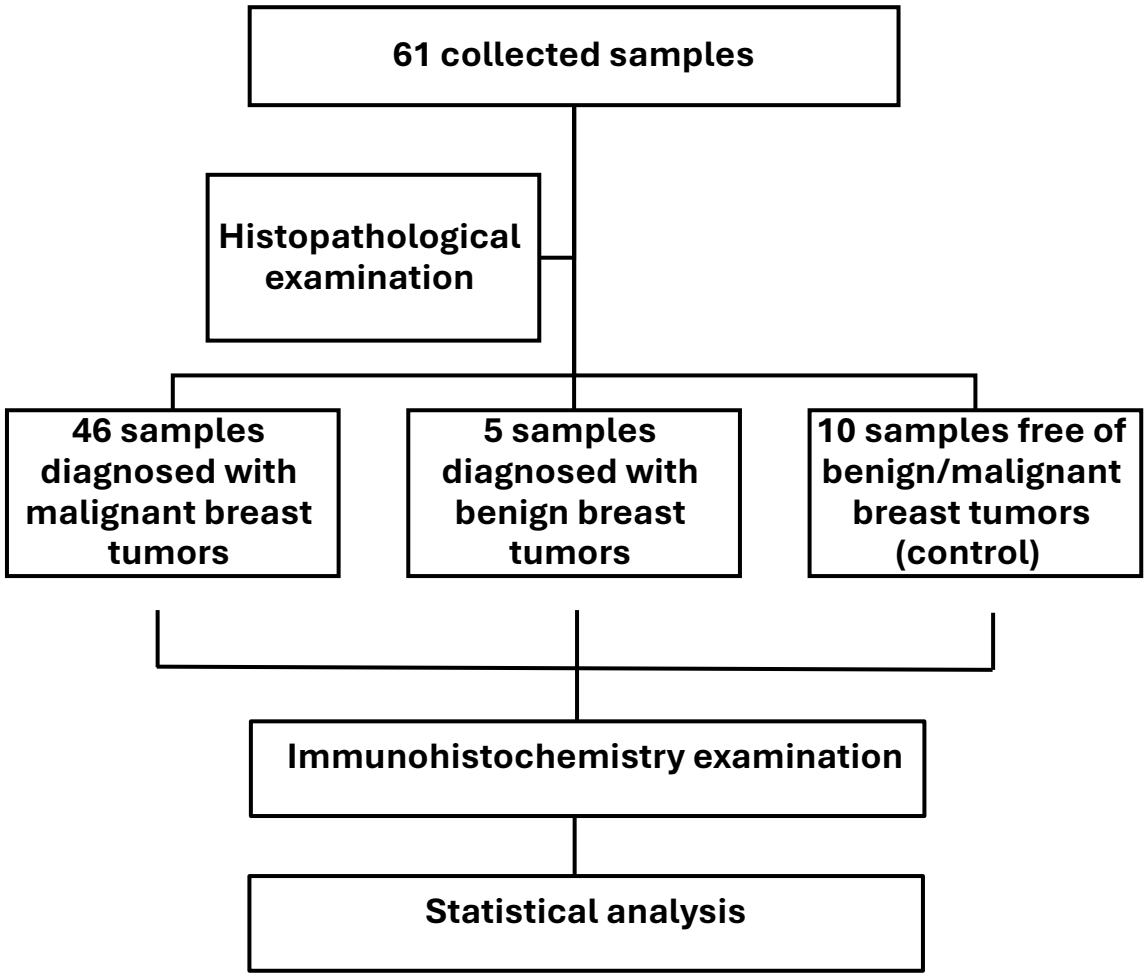

Figure 3-1: The flowchart of the experiment.

3:2:2 Histopathology procedure:

Histopathological examination was performed, according to Wick (2019), and as follows:

1- The breast tissue samples were collected and fixed in a 10% formalin solution for 48 hrs. To ensure optimal preservation of the samples, the formalin solution was replaced after the initial 24 hrs post-collection.

2- After the tissue samples were meticulously sectioned to a thickness of 0.5 cm, they were subsequently placed into plastic cassettes for dehydration and clearing, utilizing an automated tissue processor (Histo-Line ATP700, Italy). After fixation, tissues undergo gradual dehydration using changes of ethanol (70%, 90%, 95%, and 100%) to remove water and minimize distortion. Absolute ethanol is used at the end to ensure complete dehydration. Clearing is accomplished by immersing tissues in xylene 100%. Subsequently, the samples were embedded in paraffin using the standard paraffin embedding technique with a tissue embedding system (HESTION TEC2800-C, China).

3- Tissue samples were processed using a semiautomatic microtome (Histo-Line, Italy) to ensure precise trimming and sectioning at a thickness of 4 μm. The tissue sections were then carefully placed in a water bath (FALC BI, Italy) before being mounted onto glass slides using a hot plate (K & K HYSH11, Korea).

4- Tissue samples were deparaffinized using two changes of xylene for two min each. Subsequently, the samples were rehydrated through a series of ethanol dilutions (100%, 90%, and 70%) for two min each. Following rehydration, the breast sections were rinsed with tap water, stained with Harris's hematoxylin for 5 to 8 min, and washed again with tap water for two min.

5- To improve the clarity of the staining process, the tissue sections were initially differentiated in a 1% acid alcohol solution (1% HCl in 70% ethanol) for 20 sec. This step effectively removed any excess hematoxylin stain, followed by a thorough wash in tap water for 1 min. After that, the breast tissue sections were immersed in a 0.2% ammonia water solution for 1 min to bleach the hematoxylin stain further. Finally, the sections were rinsed in water for 5 min to ensure all residues were removed.

6- Breast tissue sections underwent a 20 sec rinsing process in 95% ethanol, followed by 5 min of counterstaining with eosin to enhance visualization. The tissue sections were then dehydrated using a series of ethanol dilutions-first with 70% ethanol, then 90%, and concluding with 100% ethanol, each for 2 min. The samples were cleared using two changes of xylene for 2 min each, ensuring thorough preparation for examination.

7- Eventually, to facilitate detailed observations and examinations, tissue sections were thoroughly examined using a light microscope at various magnifications, including 40X, 100X, 200X, and 400X.

3:2:3 The procedure of Immunohistochemistry:

Immunohistochemistry was performed using the Dako EnVision detection kit (Envision FLEX, Dako, K8000, Denmark). This process was conducted exactly under the manufacturer's guidelines to ensure the highest quality of results. The present study employed an anti-*T. gondii* primary antibody (Polyclonal Rabbit Antibody: MBS373041, Mybiosource, USA) to identify the presence of *T. gondii* in breast tissue samples, as follows:

1. The antibody was utilized at a dilution of 1:100, prepared using the EnVision FLEX Antibody Diluent (Dako, K8006, Denmark).
2. Paraffin-embedded breast tissue samples were sectioned at a thickness of 4 µm and then placed in a water bath (FALC BI, Italy).
3. The sections were meticulously mounted onto positively charged glass slides (Crystal Cruz® Electro-Statically Charged Micro Slides, sc-363562, SANTA CRUZ BIOTECHNOLOGY, USA) using a hot plate (K&K HYSH11, Korea).
4. The breast tissue sections were dried in the oven at 55 °C.The slides should be positioned horizontally on the oven rack or tray, ensuring the tissue section is oriented upward. Then, deparaffinization was conducted using two changes of xylene, each for 10 min.
5. The tissue was then rehydrated through a sequence of four ethanol baths: 100%, 100%, 90%, and 70%, with each stage lasting precisely 2 min. After rinsing the sections in distilled water, they were immersed in a TBS buffer bath (EnVision FLEX Wash Buffer, SM831) for 5 min, ensuring optimal conditions for the subsequent analysis.
6. The sections were placed in a glass jar containing the antigen retrieval solution (EnVision™ FLEX Target Retrieval Solution, High pH, DM828) pre-heated to 60 °C. The sections were incubated in a water bath at 97 °C for 25 min.
7. After incubation, the tissue sections were allowed to cool at RT in the glass jar for 20 min, rinsed again with distilled water, and then immersed in a TBS buffer bath for 5 min.
8. The excess buffer on the tissue sample was meticulously tapped off, and the surfaces were drained with tissue paper. The tissue sections were delineated on the glass slides with a technical wax pen technique

(Gene Tech Pen, Elabscience, E-BC-R531, China) to ensure that the reagent remained localized to the tissue; the samples then underwent rinsing and were immersed in two changes of TBS buffer bath (EnVision FLEX Wash Buffer, SM831) for five min each.

9. The excess buffer on the tissue samples was carefully removed and gently wiped away with tissue paper. The tissue sections were entirely treated with 100 µL of peroxidase block solution (EnVision FLEX Peroxidase-Blocking Reagent, SM801) as a blocking agent and incubated in a humidity container for 10 min, before rinsing and immersing the samples in two changes of TBS buffer bath (EnVision FLEX Wash Buffer, SM831), each immersion lasting 5 min.
10. The excess buffer on the tissue samples was extracted by gently tapping the slides and wiping around the tissue sections with tissue paper. Then, the samples were treated with 100 µL of anti-Anti-*T. gondii* primary antibody diluent and incubated in a humidity container at RT for 1hr.
11. The incubated sections were rinsed and immersed in two changes of TBS buffer (EnVision FLEX Wash Buffer, SM831), 5 min for each.
12. The excessive buffer on the tissue samples was extracted by gently tapping the slides and wiping around the tissue sections with tissue paper.
13. The tissue sections were treated with 100 µL of a secondary antibody linked with horseradish peroxidase (EnVision FLEX/HRP, SM802). Subsequently, the sections were incubated in a humid container at RT for 30 min.
14. The sections were carefully rinsed and immersed in two changes of TBS buffer (Envision FLEX Wash Buffer, SM831), 5 min for each.
15. The excess buffer on the tissue samples was removed by tapping and gently wiping with tissue paper.

16. The tissue sections were treated with 100 µL of a freshly prepared DAB^+ substrate-chromogen solution. This solution was made by mixing one drop of EnVision FLEX DAB^+ Chromogen (SM827) with 1 ml of EnVision FLEX Substrate Buffer (SM803).
17. The sections were then incubated in a humid container for 10 min. Then, the tissue samples were rinsed and immersed in two changes of TBS buffer bath (Envision FLEX Wash Buffer, SM831), 5 min for each.
18. The breast tissue sections were treated with Mayer hematoxylin (Mayer hematoxylin, Bio-Optica, 05-06002/L, Italy) for 3 min, then rinsed with tap water to remove excess stain.
19. The sections were treated with three successive ethanol 70%, 90%, and 100%, for 2 min each.
20. Then, the sections were immersed in two changes of xylene, 10 min for each.
21. Finally, the sections were mounted by dibutylphthalate polystyrene xylene (DPX) mounting media and covered with coverslips. The tissue samples were examined using a light microscope, with magnifications of 100X and 400X.

3:2:4 Statistical Analysis:

The data of the study were analyzed using SPSS version 24, employing the Chi-square test (χ^2) for statistical evaluation (Griffith, 2007).

Chapter Four

Results & Discussion

4:1 The infection rate of *Toxoplasma gondii*

4:1:1 The relationship between toxoplasmosis and both breast cancer and benign breast tumors:

The IHC technique for the breast tissue sections for women diagnosed with or without breast tumors in (Table 4-1) shows that the infection rate of *T. gondii* among women who have breast cancer is 47.83% (22/46), compared to 20.0% (1/5) among women who have benign breast tumors, and 0.0% of women who did not have breast cancer.

Moreover, it has been demonstrated in (Table 4-1) that 95.7 % (22/23) of women with toxoplasmosis infection are diagnosed with breast cancer compared to 4.3% (1/23) among benign breast tumors. While among *T. gondii*-negative women, 52.2% (24/46) were diagnosed with breast cancer. There is a significant difference between the relationship of toxoplasmosis with breast cancer and benign breast tumors (χ^2=8.726, p=0.013).

Table 4-1: The relationship between toxoplasmosis and both breast cancer and benign breast tumors.

Toxoplasmosis		Breast examination			Total
		Breast cancer	Benign tumors	Healthy	
Infected	**No.**	22	1	0	23
	% of Toxoplasmosis	95.7	4.3	0.0	100.0
	% of Breast cancer	47.8	20.0	0.0	67.8
	% of Total	36.1	1.6	0.0	37.7
Non infected	**No.**	24	4	10	38
	% of Toxoplasmosis	63.2	10.5	26.3	100.0
	% of Breast cancer	52.2	80.0	100.0	62.3
	% of Total	39.3	6.6	16.4	62.3

Total	**No.**	46	5	10	61
	% of Toxoplasmosis	75.4	8.2	16.4	100.0
	% of Breast cancer	100.0	100.0	100.0	100.0
	% of Total	75.4	8.2	16.4	100.0
Statistical analysis	**χ^2= 8.726, *p*= 0.013**				

4:1:2 The relationship between toxoplasmosis and breast cancer:

The data of the statistical analysis for this study shows (Table 4-2) a significant positive relationship between *T. gondii* infection and breast cancer as compared with toxoplasmosis in women free of cancer (χ^2=7.877, *p*=0.005).

Table 4-2: The relationship between toxoplasmosis and breast cancer.

Toxoplasmosis		**Breast cancer**		**Total**
		Positive	Negative	
Infected	**No.**	22	0	22
	% of Toxoplasmosis	100.0	0.0	100.0
	% of Breast cancer	47.8	0.0	39.3
	% of Total	39.3	0.0	39.3
Non infected	**No.**	24	10	34
	% of Toxoplasmosis	70.6	29.4	100.0
	% of Breast cancer	52.2	100.0	60.7
	% of Total	42.9	17.9	60.7
Total	**No.**	46	10	56
	% of Toxoplasmosis	82.1	17.9	100.0
	% of Breast cancer	100.0	100.0	100.0
	% of Total	82.1	17.9	100.0
Statistical analysis	**χ^2= 7.877, *p*= 0.005**			

4:1:3 The relationship between toxoplasmosis and benign breast tumor:

The results (Table 4-3) observed that the infection of toxoplasmosis among women who had benign breast tumors was 20% (1/5). Moreover, it has been revealed that 4.3% (1/23) of toxoplasmosis infections are observed in women who had benign breast tumors.

The data statistical analysis (Table 4-3) shows that the sample size was small, and no association was found between *T. gondii* infection and benign breast tumors, unlike the clear link observed with malignant breast tumors (χ^2=2.143, *p*=0.143).

Table 4-3: The relationship between toxoplasmosis and benign breast tumors.

Toxoplasmosis		Breast benign tumors		Total
		Positive	Negative	
Infected	**No**.	0	1	1
	% of Toxoplasmosis	0.0	100.0	100
	% of Benign Breast tumors	0.0	20.0	6.7
	% of Total	0.0	6.7	6.7
Non infected	**No**.	10	4	14
	% of Toxoplasmosis	71.4	28.6	100
	% of Benign breast tumors	100.0	80.0	93.3
	% of Total	66.7	26.7	93.3
Total	**No**.	10	5	15
	% of Toxoplasmosis	66.7	33.3	100
	% of Benign breast tumors	100.0	100.0	100
	% of Total	66.7	33.3	100
Statistical analysis	**χ^2= 2.143, *p*= 0.143**			

4:2 Immunohistochemistry technique:

The present study has been conducted to determine the possible relationship between toxoplasmosis and breast cancer among women, using the IHC technique, employing a specialized kit (Envision FLEX, Dako, K8000, Denmark) to detect *T. gondii* through the DAB^+ as a staining system. The rabbit polyclonal anti-*T. gondii* as primary antibody (Mybiosource, USA) and goat anti-rabbit IgG as the secondary antibody (EnVision FLEX, Denmark) were employed to recognize *T. gondii* positions in the malignant breast tissues.

These unique observations (Table 4-1, 4-2), corroborate the significant increase in the prevalence of *T. gondii* within breast cancer tissues, as reported by Mostafa *et al.* (2018). The elevated seroprevalence of *T. gondii* among women diagnosed with breast cancer has been observed in numerous investigations (Mostafa *et al*., 2018; Anvari *et al*., 2019; Haghbin *et al.*, 2023; Fadel *et al*., 2025).

It has been reported that an increased prevalence of serum IgG among women diagnosed with breast cancer is associated with altered levels of cytokines, specifically Interleukin-12 (IL-12) and Interleukin-23 (IL-23), in comparison to individuals who do not exhibit IgG presence (Assim & Saheb, 2018). Consistent with our results, an investigations conducted by Al-Muskakeh *et al*. (2022), Al-Sharefi *et al.*, (2023) and Ali & Khudair Khalaf (2024) demonstrate that *T. gondii* significantly facilitated the tumor growth of breast cancer in seropositive women, compared with *T. gondii* seronegative women.

The investigation using IHC of the tissue sections of women with breast cancer revealed that the chronic infection of *T. gondii* can significantly

contribute to the increase of the risk of breast cancer development and the tissues cysts of *T. gondii* are localized within various sites of breast tissues with a significant density of the parasite (Fig. 4-1, 4-2, 4-3, 4-4, 4-5).

As previously reported, *T. gondii* infection is associated with persistent inflammation in tissues, which may play a role in cancer development or exhibit anti-tumoral activity by modulating essential host signaling pathways through its ability to bypass the host's immune system (Dupont *et al*., 2023). The canonical pathways suggest several significant mechanisms that may enhance cancer development during *T. gondii* infection; these include the NFkB and Th17 pathways, the PI3K and p38-MAPK signaling pathways, and IL6, IL8, and HMGB1 signaling in the brain, for the lungs, the PKC signaling pathway is highlighted, while the STAT3 pathway, the HOTAIR regulatory pathway, and IL8 signaling are notable in the context of blood (Caner, 2021).

Toxoplasma gondii can secrete proteins that can enter the nucleus and influence the regulation of the tumor suppressor gene p53, crucial in preventing cancer, where dysfunction in this gene is correlated to the development of many types of cancers (Dupont *et al*., 2023). In a study conducted by Dupont *et al.* (2023), it is suggested that *T. gondii* can inhibit apoptosis by inactivating key proteins, including BAX/BAK and caspases; this mechanism allows infected cells to evade programmed cell death, potentially promoting an environment conducive to cancer development.

In contrast, Hosseini *et al*. (2023) revealed that toxoplasmosis-positive patient’s antibodies attached significantly to the surface of MCF-7 and 4T1 cells, with a much higher intensity than those of negative patients, employing two lines: a human cancer cell (MCF-7) and mic cancer cells (4T1), via flow

cytometry technique; these results demonstrate the possibility of utilizing antibodies against toxoplasmosis to deliver drugs directly to cancer cells.

