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Immunopathology, reproductive effects, and diagnostic challenges of *Toxoplasma gondii* infection in large animals: A narrative review

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Abstract

In large animals, *Toxoplasma gondii* infection is usually primarily a reproductive disease rather than an overt systemic illness. It is a significant cause of abortion, stillbirth, and neonatal weakness in domestic animals. This review incorporates much of the available information on the biology of the parasite and the host immune system, reproductive outcomes, and the clinical pathology of toxoplasmosis in large animals. Following consumption of sporulated oocysts, a persistent infection is initiated through the development of a tissue cyst, and a Th1-type of immunity is considered to constrain the systemic disease seen in the adult host. However, the gestational immunomodulation leads to a hospitable environment at the maternal-fetal interface, permitting vertical transmission. The reproductive disease is characterized by necrotizing placentitis, vascular damage, and fetal tissue damage, depending on the stage of gestation. There are usually minor and variable clinical pathologic alterations in adults. Therefore, blood testing has limited utility in diagnosing pathologic changes. Diagnosis is primarily based on testing of placental and fetal tissues by histopathology and molecular assays. Serological tests are mainly useful for detecting exposure, but they cannot distinguish prior infection from active reproductive disease. Improving mechanistic insight into maternal–fetal immune interactions alongside the development of reliable ante-mortem biomarkers and validated diagnostic tools is essential to better link immunopathology with clinically relevant outcomes in large animal toxoplasmosis.

Introduction

Toxoplasma gondii infection in ruminants is a prevalent but frequently overlooked parasitic infection (1, 2). Its widespread occurrence in sheep, goats, cattle, and camelids within several production systems is a consequence of oocyst contamination of the environment and the fecal-oral route of transmission (3, 4). Despite this broad distribution, systemic disease rarely occurs in adults, and infection generally persists as a subclinical, latent state in the tissues (5). Toxoplasmosis is primarily a veterinary concern in ruminants because of its effects on reproduction, rather than its effects on adult hosts (6-8). The occurrence of embryonic loss, abortion, stillbirth, or the birth of weak offspring due to transplacental infection in pregnant animals leads to significant economic losses in small ruminant production systems (9, 10). Sporadic or storm-like outbreaks of abortion are probably

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one of the most recognizable manifestations of infection at the herd level in both sheep and goats, whereas cattle and camelids tend to show more variable and less consistently documented reproductive outcomes (11-16).
The apparent contrast between widespread infection and the lack of apparent clinical signs in adults has led to an epidemiological and interpretative gap (17, 18). While the immune system may appropriately control systemic parasitic load in adults, the same immune pathways at the maternal-fetal interface fail to offer appropriate protection (19, 20). During gestation, subtle shifts in immune regulation can permit fetal infection and subsequent pathology (21). Consequently, reproductive failure occurs without readily identifiable hematological or biochemical abnormalities. Although routine clinical pathology tests have limited standalone diagnostic value, selected inflammatory and acute-phase markers may provide supportive information when interpreted alongside other diagnostic findings (22, 23).
Although seroprevalence and reproductive consequences of *T. gondii* infection in ruminants have been extensively described, most studies have focused on either reproductive performance or individual immunological or diagnostic components in isolation. This has limited the development of a coherent interpretation linking immune mechanisms with downstream clinicopathological and diagnostic manifestations across large animal species. In this context, the present review specifically emphasizes the relationships between maternal–fetal immune interactions, tissue-level pathology, and routinely applied laboratory findings, to improve the interpretation of diagnostic results within a unified biological framework.

Methods

This narrative literature review was carried out in May 2026 to summarize current knowledge about the immunopathology, reproductive consequences, clinicopathological changes, and diagnostic challenges of *T. gondii* infection in large animals. The literature search covered studies published between approximately 2005 and May 2026. Relevant articles were identified through searches of electronic databases including PubMed, Scopus, and Google Scholar using combinations of keywords such as *Toxoplasma gondii*, toxoplasmosis, ruminants, sheep, goats, cattle, camel, reproductive pathology, placentitis, clinical pathology, hematology, biochemistry, oxidative stress, immunology, and vertical transmission. Only peer-reviewed original research articles, review papers, and case reports written in English were included, while studies with insufficient methodological or diagnostic information were excluded. Special focus was given to articles that investigated maternal-fetal immune interactions and the interpretations of the results in terms of reproductive function and the interpretative value of hematological, biochemical, molecular, and pathological findings in naturally or experimentally infected animals. Selected articles were critically integrated to provide an integrated overview of the relationship between immune response, placental pathology, and clinical pathology findings in large animal toxoplasmosis. Additionally, due to limited species-specific data on certain immunological mechanisms, especially at the maternal–fetal interface, relevant findings from human studies were also consulted to complement and contextualize the interpretation of immune pathways.