Similarly, Xu *et al.* (2021) reported that the immunomodulatory effects of RH-Δompdc may represent a promising therapeutic strategy for tumor development; the direct inoculation of the uracil auxotroph RH-Δompdc into the 4T1 tumor has demonstrated significant stimulation of both anti-infection and anti-tumor immunity in murine models; this intervention inhibited tumor growth and metastasis, enhanced the survival rates of tumor-bearing mice, and promoted the increased secretion of IL-12 and IFN-γ in both the tumor microenvironment and the serum, as supported by Alipanahi *et al.* (2025). Also, the attenuated *T. gondii* has demonstrated great promise as a creative immunotherapy for breast cancer treatment (Chen *et al*., 2022; Ye *et al.*, 2023).

Ye *et al.* (2024) declared that *T. gondii* has been shown to transcriptionally regulate multiple signaling pathways by influencing key hub genes, including BRCA1, MYC, and IL-6; this regulatory mechanism may inhibit the growth and migration of breast tumors, suggesting a potential route for anti-tumor therapy.

Toxoplasma gondii has been implicated in the potential development of breast cancer, likely through the up-regulation of the PD-1 (Programmed Cell Death Protein-1) and PD-L1 (Programmed Cell Death Ligand-1) genes; the activation of the PD-1 and PD-L1 signaling pathways plays a critical role in promoting carcinogenesis by enabling cancer cells to evade detection by the immune system, however, further investigations are required to confirm these discoveries (Al-Muskakeh *et al*., 2022; Parvez *et al*., 2023; Ali & Khudair Khalaf, 2024).

The results of immunohistochemistry analysis demonstrated a high degree of accuracy in illustrating the prevalence and distribution of *T. gondii* within breast cancer tissue sections compared with those that do not contain *T. gondii*.

The analysis revealed the presence of *T. gondii* in malignant tissues at varying densities. Specific regions exhibited a low level of the parasite, such as that observed in the blood vessels of the affected cancerous tissue (Fig. 4-1). Whereas others showed moderate levels such as that

observed in the blood vessels and stroma of the affected cancerous tissue (Fig. 4-2). Furthermore, locations within the tissue exhibited very high parasite densities, such as those observed within neoplastic epithelial cells, near the blood vessels lumen and stroma of the affected cancerous tissue (Fig. 4-3).

In contrast, the immunohistochemistry analysis demonstrated that there were sections of breast cancer tissue that were devoid of the parasite, such as those in both neoplastic epithelial cell clusters and stromal tissue of the cancerous breast tissues (Fig. 4-5).

This variability may be associated with various factors such as the diverse immune responses of the host, as the parasite attempts to evade immunological defenses to ensure its survival and reproduction, eventually, understanding this relationship depends on various factors, such as the type of cancer, the parasite strain, and the host's immune status (Jung *et al.*, 2016; Nayeri *et al*., 2024).

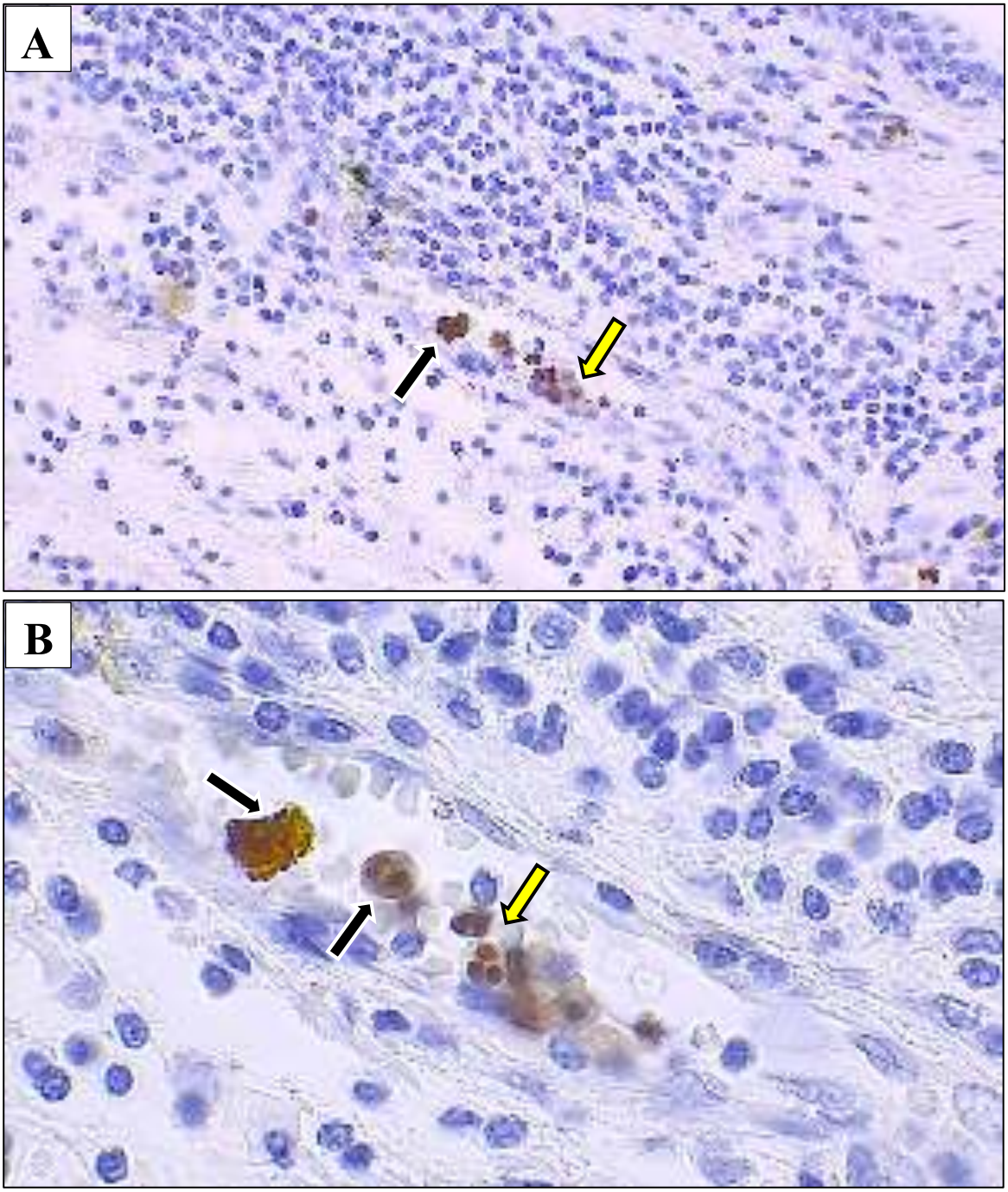

Figure 4-1: The expression of anti-*T. gondii* primary antibody was observed in the affected cancerous breast tissue indicating the mature bradyzoite cysts of *T. gondii* (black arrow) and immature bradyzoite cyst (yellow arrow) that were observed in the blood vessels of the affected cancerous tissue. Hematoxylin and DAB by IHC. A: 400X & B: 1000X. (see Appendix A & Appendix B).

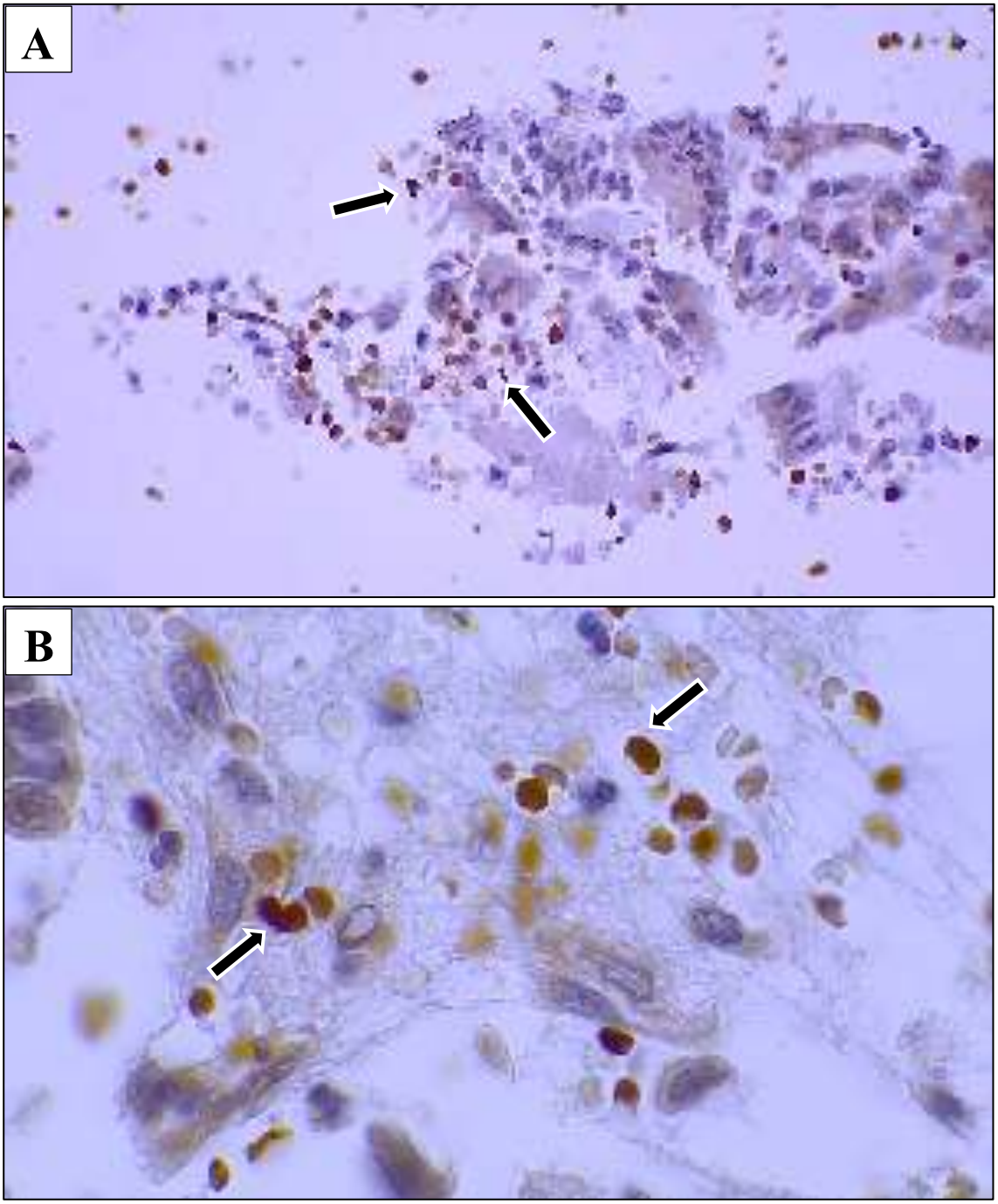

Figure 4-2: The expression of anti-*T. gondii* primary antibody was observed in the affected cancerous breast tissue indicating the bradyzoite of *T. gondii* (black arrow) observed in the blood vessels of the affected cancerous breast tissue. Hematoxylin & DAB by IHC. A: 400X & B: 1000X.

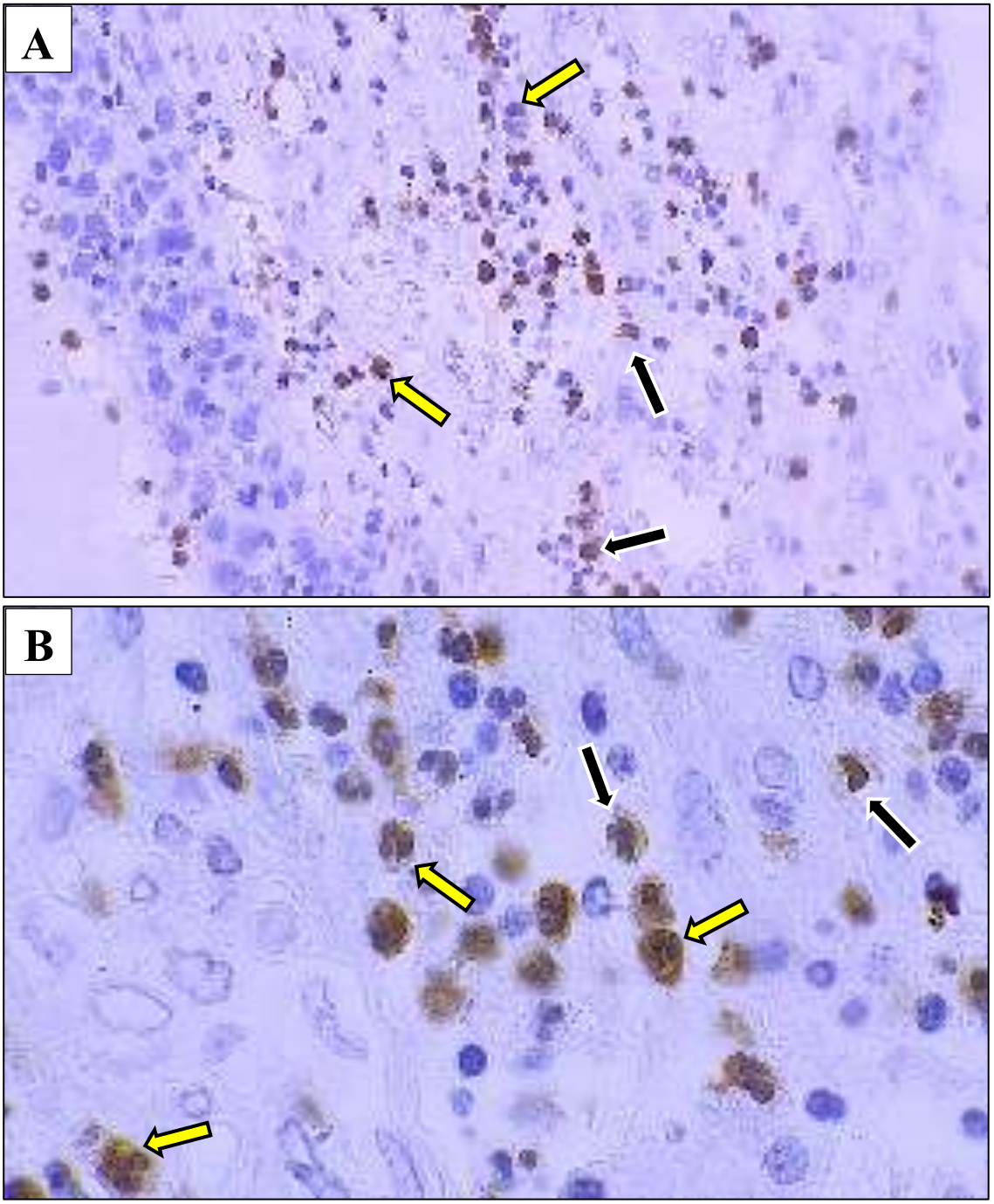

Figure 4-3: The expression of anti-*T. gondii* primary antibody was observed in the affected cancerous breast tissue indicating the mature bradyzoite of *T. gondii* (black arrow) and immature bradyzoite cyst (yellow arrow) that was observed in the blood vessels lumen and stroma of the affected cancerous tissue. However, note the severe *T. gondii* infection was noted in this case compared with other cases, where many of *T. gondii* parasites were observed. Hematoxylin & DAB by IHC. A: 400X & B: 1000X. (see Appendix E & Appendix F).

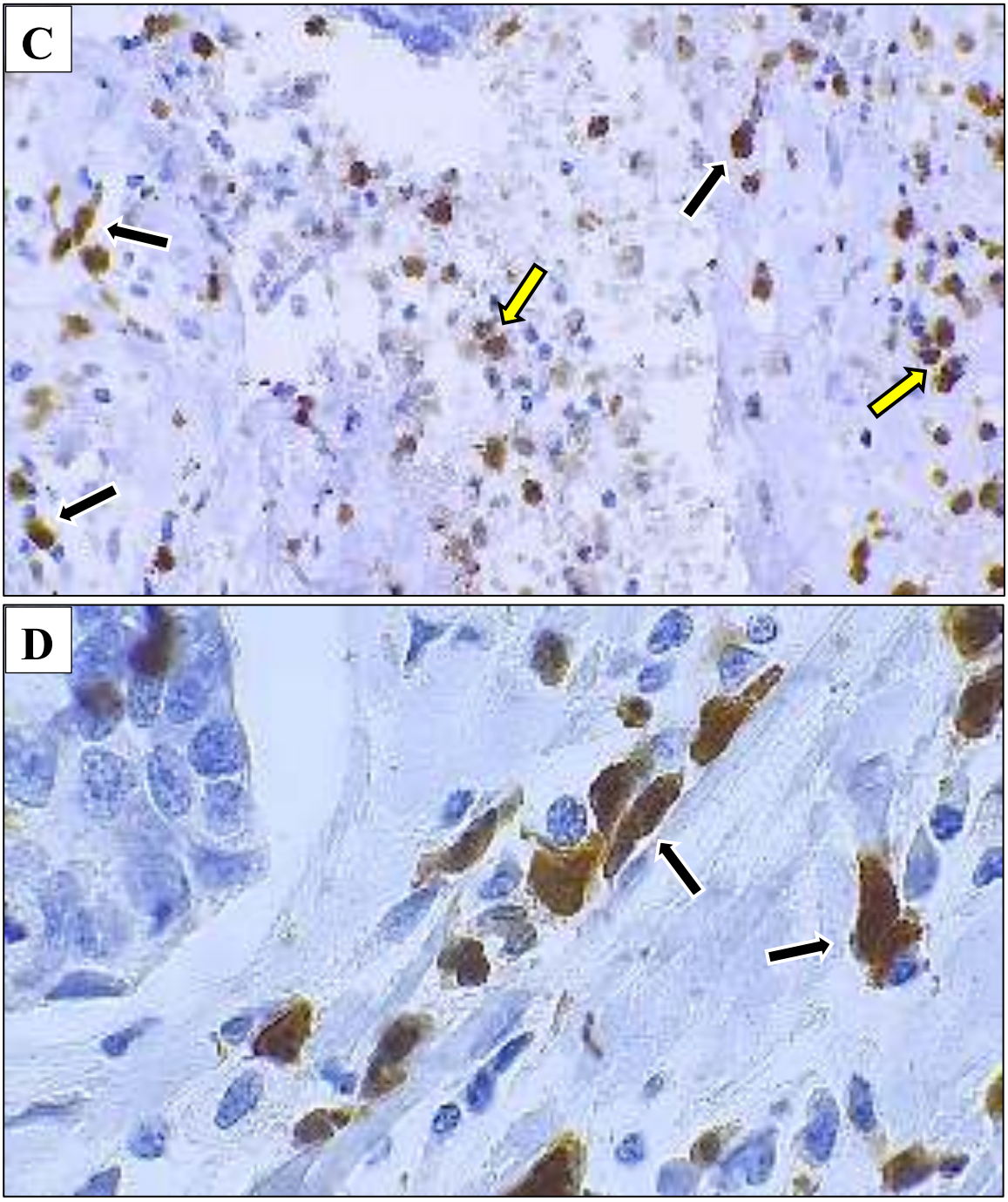

Figure 4-3: The expression of anti-*T. gondii* primary antibody was observed in the affected cancerous breast tissue indicating the mature bradyzoite of *T. gondii* (black arrow) and immature bradyzoite cyst (yellow arrow) that was observed in the blood vessels lumen and stroma of the affected cancerous tissue. However, note the severe *T. gondii* infection was noted in this case compared with other cases, where a lot of *T. gondii* parasites were observed. Hematoxylin & DAB by IHC. C: 400X & D: 1000X.