Parasite Biology and Transmission in Ruminants

The primary route of infection with *T. gondii* in livestock is environmental; transmission to animals is most likely via ingestion of sporulated oocysts in contaminated feed, water, or pasture (24, 25). After ingestion of sporulated oocysts, the parasite multiplies in an asexual form, the tachyzoite, then circulates in the bloodstream, eventually establishing long-term persistence through the formation of tissue cysts in organs such as muscle and neural tissues (26, 27). This chronic phase is typically asymptomatic in adult animals, contributing to the covert maintenance of *T. gondii* infection within herds (28, 29).

The biological importance of infection to large animals, especially ruminants, lies not with generalized systemic parasitism, but rather in reproduction-related susceptible states (18, 30). During the period of maternal parasitemia, the tachyzoites display a marked tropism for the placenta, using the maternal-fetal interface to infect fetal circulation, accompanied by focal placental necrosis and disruption of trophoblastic integrity, leading to hematogenous fetal infection (20, 31, 32).

The final pathological findings depend on the stage of gestation at the time of maternal infection (33, 34). Early gestation is characterized by limited fetal immunocompetence, resulting in either embryonic loss or non-viable developmental arrest with minimal inflammatory reaction (35). In mid-gestation, partial immune maturation allows survival with pronounced necrotizing lesions in fetal organs. Late gestational infection more frequently results in congenital persistence with viable but infected offspring, reflecting a balance between partial immune containment and incomplete parasite clearance (36, 37). Therefore, the temporal immunological window is the principal factor responsible for differing lesion patterns and epidemiology of toxoplasmosis in larger animal species (28, 38).

Host Immune Response and Pregnancy-Associated Modulation

Following the systemic dissemination of *T. gondii*, the innate immunity of the host plays a central role in large animals. Infected macrophages and dendritic cells (DCs) are both the effector cells and antigen-presenting cells (39, 40). Early pattern-recognition receptor (PRR) stimulation can induce rapid cytokine responses, particularly through natural killer (NK) cell activation, mediated by IL-12 and early production of interferon (IFN)-γ, which can inhibit tachyzoite replication in the systemic spread (41, 42). Macrophage activation toward a classically activated (M1) type allows for enhanced intracellular killing of tachyzoites via nitric oxide (NO)-dependent and reactive oxygen species (ROS)-mediated processes, forming the first defensive barrier against systemic dissemination of the parasite (43-45).

The adaptive immune system is then polarized toward a robust Th1-driven response, IFN-γ serving as the central effector cytokine mediating parasite control (45-47). This axis is critical for maintaining chronic infection in a latent bradyzoite form through ongoing $CD4^+$ Th1 and $CD8^+$ T cell activity (48-50). However, this immunological defense structure is substantially modulated during gestation (51).

Pregnancy is accompanied by a tightly regulated immunological shift at the maternal-fetal interface, characterized by a relative down-regulation of cell-mediated immunity to prevent fetal rejection (51). This state is associated with partial recalibration of Th1/Th2 signaling profiles, amplification of regulatory T cell populations, and creation of local immunosuppressive microenvironments within the placenta (37, 52). Such immune adaptations are critical for the survival of the fetus, yet paradoxically establish a privileged site for intracellular pathogens such as *T. gondii* (17, 53).

The resulting immunological paradox lies in the coexistence of effective systemic parasite containment with localized immune insufficiency at the placental interface. Therefore, while peripheral IFN-γ- mediated responses may be intact, the local downregulation of pro-inflammatory signaling at the placental interface may be detrimental for parasite clearance (20, 54). Pregnancy immune dysregulation is not a systemic failure of immunity, but rather a localized response that ultimately allows for the manifestation of the reproductive pathology (21). This compartmental immune dissociation is the determinant of vertical transmission, permitting parasites to avoid the systemic immune response and invade the fetus (55).

Reproductive Pathology

The most important clinical manifestation of *T. gondii* infection in large animals is the reproductive pathology (56). Lesion distribution and degree are primarily dependent upon tropism for the placenta and the stage of fetal

development (20, 33). At the maternal-fetal interface, the parasite induces a necrotizing placentitis, characterized by focal to multifocal necrosis of the trophoblast with variable mononuclear inflammatory cell infiltration (20, 51, 57). The cotyledonary structures are typically associated with progressive loss of villous structure, resulting in impaired placental integrity and dysfunction (58). Vascular involvement, including endothelial damage and perivascular inflammation, impairs uteroplacental perfusion and, consequently, impairs the efficient transport of nutrients and oxygen to the fetus (20, 59).

Fetal involvement arises after hematogenous spread through the compromised placental barrier. Clinical outcomes may range from embryonic resorption, abortion, or stillbirth to the delivery of weak neonates (60, 61). Histopathologic analysis of the fetus reveals multifocal foci of necrosis and inflammation, typically within rapidly developing tissues, including the liver parenchyma, central nervous system, and myocardium (50, 51, 62). Neural involvement is particularly prominent, reflecting both the tropism of *T. gondii* and the immunological immaturity of fetal brain tissue (63, 64). The severity and distribution of lesions depend on the gestational stage of infection. Earlier exposure leads to more generalized developmental failure, while later infection more commonly results in localized necrotizing lesions in partially developed fetal organs (33).

Although large animals are often discussed collectively in the context of toxoplasmosis, important interspecies differences exist. Sheep and goats are considered the most susceptible livestock species with respect to reproductive toxoplasmosis and associated economic losses. In contrast, cattle generally appear more resistant to clinical disease, and the contribution of *T. gondii* to bovine reproductive failure remains less clearly established. Information regarding camelids is comparatively limited, and current knowledge is based on a relatively small number of epidemiological and clinical studies. These differences should be considered when interpreting immunological, pathological, and diagnostic findings across species (Table 1).

Linking Immune Response to Reproductive and Pathological Outcomes

The pathogenesis of reproductive toxoplasmosis in large animals appears to be a dynamic interaction between parasite replication and the maternal immune system at the maternal-fetal interface, where immunological control and immunopathology coexist (51, 65, 66). IFN-γ has a central role, acting as both a critical anti-parasitic cytokine and a potential driver of tissue injury when tightly regulated immune balance is disrupted (47, 67). Consistent IFN-γ signals, usually supplied from Th1 CD4+ T cells and NK cells, not only boost macrophage microbicidal activity but also suppress systemic tachyzoite spread (48). However, in the placental tissue, such signaling could potentially activate extreme immune responses, causing collateral damage to trophoblastic structures and endothelial cells (20, 48, 68).

Cytokine-mediated inflammation in the placenta appears as tissue-specific expression and up-regulation of inflammatory cytokines, namely TNF-α, IL-1β, and IFN-γ-induced chemokines, which facilitate the migration of mononuclear inflammatory cells into the placental stroma (51, 67, 69). This pro-inflammatory microenvironment disrupts the tightly regulated immunological equilibrium necessary to sustain pregnancy (20, 66). Maternal-fetal exchange of oxygen and nutrients is impaired by immune-mediated vascular injury of the placenta that involves endothelial activation and microvascular injury. Concurrent trophoblastic degeneration and necrosis reflect both direct parasite replication within placental cells and indirect immune-mediated cytotoxicity (36).

An important conceptual clarification is the distinction between parasite-mediated damage and immune-mediated pathology in the host (51). While *T. gondii* tachyzoites are capable of directly invading and destroying placental and fetal cells, a considerable proportion of tissue damage takes place secondary to the host's inflammatory response (37, 70). These include immune cell–mediated cytotoxicity, oxidative damage and its subsequent cellular damage, and disruption of the placental structure due to high levels of signaling cytokines.

In many cases, the magnitude of fetal compromise exceeds what would be expected from parasite burden alone, underscoring the dominant role of immunopathological mechanisms in determining outcome (51, 70).
Therefore, fetal loss associated with toxoplasmosis cannot solely be attributed to direct parasitic invasion. It is indeed a multi-faceted process, in which placental failure, vascular compromise, thrombosis, and the loss of maternal-fetal homeostasis through inflammation stand among the mechanisms responsible for embryonic death and abortion (51, 70). This mechanism is particularly evident in cases where relatively low parasite loads are associated with profound reproductive failure, indicating that immune-mediated processes are the major determinants of pathology (51, 71). The localized immune dysregulation transforms an otherwise controlled infection into a reproductive disease. Thus, Reproductive outcome relies on the interplay of immune protection and immune tolerance at the placenta (48, 71) (Figure 1).

Table 1. Species-specific differences in the epidemiology, reproductive impact, and available evidence of *Toxoplasma gondii* infection in large animals

Species	Susceptibility	Reproductive impact	Clinical significance	Strength of evidence
Sheep	High	Frequent abortion, stillbirth, weak offspring, congenital infection	Major cause of economic loss and reproductive failure	Extensive experimental and field evidence
Goats	High	Abortion, neonatal mortality, congenital infection	Important reproductive pathogen	Moderate to extensive evidence
Cattle	Relatively low	Sporadic abortion; causal association less consistent	Usually subclinical; reproductive significance remains controversial	Moderate evidence
Camelids	Unclear/variable	Occasional reproductive losses reported	Limited understanding of clinical impact	Limited evidence

Clinical Pathology and Laboratory Findings

Unlike the pronounced reproductive lesions found at the maternal-fetal interface, the clinical pathology features of toxoplasmosis in adult large animals are often subtle, non-specific, and lack definitive diagnostic specificity (13, 30, 72). In most cases of naturally infected ruminants and camelids, peripheral hematological values are normal due to the subclinical nature of systemic disease and the containment of tachyzoite multiplication by the host cell-mediated immune system (73, 74). When hematological alterations are present, abnormal parameters are often minimal and temporary; the typical changes involve a non-specific variable neutrophilia, with or without a left shift, and occasionally mild lymphocytosis or lymphopenia, depending on the phase of infection and host immune status (23, 73, 75, 76). However, these changes lack discriminatory value and are easily confounded by concurrent inflammatory or stress-related conditions common in production systems (76). Nevertheless, acute-phase proteins and inflammatory biomarkers may provide useful adjunctive information regarding host inflammatory responses and disease activity, although their diagnostic specificity for toxoplasmosis remains limited.