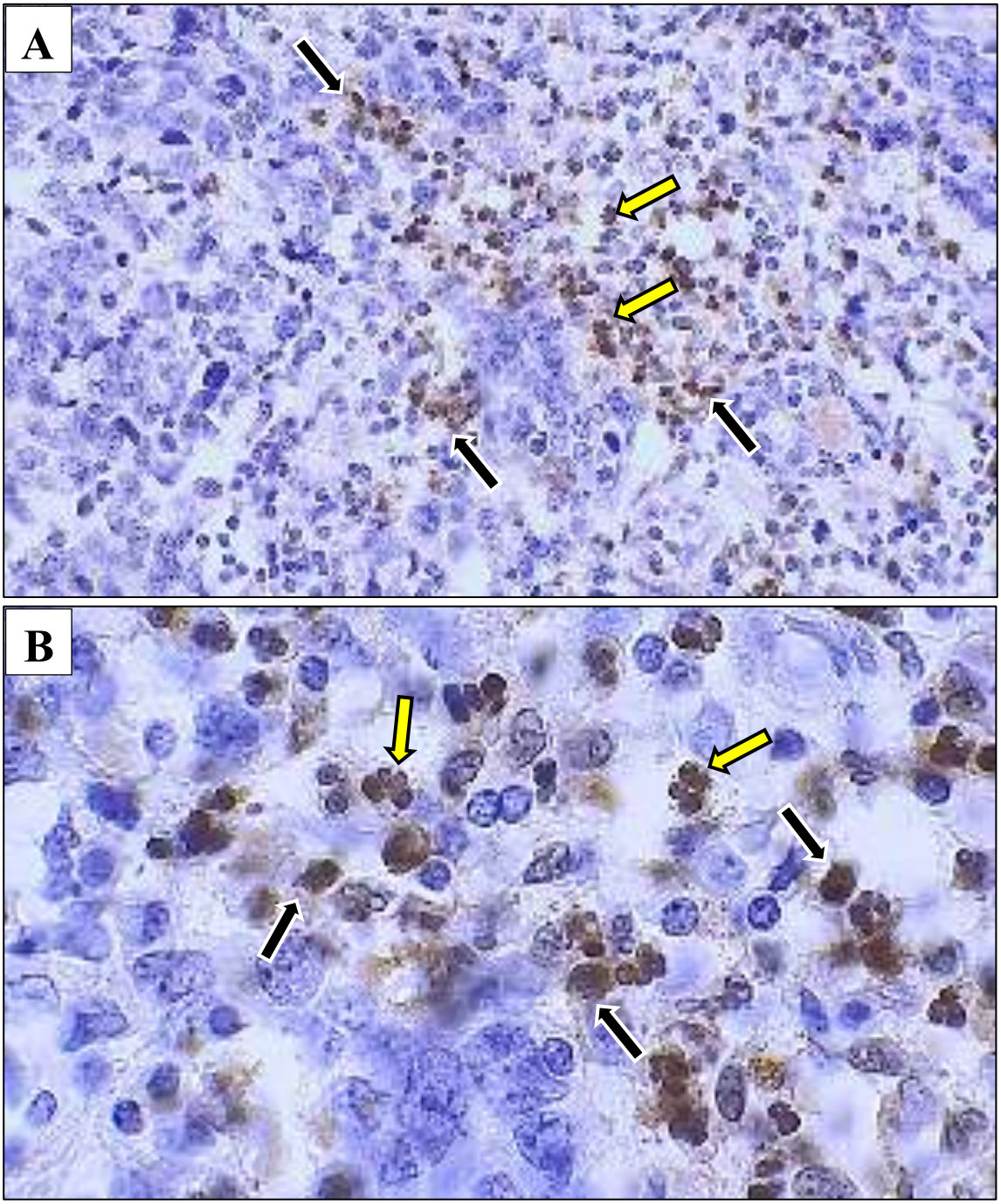

Figure 4-4: The expression of anti-*T. gondii* primary antibody was observed in the affected cancerous breast tissue indicating the mature bradyzoite cysts of *T. gondii* (black arrow) and immature bradyzoite cyst (yellow arrow) that were observed within neoplastic epithelial cell, near the blood vessels lumen and stroma of the affected cancerous tissue. Hematoxylin & DAB by IHC. A: 400X & B: 1000X. (see Appendix C & Appendix D).

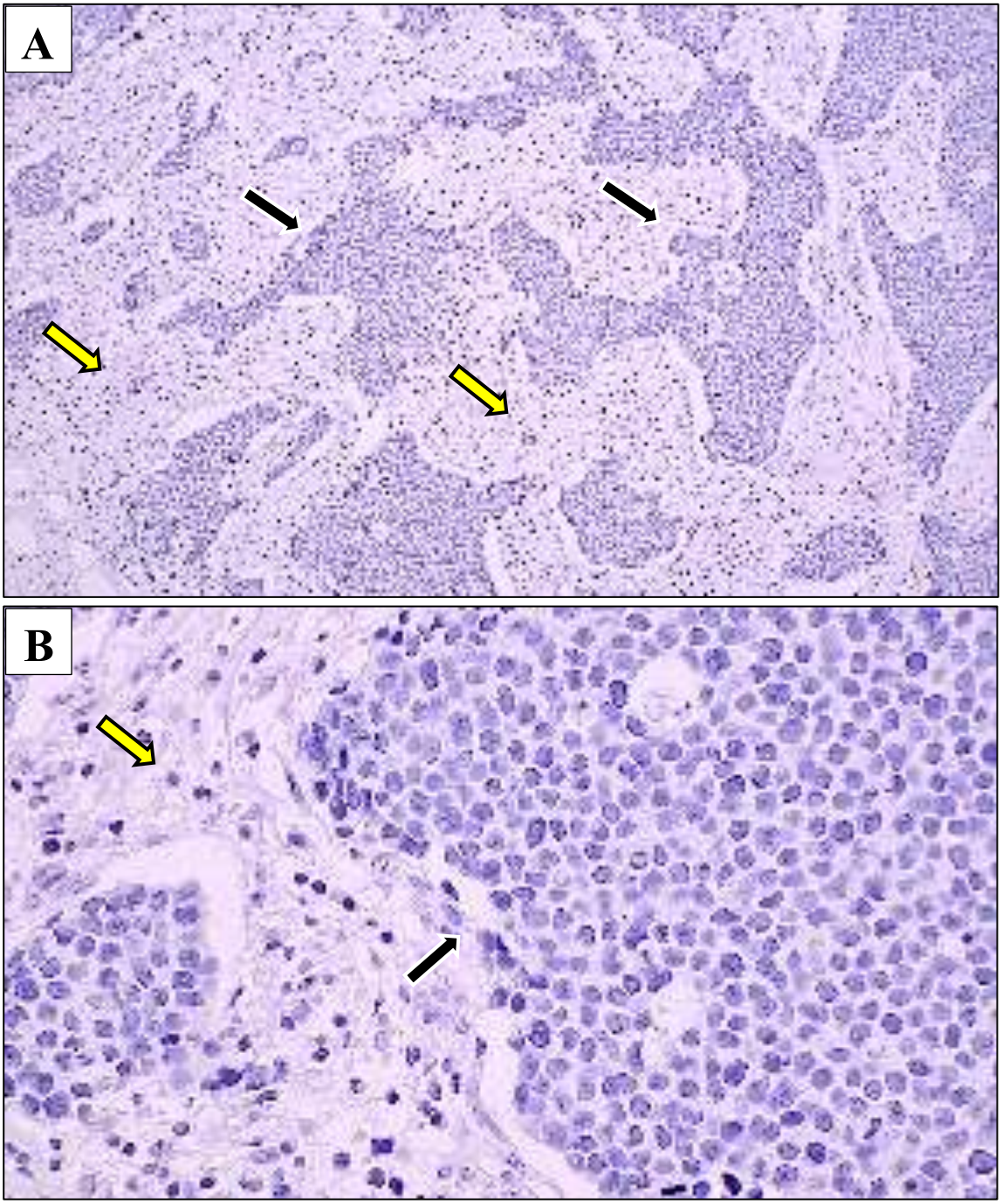

Figure 4-5: Note the negative expression of anti-*T. gondii* primary antibody, indicating the negative presence of *T. gondii* parasite or its cysts in both neoplastic epithelial cells clusters (black arrow) or stromal tissue (yellow arrow) of the cancerous breast tissue. DAB & Hematoxylin by IHC. A: 100X & B: 400X. (see Appendix I & Appendix J).

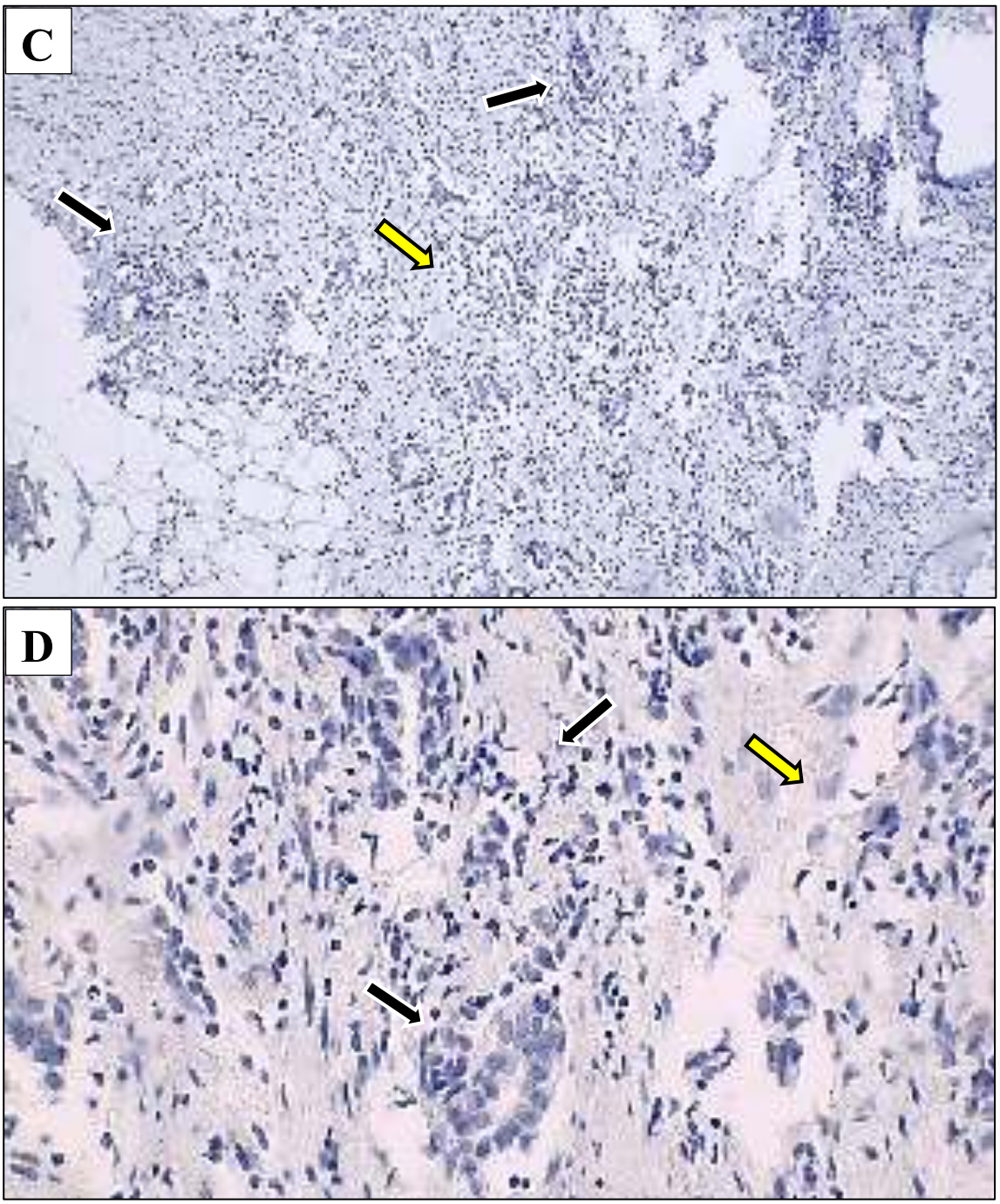

Figure 4-5: Note the negative expression of anti-*T. gondii* primary antibody, indicating the negative presence of *T. gondii* parasite or its cysts in both neoplastic epithelial cell nests (black arrow) or stromal tissue (yellow arrow) of the cancerous breast tissue. DAB and Hematoxylin by IHC. C: 100X & D: 400X. (see Appendix L, Appendix M & Appendix N).

4:3 Histopathology technique:

The identification of *T. gondii* was accomplished by applying the histopathology technique using H&E. The study demonstrates the presence of *T. gondii* in malignant tissue samples obtained from women diagnosed with breast cancer and demonstrates that the infection with *T. gondii* causes severe damage to the typical breast tissues (Fig. 4-6).

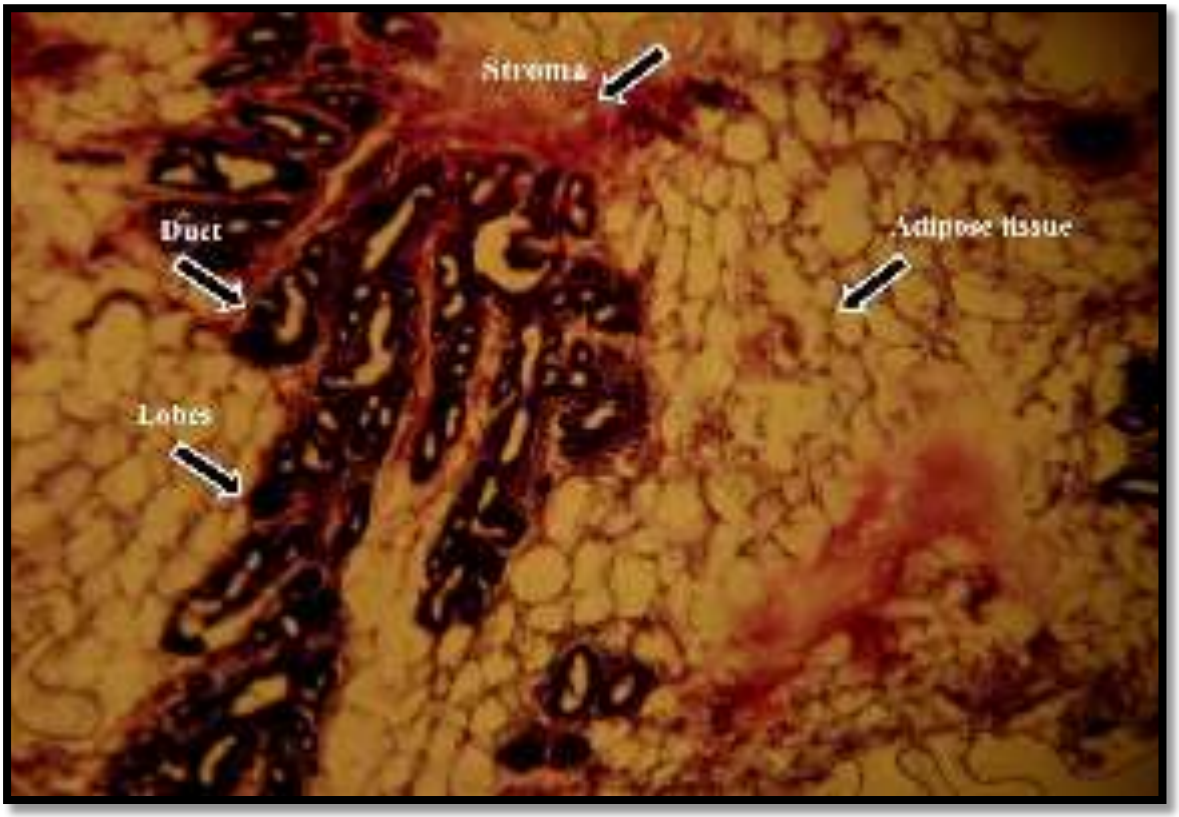

Figure 4-6: Normal microscopic appearance of the breast tissue. The ducts in the lobules with collagenous stroma extend between these structures. The adipose tissue can be seen as highly visible and admixed with these elements. H&E stain, 100X.

The microscopic examination of breast tissue sections has demonstrated the following findings:

1) Breast invasive ductal carcinoma with positive toxoplasmosis:

Histopathological section stained with H&E at (A: 40X, B: 100X, C: 400X) magnification showed invasive ductal carcinoma (IDC) of the breast with noted positive toxoplasmosis.

The milky duct contains dense collagenous fibrous tissue, among which lie small irregular nests and cords, or glands of cancer cells. The ducts and the veins incorporated into the tumor often become encased in a thick layer of elastic tissue. Dense fibrotic stroma indicates a desmoplastic response, and myofibroblasts and collagen deposition give the cancer a firm consistency. The typical lobular and ductal structures are replaced by invasive tumor cells.

The results observed the presence of chronic inflammation and tissue changes, which may be due to toxoplasmosis, which is a known contributor to tumor progression, where the infected tissues may contain tachyzoites or tissue cysts, though usually these are difficult to detect in routine H&E unless extensive. The tumor grade is moderate to high based on nuclear atypia and mitotic count observed in tissue sections and *T. gondii* evidence, which is not more visible histologically in H&E staining; the implication of aggressive behavior and potential immune modulation if co-infected with toxoplasmosis (Fig. 4-7).