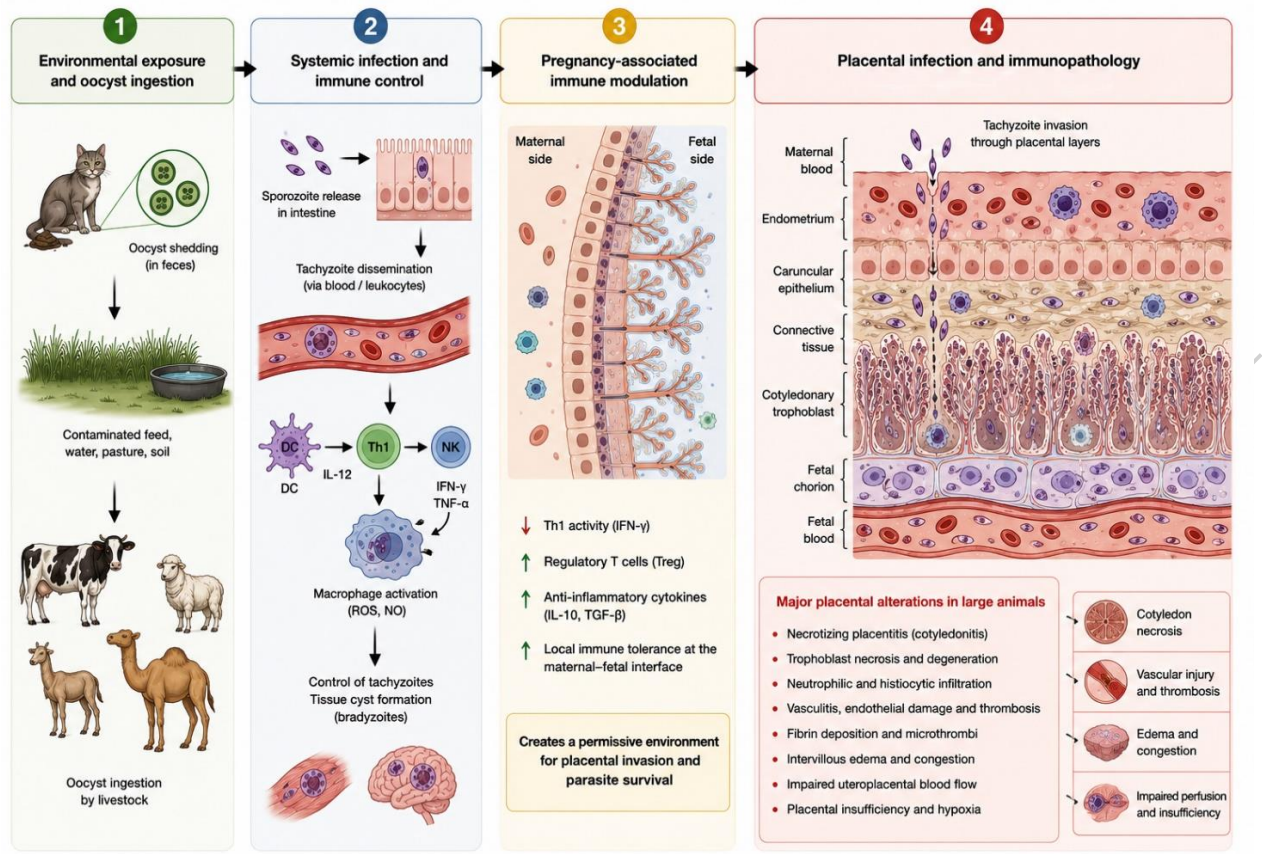

Fig. 1. Schematic summary of immunopathogenesis of *Toxoplasma gondii* infection in large animals. Following environmental exposure and ingestion of sporulated oocysts from contaminated feed, water, and pasture, sporozoites are released in the intestinal tract and transform into systemically spreading tachyzoites which can be disseminated via blood and leukocyte migration. In adult animals, Th1-mediated immunity initiated by dendritic cell (DC) activation and subsequent IL-12-dependent Natural killer (NK) cell activation and IFN-γ secretion is thought to control the systemic infection. When activated, macrophages inhibit intracellular parasite replication via reactive oxygen species (ROS) and nitric oxide (NO) production to limit tachyzoite development and promote the formation of persistent tissue cysts. Partial suppression of Th1 and enhancing regulatory immune signals in the restricted site of immune modulation within the maternal-fetal interface create a supportive placental microenvironment enabling parasite replication and transplacental transfer during pregnancy. Invading tachyzoites in the complex structure of the ruminant placenta resulted in inflammation and vascular damage, characterized by trophoblast degeneration, necrotizing placentitis, endothelial damage, thrombosis, edema and reduced uteroplacental blood flow. Combined parasitic and immune-induced damage result in placental dysfunction and fetal injury. *(Illustration generated using an AI-based tool (DALL·E) and compiled by the authors)*

Similarly, biochemical profile results have limited diagnostic value. In large animal toxoplasmosis, especially ruminants, serum biochemical alterations reflect a combination of hepatic injury, muscle involvement, and systemic inflammatory response induced by *T. gondii* (77). Among liver-associated enzymes, aspartate aminotransferase (AST) is a better indicator of tissue damage than the other liver enzymes, because it is not liver-specific and is also abundantly present in skeletal and cardiac muscle; thus, AST increase may reflect both hepatocellular injury and muscular damage resulting from parasite invasion or immune-mediated degeneration of myocytes (77-79). Alanine aminotransferase (ALT) alterations have also been reported in the cases of toxoplasmosis, but have little diagnostic value in large animals because ALT is not liver-specific and levels can

fluctuate greatly (73, 80-82). For these reasons, AST is the most relevant marker of hepatocellular leakage in toxoplasmosis. Muscle involvement in the host can be inferred from concurrent increases in creatine kinase (CK) and lactate dehydrogenase (LDH), indicating myofiber disruption (23, 73, 83).

Occasional increases in CK, LDH, and AST in large animal toxoplasmosis may reflect transient muscular and systemic inflammatory injury rather than primary hepatic disease (23, 77, 79). During acute tachyzoite dissemination, invasion of myocytes and localized inflammatory responses can induce mild myocellular damage, resulting in leakage of CK and AST into circulation. In addition, cytokine-mediated oxidative stress and macrophage-driven inflammatory mechanisms may contribute to secondary tissue injury (51, 71, 84). Because AST is also present in skeletal and cardiac muscle, mild elevations may occur in association with muscular involvement or fetal–placental inflammatory pathology rather than direct hepatocellular damage (77, 82, 85). In terms of plasma protein alterations, Toxoplasmosis can cause changes in acute-phase proteins. The response is clearly demonstrated in ruminants and camelids, in which a typical acute-phase reaction is observed (66). Increases in haptoglobin, serum amyloid A (SAA), and fibrinogen are commonly observed in the acute phase of infection in cattle and sheep, a phenomenon explained by IL-1-, IL-6-, and TNF-α- driven hepatic stimulation of acute-phase protein synthesis (86-90). In camels, increases of haptoglobin and fibrinogen were also shown to be the most significant positive acute-phase protein levels (66, 91). Alterations in acute-phase proteins occur as part of a systemic inflammatory reaction in which hepatic protein synthesis shifts away from constitutive proteins such as albumin toward acute-phase reactants, resulting in dysproteinemia and hypoalbuminemia in the infected hosts (86, 89, 92).

Oxidative stress is widely recognized as a significant component of host-parasite interactions and the pathogenesis of toxoplasmosis, reported in both experimental and clinical settings (84, 93). Infection with *T. gondii* is known to induce strong Th1-type immune responses that result in the activation of macrophages and overproduction of ROS and reactive nitrogen species (RNS), particularly through the stimulation of NADPH oxidase and induction of inducible nitric oxide synthase (iNOS) (94, 95). Although controlling parasite replication is advantageous, excessive and sustained production of ROS/RNS overwhelms the host's antioxidant defense system, causing oxidative stress that damages lipids, proteins, and nucleic acids (93). Accordingly, in the affected animal serum and tissues, the level of malondialdehyde (MDA), a major end-product of lipid peroxidation, was significantly elevated (96). At the same time, the activity of the main antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), was significantly decreased, and the reduced glutathione (GSH) and total antioxidant capacity (TAC) were depleted (93, 95, 96). Furthermore, the mitochondrial dysfunction at the cellular level leads to further acceleration of oxidative stress, since ROS production further amplifies oxidative stress by impairing electron transport chain function and promoting additional radical generation (71, 84) (Figure 2).

The dissociation between systemic immune control and localized pathology in the placenta means that peripheral blood-based diagnostics are fundamentally insufficient for appropriately defining the disease process in reproductive toxoplasmosis (97, 98). Reported hematological and biochemical alterations have not been entirely consistent across studies. While some investigations documented measurable changes in inflammatory and oxidative stress markers, others reported minimal or nonspecific alterations. These discrepancies highlight the limited diagnostic specificity of clinicopathological findings and emphasize the need for interpretation alongside serological, molecular, and pathological evidence. The lack of reproducible and consistent hematological and biochemical signatures indicates that systemic laboratory findings do not reliably reflect the pathological events at the maternal-fetal interface (23, 37).