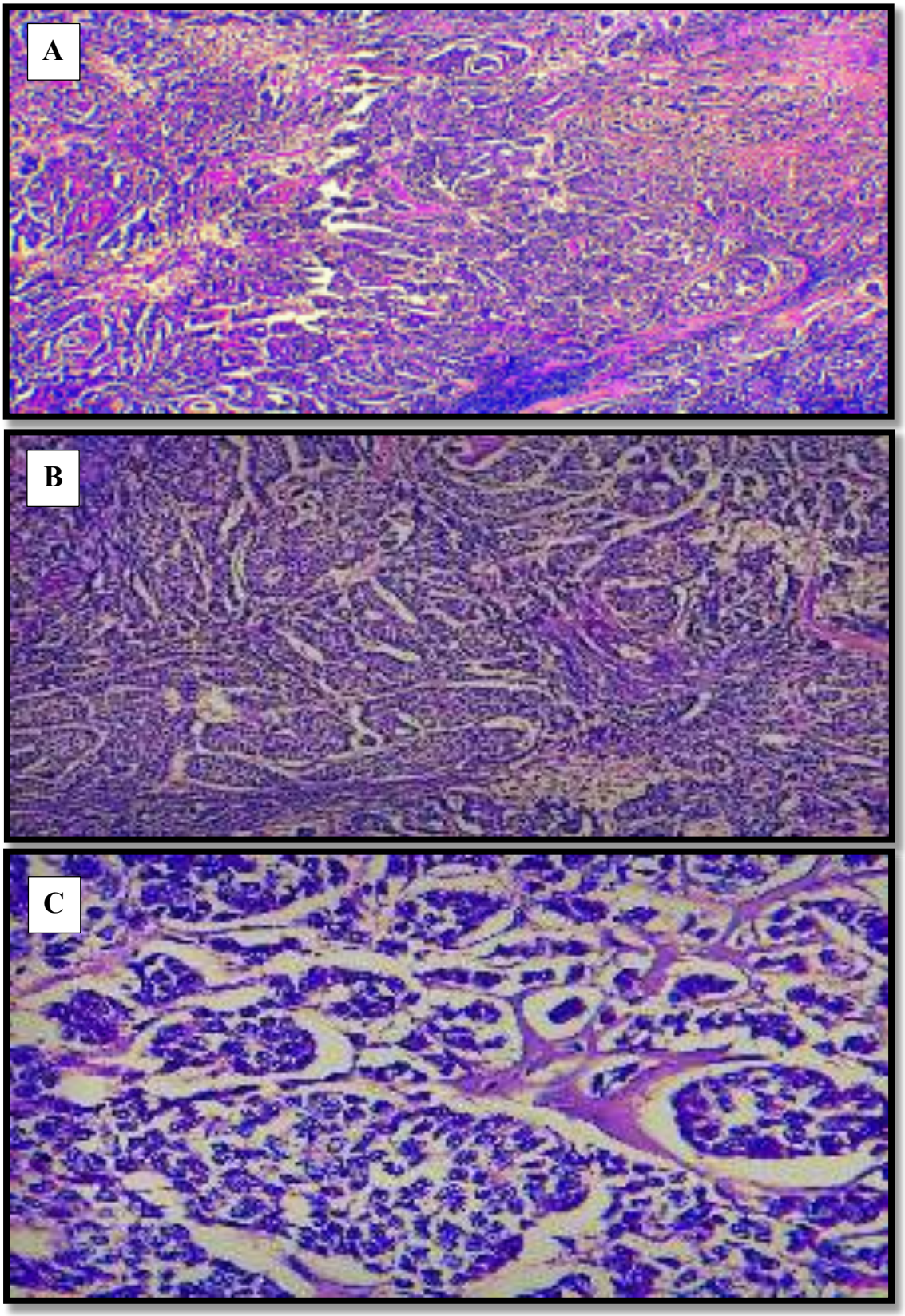

Figure 4-7: Breast invasive ductal carcinoma with positive toxoplasmosis. Infiltrative ducts and nests of malignant ductal epithelial cells within desmoplastic stroma. H&E. A: 40X, B: 100X & C: 400X.

2) Benign fibroadenosis with positive toxoplasmosis:

The high-resolution histological section (H&E stained, at 40X magnification) was found to be benign fibroadenosis with positive toxoplasmosis. Based on the histopathological changes, there is proliferation of small ducts or acini with dilated ducts or cysts within the lobules. A dense fibrous layer surrounds the glandular tissue, compressing and distorting it. The fibroblastic stroma found between the ducts may appear hyalinized.

Inflammatory changes observed by scattered mononuclear inflammatory infiltrates may include lymphocytes, plasma cells, and possibly macrophages in the interstitial stroma, which may be secondary to toxoplasmosis (Fig. 4-8).

The histological section (100X magnification) observed that the lobules are enlarged and consist of benign glandular and ductular elements, the ductules are found lined by a dual layer of epithelium (inner luminal epithelial and outer myoepithelial cells), which confirms the benign nature. Scatter some chronic inflammatory cells, primarily lymphocytes and plasma cells, are found around ducts and in the stroma (Fig. 4-8).

The histological section (400X magnification) shows that proliferation of lobules and ducts with regular arrangement, without cellular abnormalities or architectural distortions, indicates a tumor or malignant cells. The ductal epithelial cells were found cuboidal to columnar, with uniform, round nuclei and no pleomorphism, and the myoepithelial cells that support the epithelial cells are often present but less prominent.

Some ductules show increased epithelial layers, consistent with benign epithelial hyperplasia, usually seen in fibroadenosis. The interlobular stroma contains scattered lymphocytes and plasma cells, which indicate

chronic inflammation. This inflammatory response may be due to *T. gondii* infection, which can stimulate a localized immune reaction (Fig. 4-9).

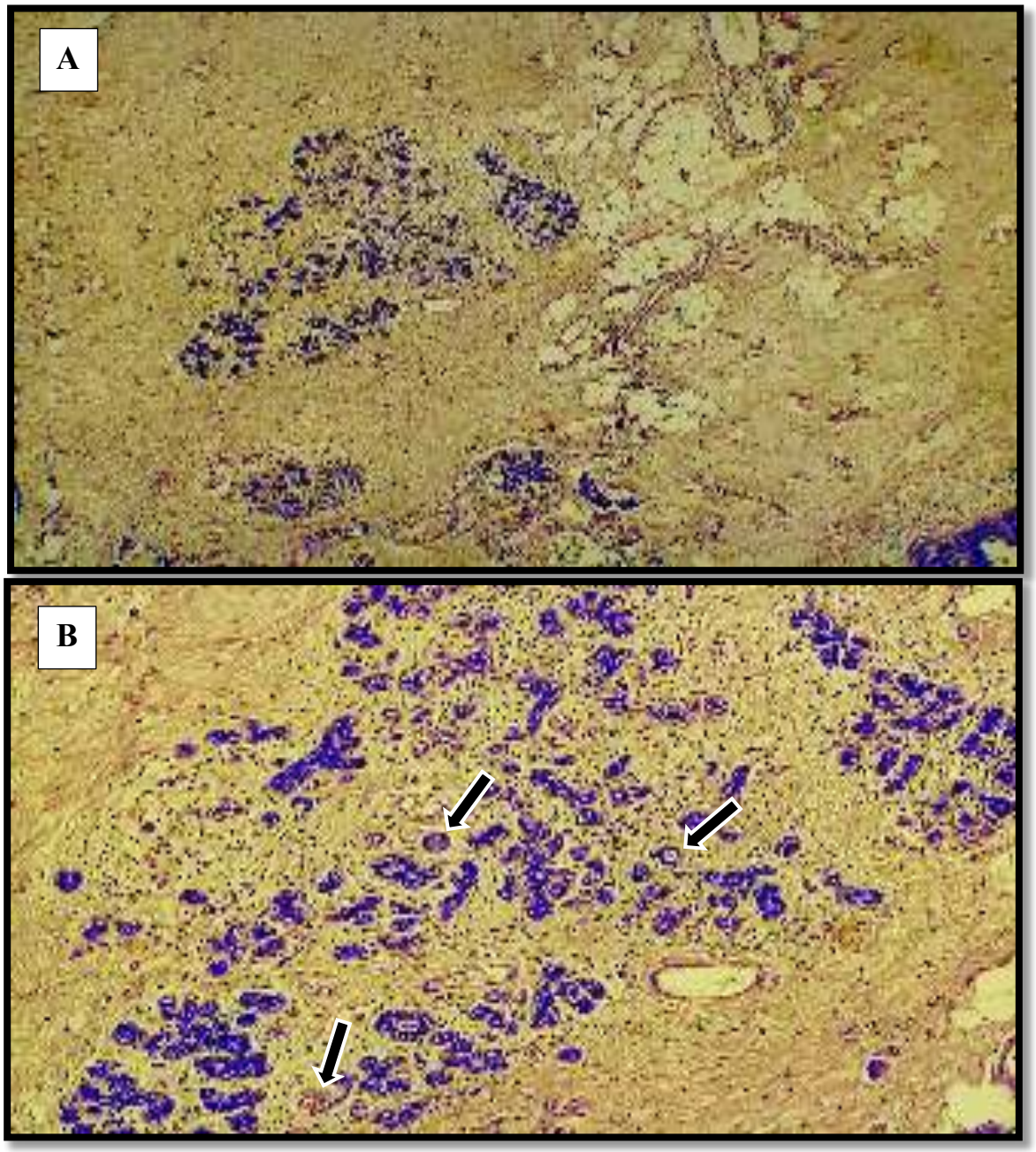

Figure 4-8: Benign fibroadenosis with positive toxoplasmosis. Expanded lobules of benign glands and ductules within fibrous stroma. H&E. A: 40X & B: 100X.

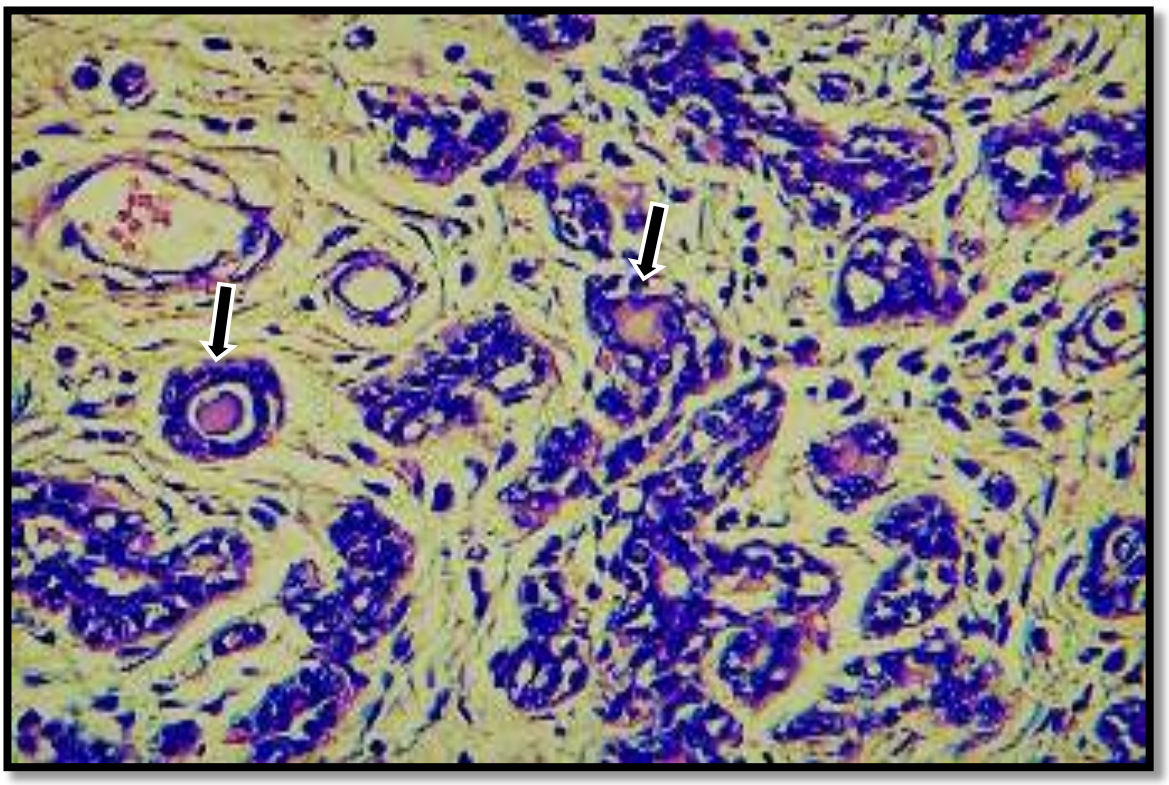

Figure 4-9: Benign fibroadenosis with positive toxoplasmosis. Expanded lobules of benign glands and ductules within fibrous stroma with eosinophilic secretion in the lumens. H&E. 400X.

3) Breast invasive ductal carcinoma with negative toxoplasmosis:

The Figure (4-10A) shows breast invasive ductal carcinoma with negative toxoplasmosis (40X magnification), characterized by malignant ductal epithelial cells arranged in irregular ducts, tubules, and nests infiltrating the surrounding breast tissue. The surrounding stroma is fibrotic and reactive, forming desmoplasia, which is considered a typical response to invasive carcinoma. The malignant epithelial cells show pleomorphism with hyperchromatic nuclei and high nuclear-to-cytoplasmic ratios, with mitoses that may be visible, indicating active proliferation. No granulomatous changes or surrounding inflammatory infiltrate, suggestive of *T. gondii* infection, which supports a histological toxoplasmosis-negative result.

The (Fig. 4-10B) provided shows classic features of breast invasive ductal carcinoma with negative toxoplasmosis (100X magnification), which

shows infiltrating malignant cells into the ducts and tubules, invading the surrounding breast parenchyma with irregular shapes, and showing a randomized arrangement, indicating loss of standard ductal architecture. The stroma is markedly desmoplastic with dense, pink, eosinophilic collagen deposition and scattered fibroblasts with myofibroblasts. Histologically, the absence of granulomatous inflammation or necrosis indicates that no *T. gondii* cysts or tachyzoites are seen, supporting the negative toxoplasmosis status.

The (Fig. 4-10C) provided shows classic features of invasive ductal carcinoma of the breast with negative toxoplasmosis, which is characterized by infiltrative nests and irregular ducts composed of malignant ductal epithelial cells. The tumor cells have hyperchromatic, pleomorphic nuclei and prominent nucleoli, indicating high-grade malignancy. The tumor cell nests are surrounded by desmoplastic stroma of dense fibrous tissue, which appears as pinkish, eosinophilic fibrotic areas that separate the nests of tumor cells.

4) Benign fibroadenosis with negative toxoplasmosis:

The Figure (4-11A) observed breast with benign fibroadenosis or fibrocystic changes with negative toxoplasmosis (40X magnification). Where found, lobular units are enlarged, showing proliferation of glandular structures (benign acini and ductules), and this expansion is typical of fibrocystic changes. The ductules are lined by two layers of luminal epithelial cells and myoepithelial cells, indicating benign architecture without cellular atypia, mitotic activity, and necrosis. There is increased dense collagenous stroma between the glandular tissues; there are no granulomas, necrosis, or parasitic cysts, which supports the negative toxoplasmosis status, as there is no histological evidence of *T. gondii* infection or inflammation.

The (Fig. 4-11B) observed benign fibroadenosis or fibrocystic changes with negative toxoplasmosis under (100X magnification), which are characterized by expanded lobules with benign glands and ductules and observed clusters of glandular and ductal epithelial structures arranged in lobular patterns. The epithelial cells lining those ducts were uniform, lacked significant atypia, and had round to oval nuclei with regular chromatin. The stroma is abundant and appears collagenous and hypocellular, which is typical of fibroadenosis, where the dense stroma surrounds and separates the ductal and glandular structures. Given the absence of inflammatory or infectious features, with no granulomas, necrosis, or visible organisms, and the diagnosis of negative toxoplasmosis, there are no signs of the parasitic infection.

The (Fig. 4-11C) shows benign fibroadenosis or fibrocystic change with negative toxoplasmosis (400X magnification) which is a common benign breast condition and characterized by enlarged mammary lobules with increased numbers of acini, the lobules appear more prominent than normal breast tissue, multiple small ducts and ductules are visible throughout the tissue, the glands are lined by benign epithelial cells and these epithelial cells maintain their standard outer structure. Dense fibrous connective tissue appears to surround and separate the glandular structures with a pink to eosinophilic stain. This fibrosis is a characteristic feature of fibroadenosis and confirms the benign nature of the lesion with no evidence of atypia, malignancy, or infectious organisms. No granulomas, necrosis, or parasitic cysts are present, which supports the negative toxoplasmosis status, as there is no histological evidence of *T. gondii* infection or inflammation.