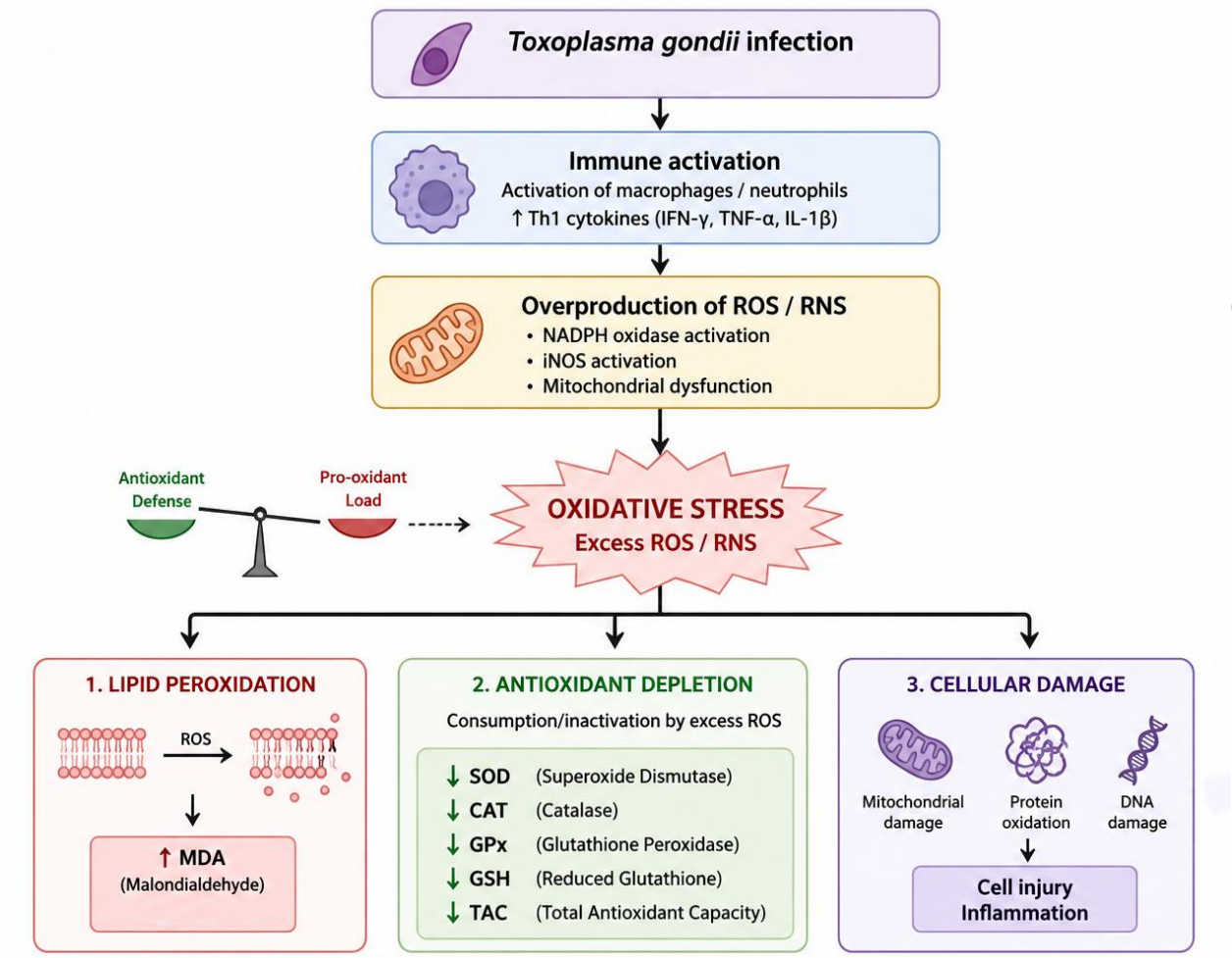

Fig. 2. Mechanisms underlying oxidative stress and oxidative biomarker alterations during *T. gondii* infection. Infection with *T. gondii* activates macrophages and neutrophils and stimulates a Th1-type inflammatory response characterized by increased cytokine production, including IFN-γ, TNF-α, and IL-1β. Subsequent activation of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), inducible nitric oxide synthase (iNOS), and mitochondrial dysfunction promotes excessive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to oxidative stress. Increased oxidative burden induces lipid peroxidation, reflected by elevated malondialdehyde (MDA), while antioxidant defense mechanisms become depleted, resulting in reduced superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), reduced glutathione (GSH), and total antioxidant capacity (TAC). Oxidative damage to mitochondria, proteins, and DNA ultimately contributes to cellular injury, inflammation, and dysfunction of affected tissues. *(Illustration generated using an AI-based tool (DALL·E) and compiled by the authors)*

The clinicopathological signs of large animal toxoplasmosis are generally not definitive, although they may provide indirect evidence of the inflammatory and damage responses exerted on tissues in acute and reproductive infections (97). Their greatest value lies in supporting clinical interpretation, prioritizing differential diagnoses, and complementing pathological and molecular findings during reproductive investigations (99, 100).