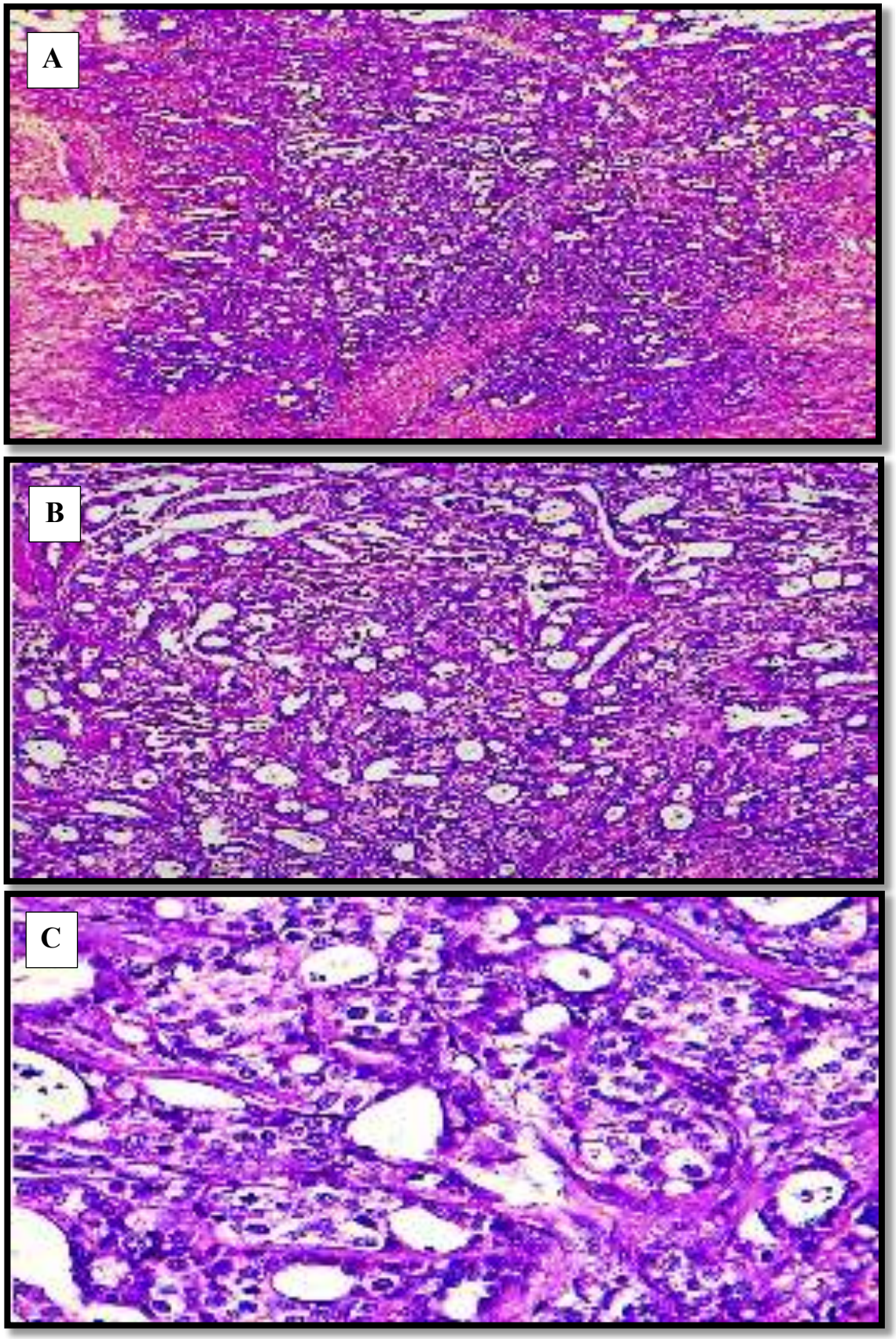

Figure 4-10: Breast invasive ductal carcinoma with negative toxoplasmosis. H&E. A: 40X, B: 100X & C: 400X.

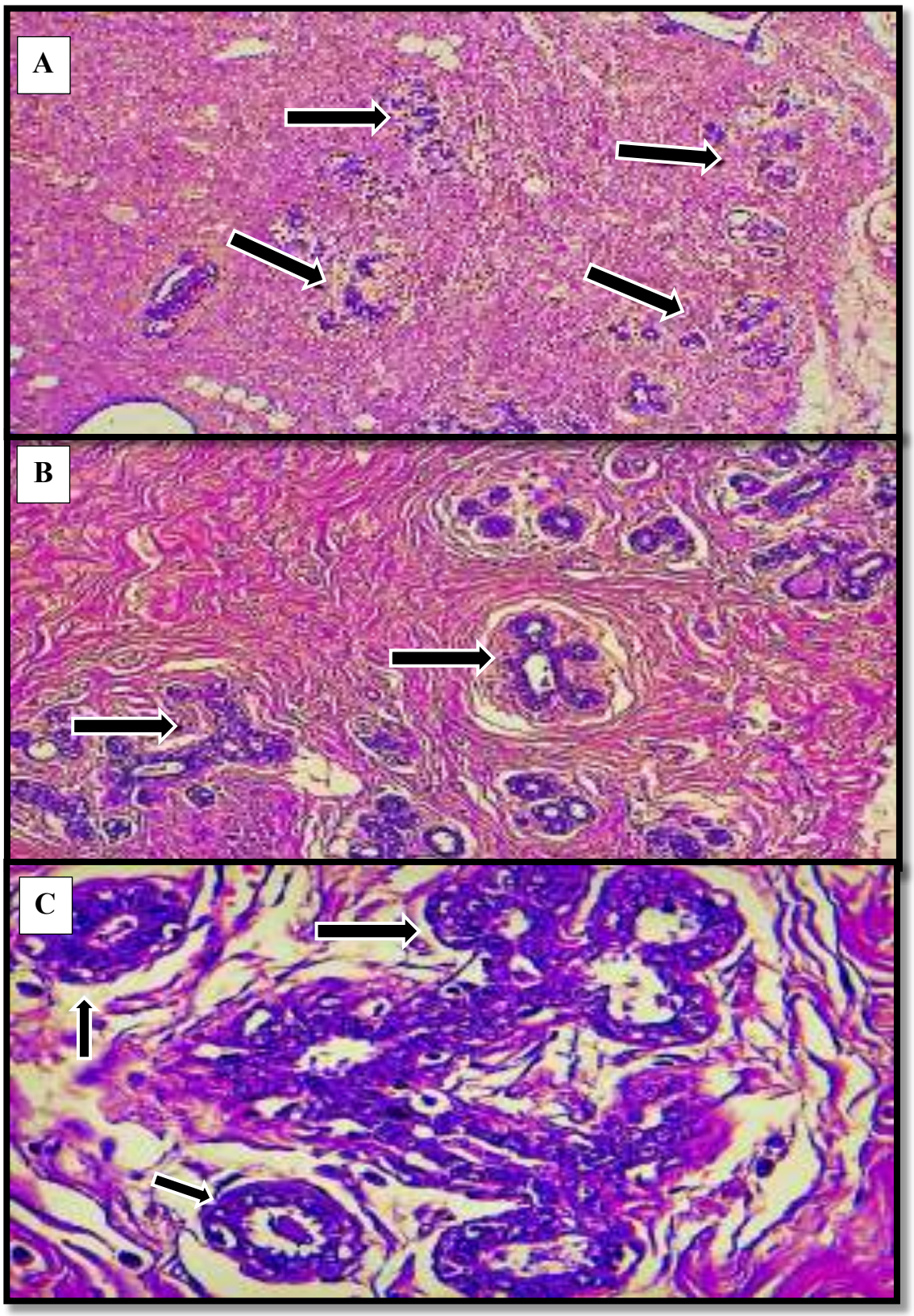

Figure 4-11: Benign fibroadenosis with negative toxoplasmosis. Expanded lobule of benign glands and ductules within fibrous stroma and lined by benign ductal epithelial cells (black arrows). H&E. A:40X, B: 100X & C: 400X.

The present study demonstrated the presence of *T. gondii* in malignant breast tissue among women and identified particular histological alterations resulting from its impact; consistent with our results, numerous studies have reported that *T. gondii* is present in malignant breast tissue (Kalantari *et al.*, 2017; Ameer *et al*., 2022; Al-eadani *et al.*, 2025).

The results of this study indicate that chronic inflammation and tissue alterations are due to the presence of *T. gondii* in the tissue, and these indications resemble those in malignant brain tissue (Yang *et al.*, 2024). Also, the study found implications of aggressive behavior and potential immune modulation of toxoplasmosis, as Jung *et al.* (2022) reported, *T. gondii* can modulate host biological processes for its survival strategy in the malignant brain tumors.

The study observes inflammatory changes represented by accumulation of lymphocytes and macrophages which are essential in defense against toxoplasmosis, and this agrees with studies have been showed that the infection with *T. gondii* presents a complex interaction between the parasite and the host's immune response, also indicates that $CD4^+$ and $CD8^+$ T cells play vital roles in managing the infection by releasing the cytokine IFN-γ, which stimulates macrophages to eliminate the parasite effectively (Dupont *et al*., 2012; Sturge & Yarovinsky, 2014; Khan *et al*., 2019).

Stachs *et al.* (2019) reported that certain ductules exhibit increased epithelial layering, consistent with benign epithelial hyperplasia, a feature commonly observed in fibroadenosis.

The stroma may be fibrotic and reactive, producing desmoplasia, which is viewed as a typical response to invasive carcinoma (Fig. 4-10); as

Kumar *et al.* (2021) reported, desmoplasia refers to the growth of dense fibrous connective tissue around a tumor or area of injury, it is characterized by the excessive production of collagen and extracellular matrix by activated fibroblasts, resulting in a fibrotic reaction; this process often forms a stiff tissue stroma around malignant cells and is considered an essential component of the TME; Desmoplasia can influence tumor progression, invasion, and response to therapy.

The absence of granulomatous inflammation and necrosis in the breast tissues indicates that *T. gondii* is not observed (Fig. 4-10), thereby supporting the negative diagnosis of toxoplasmosis in the breast tissue; these findings are supported by a study conducted by Weiss & Dubey (2009), which shows that the presence of granulomatous inflammation in histological examination of tissue samples suspected of being infected with *T. gondii* is an important indicator that the body is attempting to contain the infection and provides histopathological evidence supporting the diagnosis.

The current study demonstrate that the infection of *T. gondii* has caused severe damage to the typical breast tissues (Fig. 4-7), including necrosis, destruction in infected tissues, and a significant presence of *T. gondii* at the site of infection; these findings agree with the study of Xu *et al.* (2021), which declared the detection of *T. gondii* in malignant tissues and suggested its potential impact on the tumor microenvironment.

While our study revealed histological changes, Al-eadani *et al.* (2025) reported that *T. gondii* infection did not induce significant alterations in the examined breast tissue sections.

Chapter Five

Conclusion

&

Recommendations

5. Conclusion and Recommendations

5:1 Conclusions:

The *Toxoplasma* parasite is a possible causes that increase the risk of developing cancer, especially with taking Chemotherapy and radiotherapy doses and treatments that suppress the body's immunity, which makes the parasite in a position to transform from the chronic disease in which the stage is bradyzoite of the slow growing phase to tachyzoite which is the rapidly growing that responsible for the acute condition in the absence of immune control, which makes this parasite a cause of mortality for most of the treated cases and therefore conclude that the parasite and its toxic secretions have a significant role in stimulate the growth and development of malignant tumors, especially brain tumors, and are also responsible for the deterioration of the patient's condition.

From the findings of the current study, it can be concluded the following:

1) Statistical analysis revealed a strong positive correlation between *T. gondii* infection and breast cancer, but no significant association with benign breast tumors. *T. gondii* can evade immune responses, inhibit apoptosis, promote the proliferation of infected cells, and alter the cellular microenvironment.

2) Immunohistochemistry is a distinct and highly sensitive technique for cancer diagnosis and the detection of *T. gondii* antigens in malignant breast tissue. This method provides excellent visualization of biomolecular distribution and effectively highlights molecular alterations.

3) The infection rate of *T. gondii* was significantly higher in malignant breast tissues (47.83%) compared to benign breast tumors (20%) and was absent in women without malignant or benign breast tumors.

4) Immunohistochemistry and histopathological examinations supported the role of *T. gondii* in inducing chronic inflammation, tissue damage, and desmoplastic reactions, which may contribute to tumor progression.

5:2 Recommendations:

1) Given the limited scope of previous research on this topic, several cross-sectional investigations should be conducted utilizing histological, immunological, and serological techniques to determine the parasite's role as a potential carcinogenic agent and the overall prevalence of *T. gondii* in Iraq, especially among cancer patients.
2) Routine serological testing and tissue screening for *T. gondii* infection are recommended for women diagnosed with breast cancer.
3) Incorporating PCR testing alongside IHC may improve the sensitivity of *T. gondii* detection in malignant tissues.
4) Oncologists are advised to consider *T. gondii* infection when treating cancer patients, as immunosuppressive therapies may exacerbate the infection.
5) Further experimental studies are recommended to evaluate the potential use of alternative *T. gondii* strains or parasite-derived proteins as cancer immunotherapy.
6) Promote health awareness within Iraqi society, focusing on women, regarding the risks associated with toxoplasmosis. This can be achieved through seminars and awareness programs utilizing various platforms, including print, audio, visual, and social media.
7) Developing a healthcare program for women with breast cancer and performing the serological examination for early detection of toxoplasmosis.

8) Early and regular breast cancer screening, including self-exams and clinical exams, is vital for awareness and early detection. Women aged 40-70 should have a mammogram every two years. These strategies help in early detection and managing breast cancer effectively.
9) Conducting clinical studies that focus on various *T. gondii* strains and stages to evaluate the potential of *T. gondii* to induce a range of tumor types.
10) Preventive measures and health awareness are crucial factors in preventing and reducing the risk of both toxoplasmosis and breast cancer, particularly by dealing with parasite transmission routes and adopting healthy lifestyles such as breastfeeding, exercising, avoiding radiation exposure, and limiting the use of hormonal treatments.
11) Future studies should investigate the role of *T. gondii* in carcinogenesis across various tissues, which is essential for understanding its overall impact. Such studies should be comprehensive to identify factors linking toxoplasmosis and cancer.

Chapter Six

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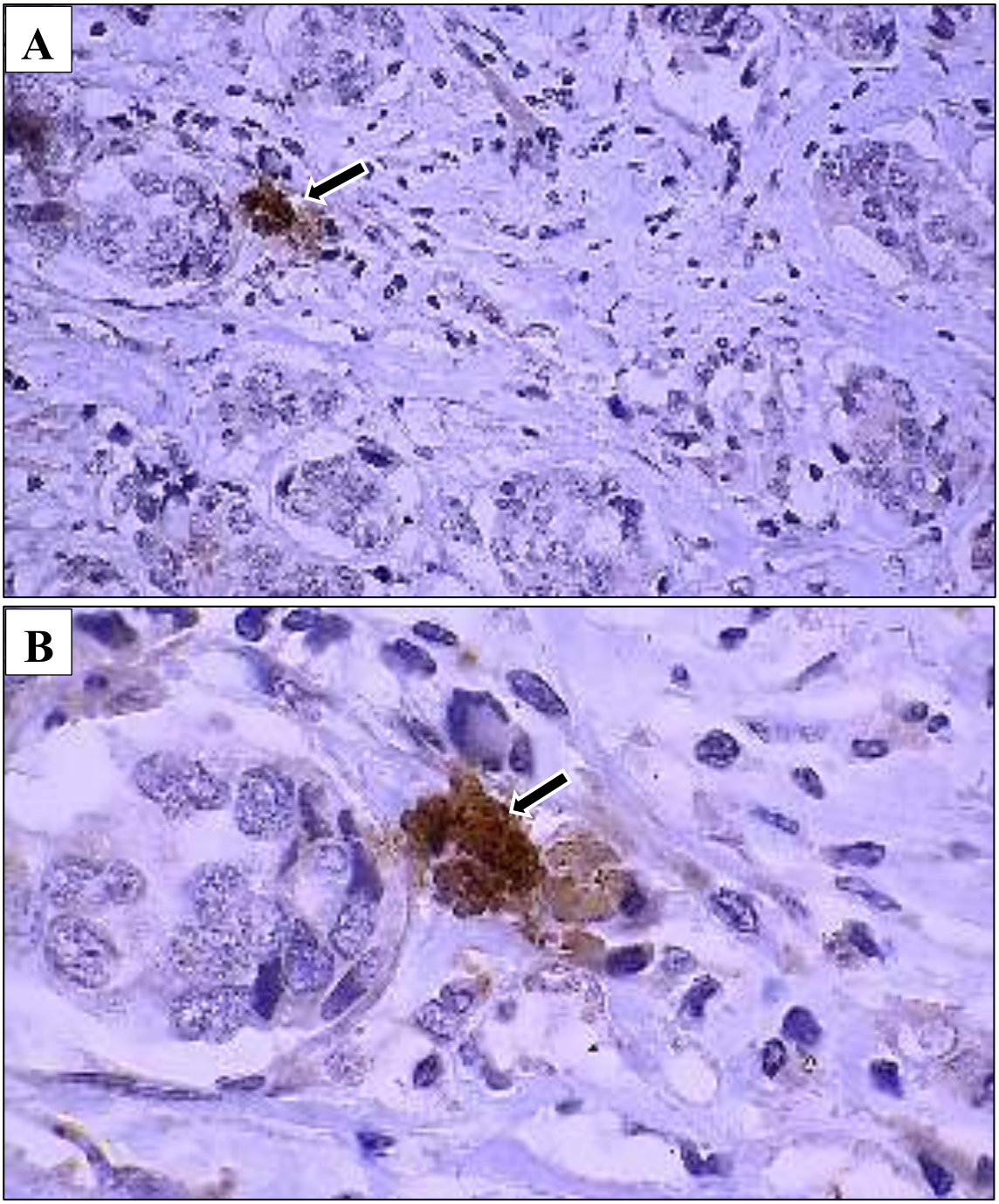
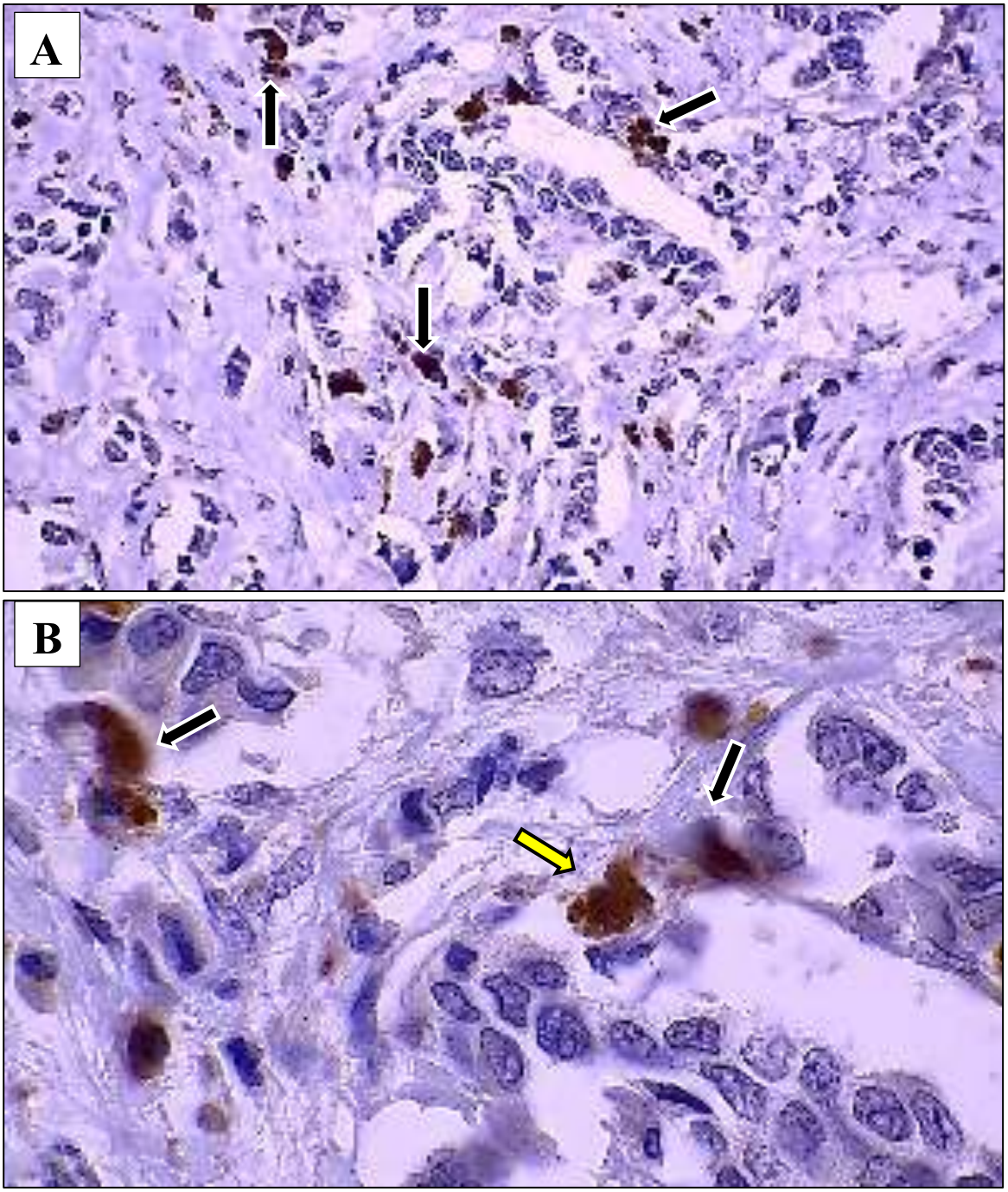
Appendices



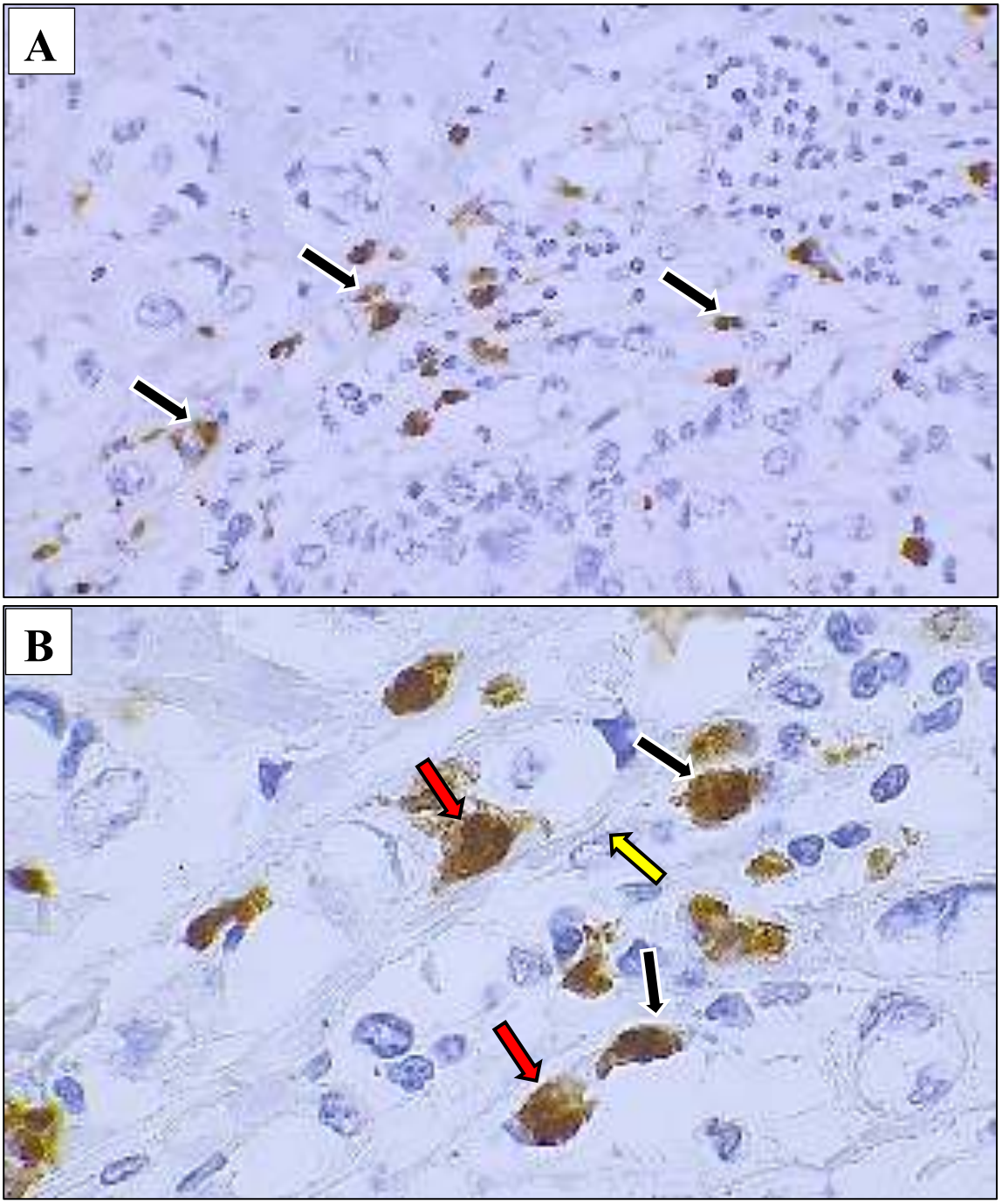
Appendix (A): Photomicrograph of breast cancer tissue.

The expression of anti-*T. gondii* primary antibody was observed in the affected cancerous tissue indicating the mature bradyzoite cysts of *T. gondii* (black arrow) that were observed in the small blood vessels of the affected cancerous tissue. Hematoxylin and DAB by IHC. A: 400X & B: 1000X.



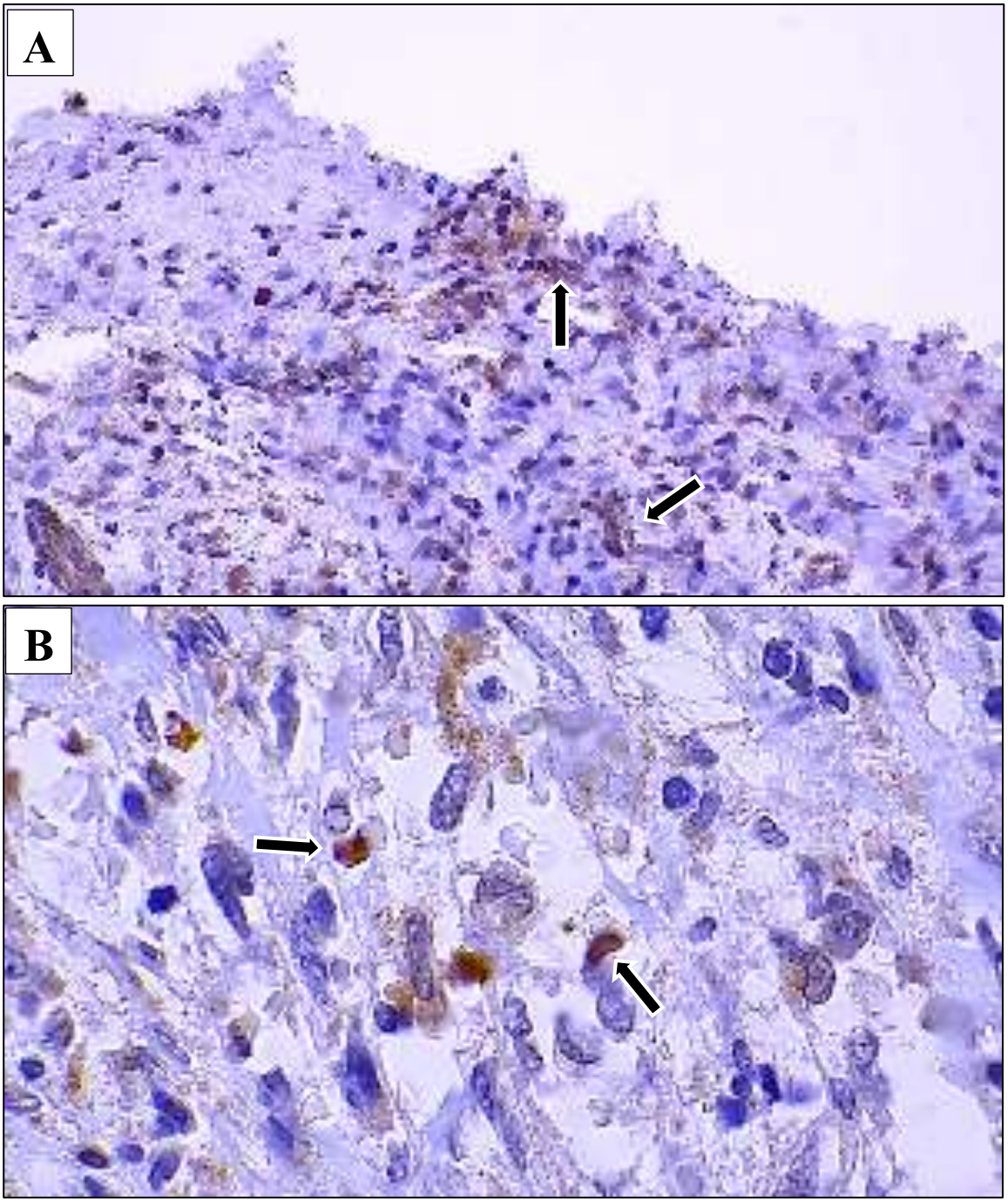
Appendix (B): Photomicrograph of breast cancer tissue.

The expression of anti-*T. gondii* primary antibody was observed in the affected cancerous tissue indicating the bradyzoite of *T. gondii* (black arrow) and mature bradyzoite cyst (yellow arrow) that was observed in the blood vessels of the affected cancerous tissue. Hematoxylin and DAB by IHC. A: 400X & B: 1000X.



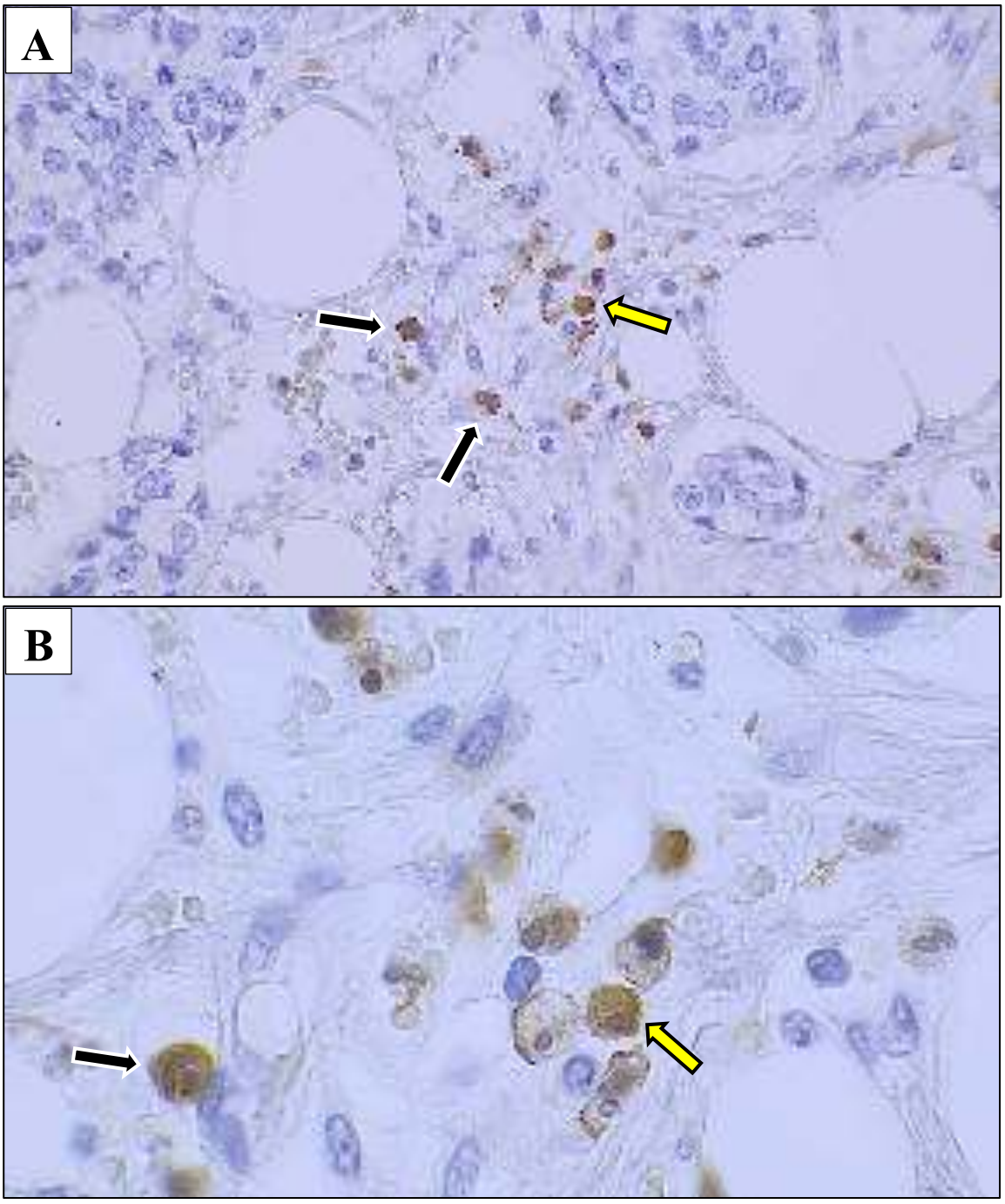
Appendix (C): Photomicrograph of breast cancer tissue.

The expression of anti-*T. gondii* primary antibody was observed in the affected cancerous tissue indicating the immature bradyzoite of *T. gondii* (black arrow) that was observed within the stroma of these cancerous tissues especially near small blood vessels (yellow arrow). However, some bradyzoite cysts were ruptured (red arrow), but their bradyzoite still inside the cyst. Hematoxylin and DAB by IHC. A: 400X & B: 1000X.



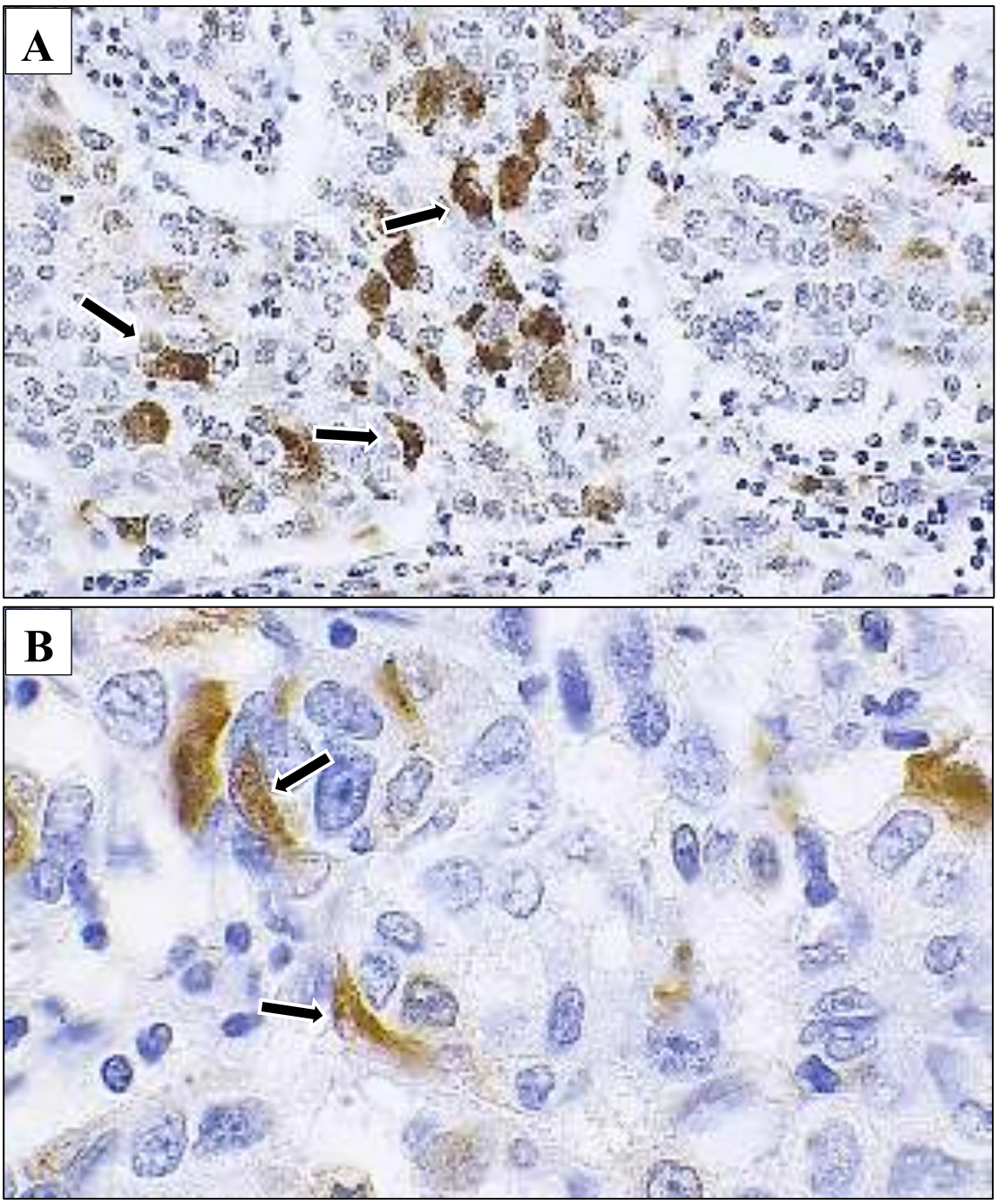
Appendix (D): Photomicrograph of breast cancer tissue.

The expression of anti-*T. gondii* primary antibody was observed in the affected cancerous tissue indicating the mature bradyzoite of *T. gondii* (black arrow) observed within neoplastic epithelial cells, near the blood vessels lumen and stroma of the affected cancerous tissue. Hematoxylin and DAB by IHC. A: 400X & B: 1000X.