Definitive diagnosis is therefore dependent on the direct examination of the placental and fetal tissues, with far more diagnostic specificity in terms of parasite identification and description of lesions compared to evaluation of blood and body fluids (101, 102). Confirmatory diagnosis is based on the combination of histopathology and

molecular tests, such as polymerase chain reaction (PCR), using placental cotyledons or fetal organs as substrates (1, 102, 103). This tissue-based diagnostic approach reveals an underlying pitfall in current clinical pathology methodologies and further underscores the necessity for a united diagnostic protocol that prioritizes reproductive tissues over peripheral blood analysis in suspected cases of toxoplasmosis in large animals.

Diagnostic and Interpretative Challenges

In the diagnostic work-up of abortion associated with *T. gondii* in large animals, a structured differential diagnostic approach is essential due to overlapping clinical and pathological presentations with other abortifacient agents. Important differentials include *Neospora caninum*, *Brucella* spp., *Chlamydia abortus*, *Coxiella burnetii*, and *Campylobacter* spp., all of which may induce abortion and placental lesions of varying severity (104). Differentiation is primarily based on a combination of pathogen-specific serology and molecular assays, supported by distinct histopathological patterns (105).

The diagnosis of reproductive toxoplasmosis in large animals is fundamentally limited by the dissociation between exposure to infection via the immune system and localized pathology on the placental-fetal aspect (106, 107). Serology tests, including enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFAT), have a wide application for diagnosis on the herd level, but limited interpretative value, as IgG titers generally represent only prior exposure and do not represent active infection or reproductive disease (1, 108, 109, 110). Even in cases of recent seroconversion, serology alone cannot reliably differentiate between transient infection, chronic tissue cyst carriage, or infection temporally associated with gestational loss (106, 109, 111). IgM-based assays are theoretically indicative of recent infection but are often inconsistent in sensitivity and may fluctuate or persist unpredictably, further complicating clinical interpretation (112, 113).

Molecular diagnostics, namely PCR detection of parasitic DNA, can achieve a much greater level of specificity but are highly dependent on sample type and sampling time (1, 114). Placental cotyledons and fetal organs such as the brain and liver provide the highest diagnostic yield due to preferential parasite localization (101, 115, 116). However, the uneven distribution of organisms and post-mortem degradation of the fetal tissues mean that results can still be falsely negative (97, 117). Blood-based PCR assays in adult animals are generally of limited value due to transient parasitemia and rapid immune clearance of circulating tachyzoites (118-121).

No single diagnostic method is sufficient in all clinical settings, and the selection of an appropriate diagnostic approach should be based on the stage of infection, sample type, and diagnostic objective. Combining serological, molecular, and pathological findings often provides the most reliable assessment (Table 2).

A significant, unresolved limitation in clinical pathology is the lack of robust, reproducible blood-based biomarkers that correlate with the expression of reproductive toxoplasmosis. While experimental studies have identified potential inflammatory mediators and acute-phase proteins associated with infection, further validation is required before they can be routinely applied as diagnostic tools in field conditions (88, 90, 122). Consequently, laboratory findings in peripheral blood provide only a partial representation of the pathological processes occurring at the maternal–fetal interface. Nevertheless, selected inflammatory mediators, acute phase proteins, and oxidative stress biomarkers may serve as valuable adjunctive indicators of host responses when interpreted in combination with serological, molecular, and pathological findings (30, 31, 66). Such diagnostic constraints pose major hazards of interpretation at the herd level. Thus, seropositive animals may be incorrectly classified as causally associated with abortion events, while seronegative but recently infected individuals may be undetected (5, 123). This diagnostic ambiguity is particularly problematic in screening programs aimed at identifying sources of reproductive loss, where reliance on single-modality testing can lead to erroneous epidemiological conclusions (106, 124). Hence, integrated use of serological screening, molecular identification and histopathological examinations of the placenta and fetal tissues is essential for definitive diagnosis (22, 125,

126). Through the convergence of these complementary diagnostic modalities, the true relationship between infection, immune response, and reproductive pathology could be established in large animal toxoplasmosis.

Table 2. Comparison of commonly used serological and molecular diagnostic methods for *Toxoplasma gondii* infection

Method	Target	Advantages	Limitations	Typical application
ELISA	Anti-*T. gondii* antibodies	High throughput, cost-effective, suitable for herd screening	Cannot reliably distinguish active from past infection	Epidemiological surveys and screening
IFAT	Anti-*T. gondii* antibodies	Good sensitivity and specificity	Interpretation may vary with cut-off values and infection stage	Confirmation of exposure
IgM-based serology	Recent antibody response	May indicate recent exposure	Persistence and false-positive reactions may complicate interpretation	Supportive evidence of recent infection
Conventional PCR	Parasite DNA	Direct detection of infection	Lower sensitivity in samples with low parasite burden	Tissue and abortion investigations
Real-time PCR	Parasite DNA	High sensitivity, specificity and quantification capability	Requires specialized equipment and expertise	Confirmation of infection in tissues and fetal samples
Histopathology	Tissue lesions and parasite structures	Provides pathological context	Limited sensitivity when the parasite burden is low	Investigation of abortion cases

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IFAT, indirect fluorescent antibody test; IgM, immunoglobulin M.

Conclusion and Future Directions

In large animal species, toxoplasmosis must primarily be considered a reproductive disease with pathogenesis determined by the complex interplay between parasite persistence and the host's immune-mediated injuries at the maternal-fetal interface. Failure of immune regulation at the site underlies the characteristic clinical signs, including embryonic death, abortion, and congenital infection. A key unresolved feature of this infection is the notable dissociation between the host's general immunological competence and the reproductive outcome. In general, adult hosts mount effective, Th1-polarized responses that control peripheral parasite infections and maintain chronic infection in a latent state. This control is systemic and does not confer protection on the placenta and fetus, where localized immune modulation can still result in parasite establishment and inflammation.

There is currently no existing laboratory tool available for accurate case attribution for reproductive failure from a diagnostic perspective. Serological tests identify previous exposure but do not demonstrate causality, while definitive molecular and histopathological diagnosis is limited to post-abortion tissues. The absence of blood-based biomarkers sensitive to active placental infection or fetal involvement, and their validation, continues to limit ante-mortem diagnosis and herd-level decision-making. Sensitive and specific ante-mortem diagnostic assays that distinguish between active infection, prior exposure and reactivation are needed in the field. Standard serological tests are often unreliable for differentiating stages of infection, so they should preferably be supplemented with antigen-detection methods, more sensitive molecular assays in tissue or blood, and possibly

host-response biomarkers. Standardized assay platforms across multiple large-animal species are needed to improve comparability and diagnostic accuracy under field conditions.

Future progress should move beyond descriptive epidemiology to a mechanistic integration of immunology and reproductive pathology, focusing on improving mechanistic understanding of *T. gondii* infection at the maternal–fetal interface through advanced molecular approaches. Priority areas include the immunological signatures of placental infection, the creation of composite host-pathogen biomarker arrays and characterization of immune regulation at the maternal-fetal interface during gestation in large domestic animals. In particular, placental transcriptomic and proteomic studies, together with cytokine and immune profiling, may help clarify host–pathogen interactions underlying reproductive failure. The integration of multi-omics approaches is also expected to facilitate the identification of reliable biomarkers associated with infection severity and pregnancy outcome. Furthermore, refined experimental models could be established to elucidate how systemic immune control is locally subverted within reproductive tissues. Such advances are essential for translating immunopathological insights into clinically actionable diagnostic frameworks for large animal toxoplasmosis.

Finally, ongoing research into the development and field evaluation of effective vaccine candidates targeting *T. gondii* in ruminants and other large-animal species is required to minimize vertical transmission and improve pregnancy and subsequent economic outcomes.

Abbreviations

AST = Aspartate Aminotransferase
ALT = Alanine Aminotransferase
CAT = Catalase
$CD4^+$ = Cluster of Differentiation 4 Positive T Lymphocyte
$CD8^+$ = Cluster of Differentiation 8 Positive T Lymphocyte
CK = Creatine Kinase
DC = Dendritic Cell
ELISA = Enzyme-linked Immunosorbent Assay;
GPX = Glutathione Peroxidase
GSH = Reduced Glutathione
IFAT = Indirect Fluorescent Antibody Test
IFN-γ = Interferon Gamma
IgG = Immunoglobulin G
IgM = Immunoglobulin M
IL-1β = Interleukin 1 Beta
IL-12 = Interleukin 12
iNOS = Inducible Nitric Oxide Synthase
LDH = Lactate Dehydrogenase
M1 = Classically Activated Macrophage Phenotype
MDA = Malondialdehyde
NADPH = Nicotinamide Adenine Dinucleotide Phosphate
NK = Natural Killer Cell
NO = Nitric Oxide
PCR = Polymerase Chain Reaction
PRR = Pattern Recognition Receptor
RNS = Reactive Nitrogen Species

ROS = Reactive Oxygen Species
SAA = Serum Amyloid A
SOD = Superoxide Dismutase
TAC = Total Antioxidant Capacity
Th1 = T Helper 1
Th2 = T Helper 2
TNF-α = Tumor Necrosis Factor Alpha

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Conflict of Interest
The authors declare no conflict of interest.

Ethical Approval
All procedures used in this study were approved by the Shiraz University, School of Veterinary Medicine, ethical committee and were compatible with Directive 2010/63/EU on the protection of animals used for scientific purposes.

Artificial Intelligence Statement
The authors did not use the assistance of any Artificial Intelligence Generated Content (AIGC) tools to develop any portion of the manuscript. Figures were created by the authors using original designs and layouts, with licensed graphical elements obtained from *DALL.E*.

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