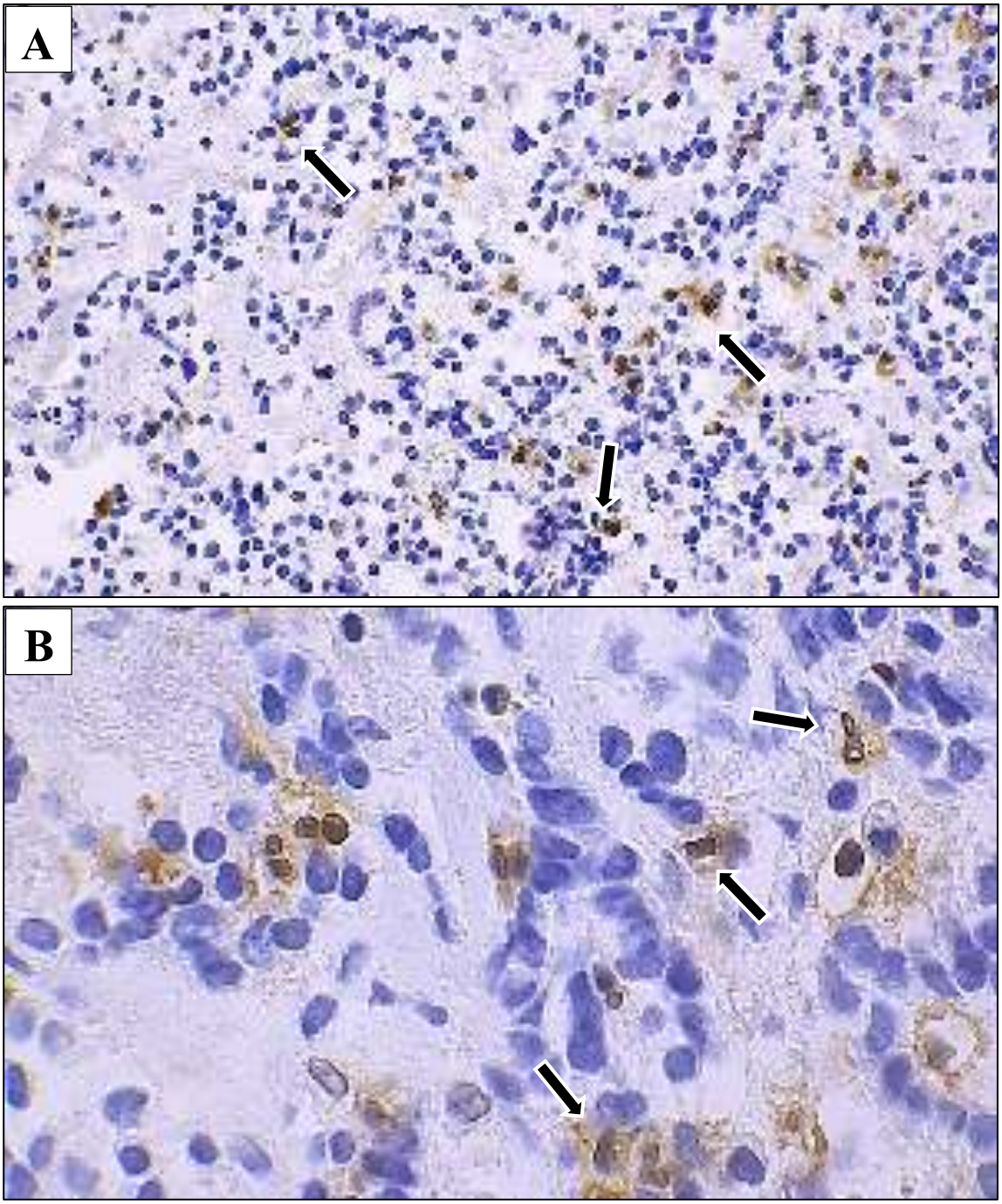
Appendix (E): Photomicrograph of breast cancer tissue.

The expression of anti-*T. gondii* primary antibody was observed in the affected cancerous tissue indicating the mature bradyzoite of *T. gondii* (black arrow) and immature bradyzoite cyst (yellow arrow) that was observed within the stromal tissue of the cancerous tissue. Hematoxylin and DAB by IHC. A: 400X & B: 1000X.



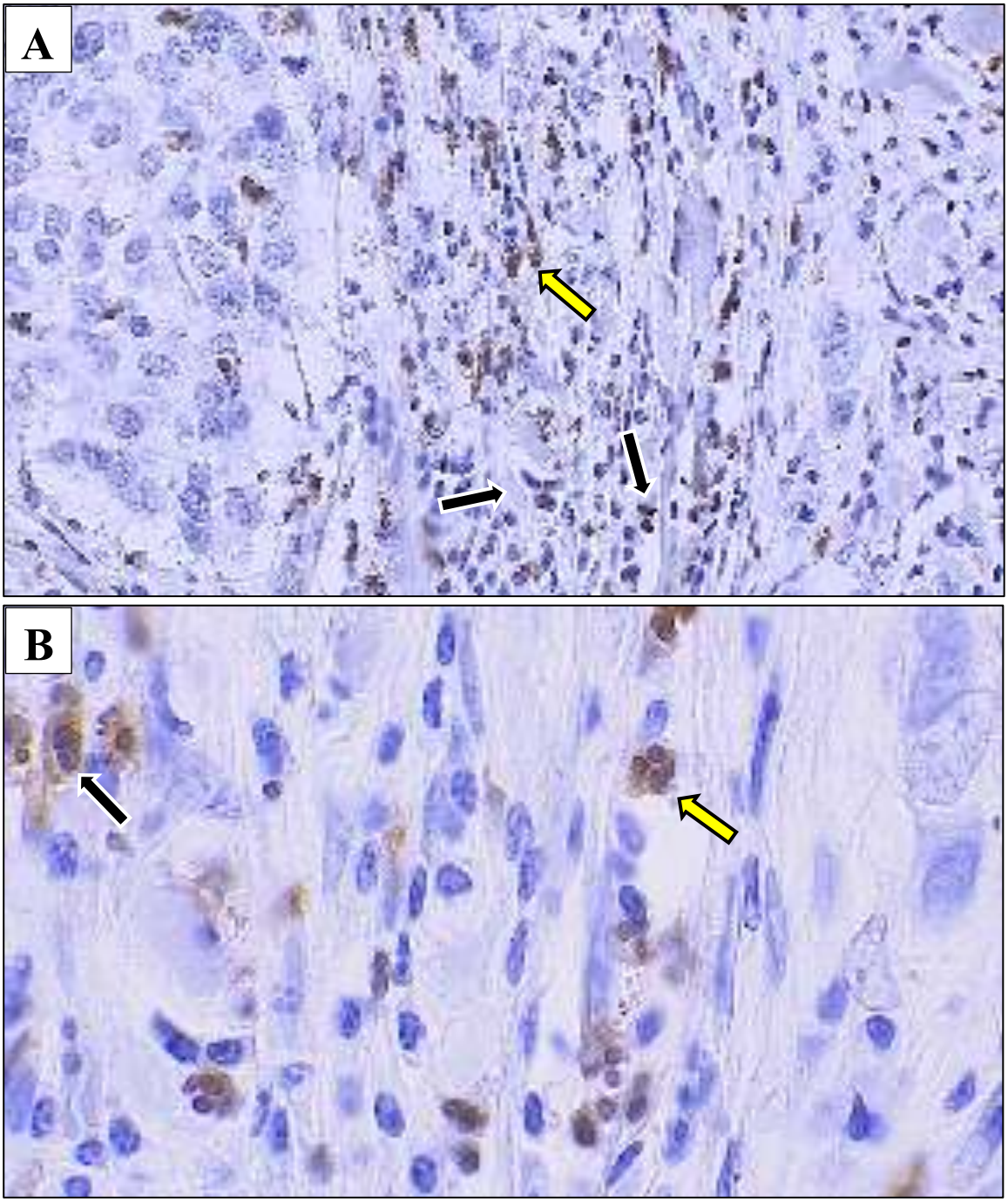
Appendix (F): Photomicrograph of breast cancer tissue.

The expression of anti-*T. gondii* primary antibody was observed in the affected cancerous tissue indicating the mature bradyzoite cysts of *T. gondii* (black arrow) that were observed within the neoplastic epithelial cells of cancerous tissue. Hematoxylin and DAB by IHC. A: 400X & B: 1000X.



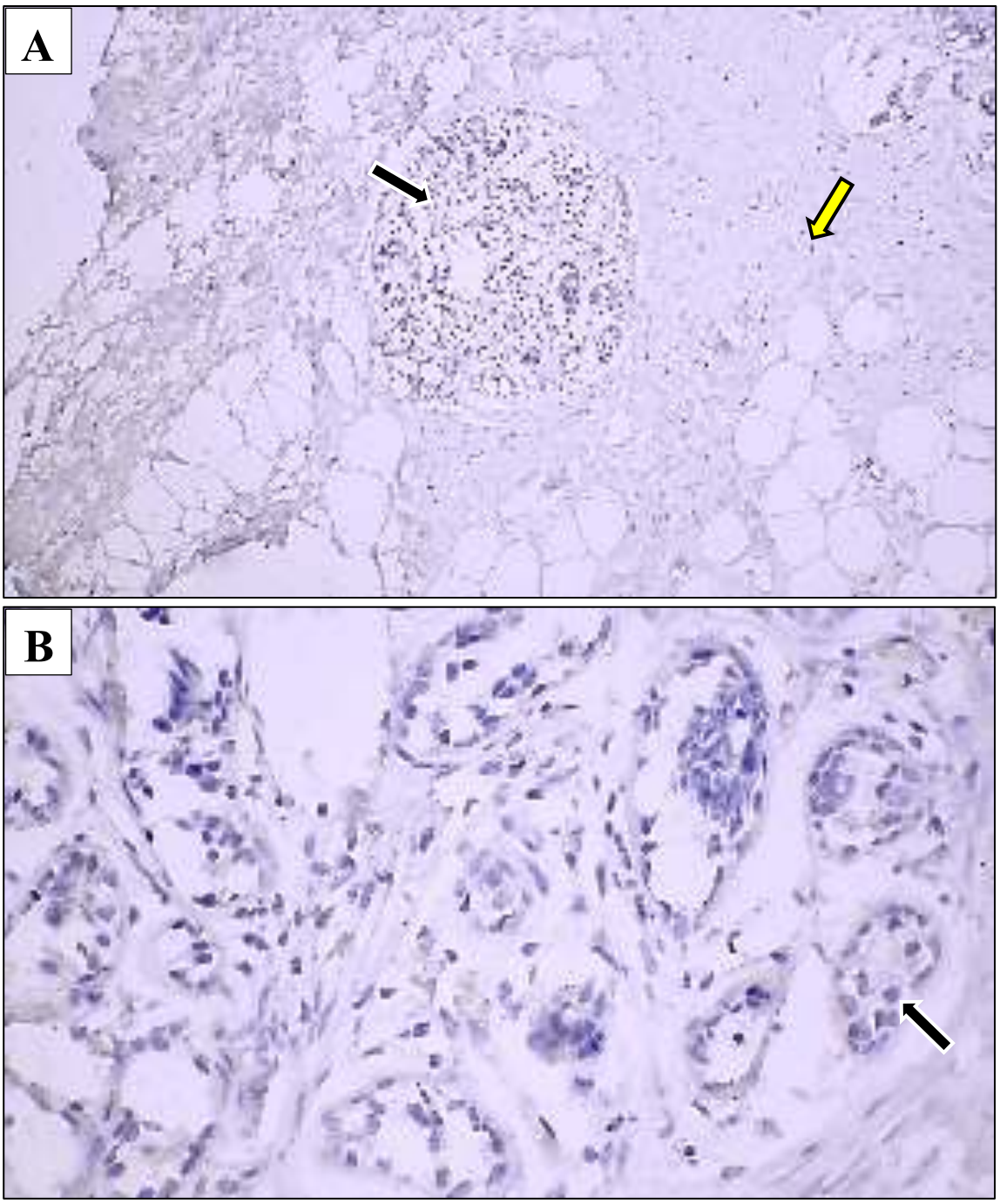
Appendix (G): Photomicrograph of breast cancer tissue.

The expression of anti-*T. gondii* primary antibody was observed in the affected cancerous tissue indicating the mature bradyzoite of *T. gondii* (black arrow) and that observed within neoplastic epithelial cells of the affected cancerous tissue. Hematoxylin and DAB by IHC. A: 400X and B: 1000X.



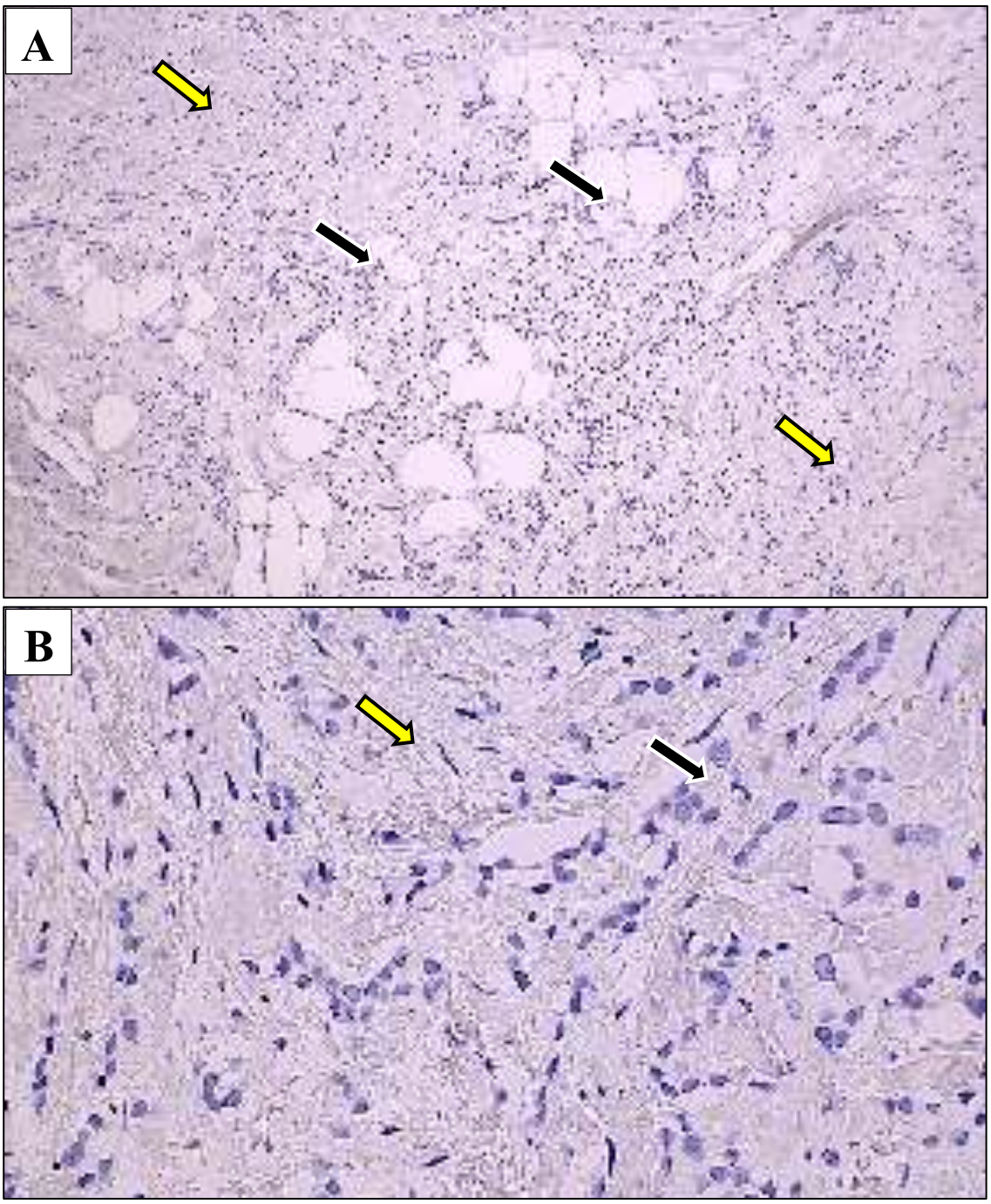
Appendix (H): Photomicrograph of breast cancer tissue.

The expression of anti-*T. gondii* primary antibody was observed in the affected cancerous tissue indicating the mature bradyzoite of *T. gondii* (black arrow) and immature bradyzoite cyst (yellow arrow) that were observed in the blood vessels lumen and stroma of the affected cancerous tissue. Hematoxylin and DAB by IHC. A: 400X & B: 1000X.



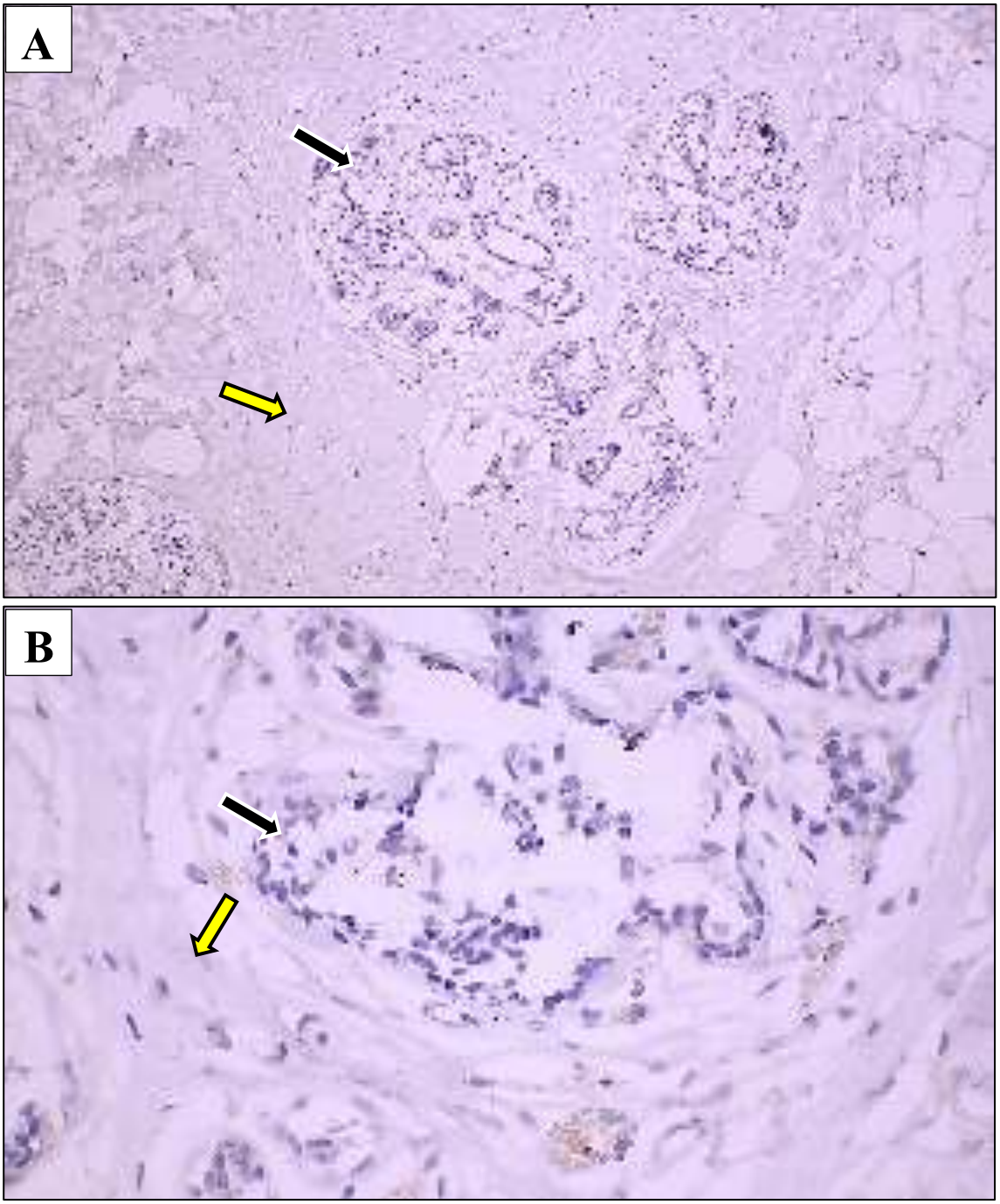
Appendix (I): Photomicrograph of benign breast tissue.

Note the negative expression of anti-*T. gondii* primary antibody, indicating the negative presence of *T. gondii* parasite or its cysts in both acinar glandular tissue (black arrow) or stromal tissue (yellow arrow) of the benign breast tissue. DAB and Hematoxylin by IHC. A: 100X & B: 400X.



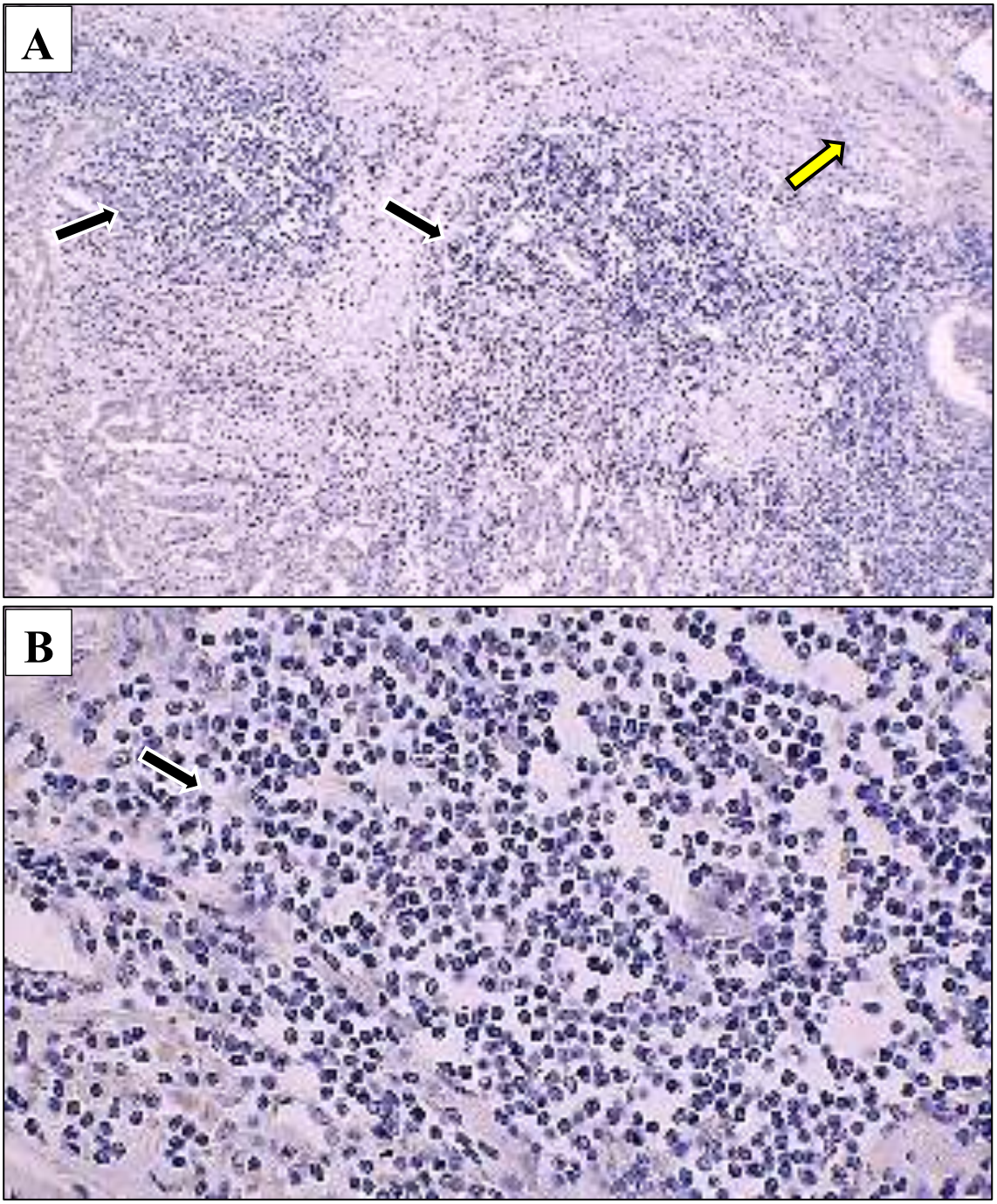
Appendix (J): Photomicrograph of breast cancer tissue.

Note the negative expression of anti-*T. gondii* primary antibody, indicating the negative presence of *T. gondii* parasite or its cysts in both neoplastic epithelial cell nests (black arrow) or stromal tissue (yellow arrow) of the cancerous breast tissue. DAB and Hematoxylin by IHC. A: 100X & B: 400X.



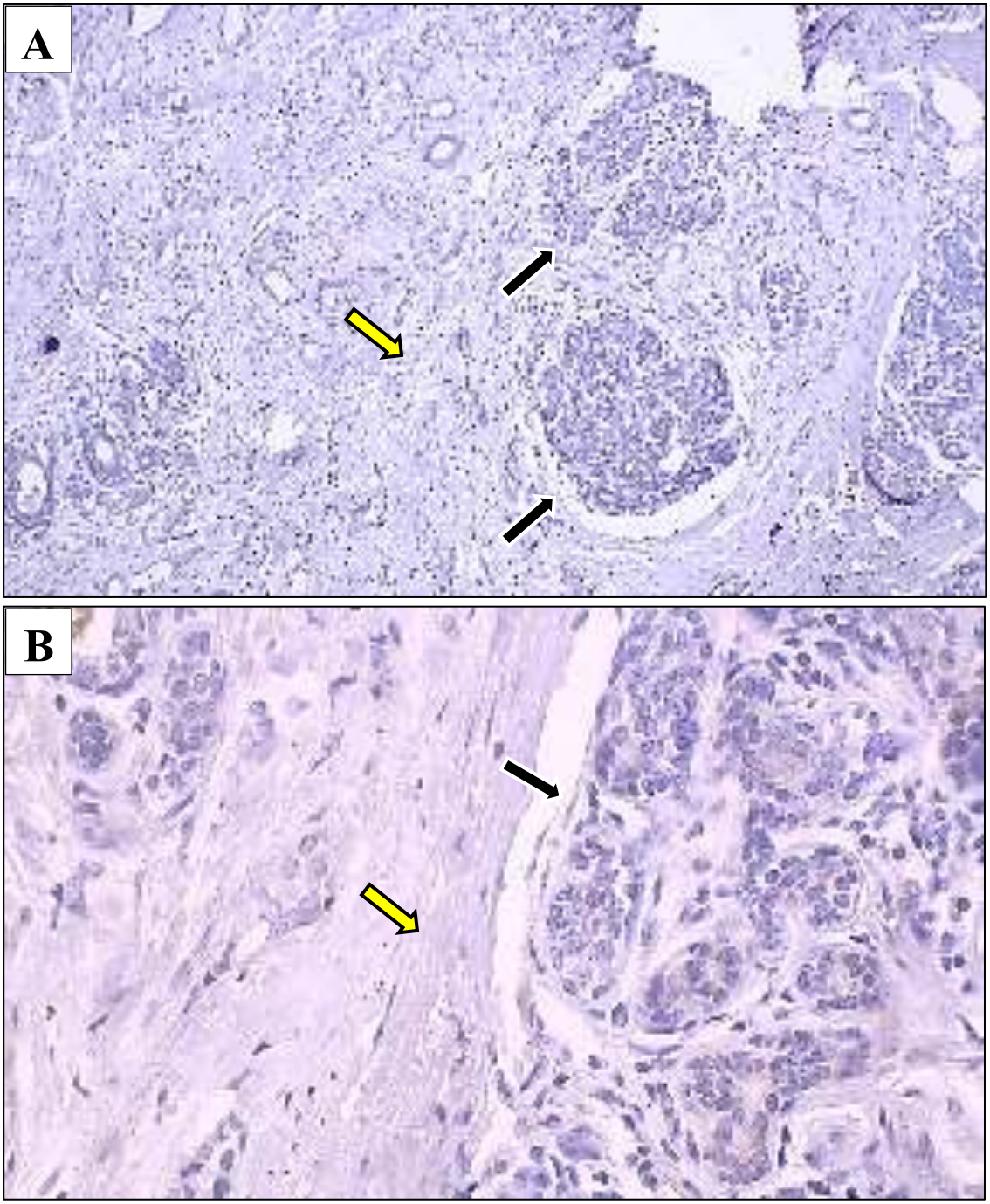
Appendix (K): Photomicrograph of benign breast tissue.

Note the negative expression of anti-*T. gondii* primary antibody, indicating the negative presence of *T. gondii* parasite or its cysts in both acinar glandular tissue (black arrow) or stromal tissue (yellow arrow) of the benign breast tissue. DAB & Hematoxylin by IHC. A: 100X & B: 400X.



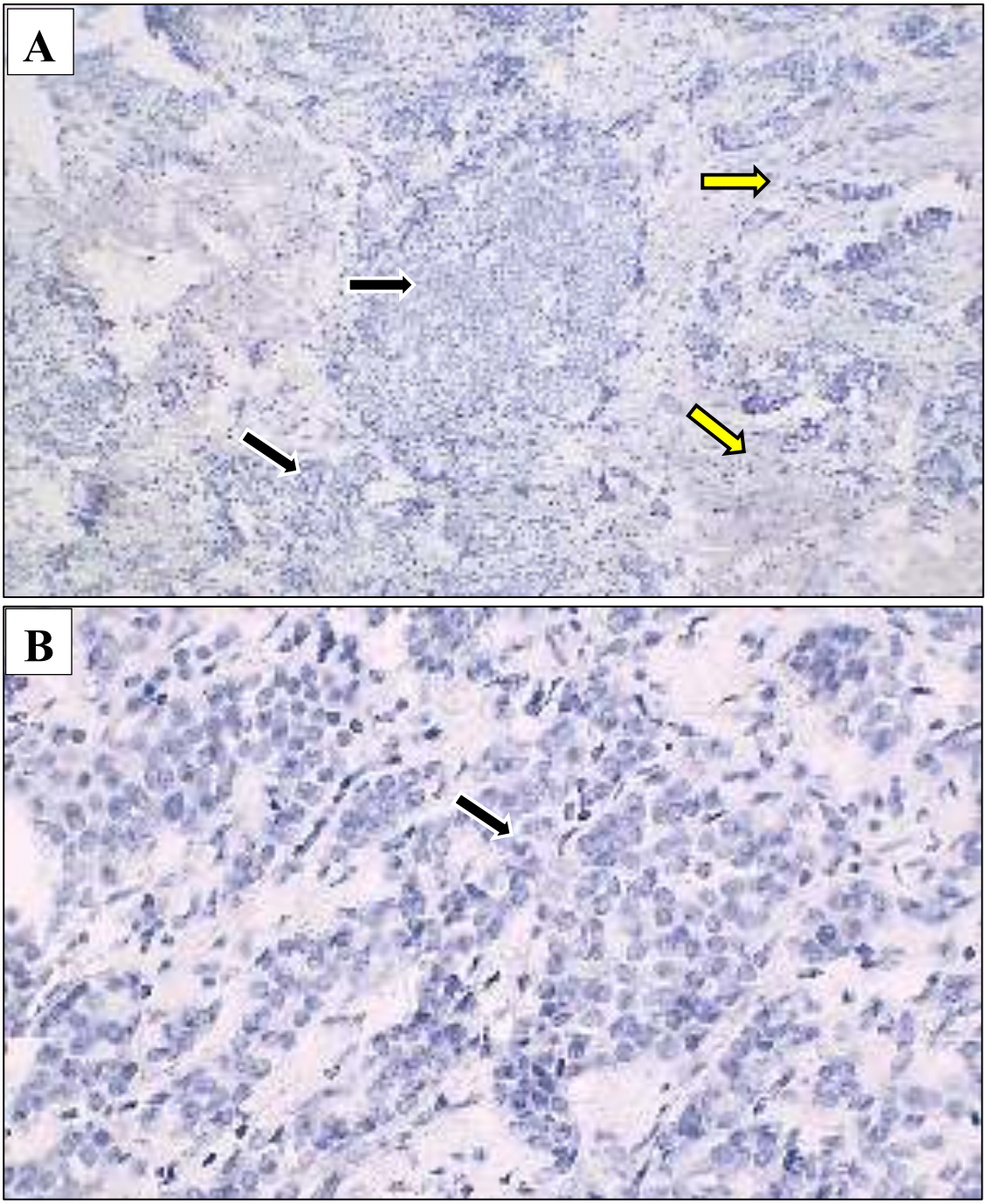
Appendix (L): Photomicrograph of breast cancer tissue.

Note the negative expression of anti-*T. gondii* primary antibody, indicating the negative presence of *T. gondii* parasite or its cysts in both neoplastic epithelial cell clusters (black arrow) or stromal tissue (yellow arrow) of the cancerous breast tissue. DAB and Hematoxylin by IHC. A: 100X & B: 400X.



Appendix (M): Photomicrograph of breast cancer tissue.

Note the negative expression of anti-*T. gondii* primary antibody, indicating the negative presence of *T. gondii* parasite or its cysts in both neoplastic epithelial cell nests (black arrow) or stromal tissue (yellow arrow) of the cancerous breast tissue. DAB and Hematoxylin by IHC. A: 100X & B: 400X.



Appendix (N): Photomicrograph of breast cancer tissue.

Note the negative expression of anti-*T. gondii* primary antibody, indicating the negative presence of *T. gondii* parasite or its cysts in both neoplastic epithelial cells nests (black arrow) or stromal tissue (yellow arrow) of the cancerous breast tissue. DAB and Hematoxylin by IHC. A: 100X & B: 400X.

ملخص:

بعض الطفيليات قد تكون عوامل مسرطِنة محتملة نظرًا لتأثيرها الكبير في صحة الإنسان. يُعَدّ *T. gondii* أحد أكثر الطفيليات داخل الخلايا إنتشارًا، حيث يُصيب أكثر من 30% من سكان العالم. ويُعَدّ الإنسان واحدًا من بين أكثر من 300مضيف وسيط لهذا الطفيلي، الذي يمتلك القدرة على إصابة جميع الخلايا المُنواة تقريبًا في الحيوانات ثابتة الحرارة.

يُعَدّ سرطان الثدي أكثر الأورام الخبيثة شيوعًا بين النساء عالميًا. وعلى الرغم من أنّ التغيرات الجينية المتأثرة بالعوامل البيئية والهرمونية تُعَدّ السبب الرئيسي في تَكَوُّنه، إلا أنّ احتمال مساهمة العدوى الطفيلية، مثل *T. gondii*، في إحداث أو تسريع هذه التغيرات الخلوية يستدعي دراسة معمّقة.

أُجريت هذه الدراسة خلال الفترة بين كانون الأول 2023 وأيار 2025 لإستكشاف العلاقة بين عدوى *T. gondii* وأورام الثدي لدى النساء باستخدام تقنيتين تشخيصيتين متكاملتين: علم الأنسجة المرضي (Histopathology) والكيمياء النسيجية المناعية (Immunohistochemistry). تم جمع 61 عينة من أنسجة الثدي من مختبر خاص تحت إشراف مختص. من بين هذه العينات، شُخّصت 46 عينة بسرطان الثدي، و 5 عينات بأورام ثدي حميدة، و10 عينات خالية من الأورام الخبيثة أو الحميدة.

أظهرت النتائج أنّ تقنية المناعة النسيجية الكيميائية كانت أكثر حساسية وموثوقية من علم الأنسجة المرضي في الكشف عن *T. gondii*. تسبّبت العدوى بـ *T. gondii* في حدوث أضرار نسيجية شديدة، بما في ذلك النخر والتدمير البنوي. وبلغ معدل إنتشار عدوى *T. gondii* نسبة 47.83% في أنسجة سرطان الثدي، و 20% في الأورام الحميدة، في حين لم تُسجَّل أي إصابة 0.00% في الأنسجة الخالية من الأورام.

كشفت التحليلات الإحصائية عن وجود إرتباط ذي دلالة إحصائية بين عدوى *T. gondii* وسرطان الثدي (χ^2=7.877, p=0.005)، بينما لم يُلاحظ إرتباط ذو دلالة إحصائية مع الأورام الحميدة (χ^2=2.143, p=0.143).

تُبرز هذه النتائج الدور المحتمل لعدوى *T. gondii* في نشوء سرطان الثدي، مع الأخذ في الإعتبار أنّ عوامل مثل نوع السرطان، وسلالة الطفيلي، وإستجابة الجهاز المناعي للمضيف قد تؤثر في هذه العلاقة. ويُوصى بإجراء المزيد من الدراسات لتوضيح الآليات الكامنة وراء إرتباط عدوى *T. gondii* بتكوّن سرطان الثدي.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ميسان

كلية العلوم

قسم علوم الحياة

العلاقة بين الإصابة بالمقوسة الغوندية وسرطان الثدي بين النساء

رسالة مقدمة

إلى مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قِبل الطالبة

إيلاف غالي غضيب الزيدي

بكالوريوس علوم الحياة / كلية العلوم / جامعة ميسان 2021

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