Review

The Neospora Paradox: Why Does the "Silent" Infection in Buffaloes Hold the Key to Beating Abortion Storms in Cattle?

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The Neospora Paradox: Why Does the "Silent" Infection in Buffaloes Hold the Key to Beating Abortion Storms in Cattle?

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Abstract

Neospora *caninum* is a master of reproductive disruption, causing devastating abortion storms in cattle and inflicting annual billion-dollar losses on the global livestock industry. Yet, in water buffaloes (*Bubalus bubalis*)—a phylogenetically close relative often raised in the same environments—the same parasite often takes on a different role: a silently persisting infection with significantly lower rates of clinical abortion. This review inverts the traditional narrative. Instead of focusing solely on the susceptible host (cattle), we argue that the key to unlocking next-generation control strategies lies in understanding the resistant host (buffalo). By dissecting this "*Neospora* paradox," we explore the cutting-edge molecular and immunological crosstalk that dictates pregnancy outcomes. We journey from the parasite's sophisticated arsenal of invasion proteins, revealed by CRISPR-Cas9 screens, to the maternal–fetal interface, where the battle between immune tolerance and parasite control is won or lost. We further examine the intriguing relationship between *N. caninum* and its similar *Toxoplasma gondii*, revealing how differential host immune recognition determines infection outcomes. Ultimately, we propose that deciphering the buffalo's successful equilibrium with *N. caninum* could illuminate novel pathways for vaccines and immunotherapeutic strategies, transforming the management of neosporosis worldwide.

Keywords: *Neospora caninum*; cattle; buffalo; abortion; vaccine; *Toxoplasma gondii*

1. Introduction: Setting the Scene with a Paradox

1.1. The Known Crisis

Neosporosis stands as the most frequently diagnosed cause of bovine abortion worldwide, representing a formidable challenge to global livestock productivity [1]. The economic toll is staggering—conservative estimates place annual losses to the global cattle industry at approximately $1.3 billion, stemming from direct abortion losses, premature culling of infected animals, reduced milk production, and diminished genetic progress [2]. In endemic regions, seroprevalence rates in dairy herds can exceed 80%, with abortion storms capable of eliminating 10–30% of a season's calf crop within weeks [3].

Picture a dairy farmer walking through barns once filled with the promise of a new generation, now confronting empty stalls and the hollow echo of economic devastation—this is the reality of neosporosis in cattle operations globally.

1.2. Introduce the Enigmatic Host

Now contrast this grim picture with the water buffalo (*Bubalus bubalis*). Despite sharing pastures, management systems, and geographic regions with cattle across Asia, the Mediterranean, and parts

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of South America, buffaloes present a fundamentally different clinical picture. Extensive seroepidemiologic surveys reveal that buffaloes are frequently exposed to *N. caninum*—indeed, seroprevalence rates often equal or exceed those in co-located cattle populations. A comprehensive review of studies from Iran reported seroprevalence ranging from 19.2% to 55.9% in water buffaloes [4,5]. Similar findings emerge from Italy [6], Brazil [7], and Egypt [8]. Yet, despite this high exposure, the associated reproductive pathology is remarkably muted. Clinical abortion outbreaks in buffalo herds are rare, and when they occur, *N. caninum* is seldom identified as the primary etiological agent [9].

This presents us with the central paradox: why does the same parasite produce devastating disease in one closely related ruminant species while establishing a seemingly benign coexistence in another? Is the buffalo merely more resistant, or has it struck a fundamentally different bargain with the parasite—one characterized by equilibrium rather than pathology?

1.3. Thesis Statement

This review argues that comparative biology—contrasting the susceptible bovine host with the resistant buffalo host—represents the most powerful, and currently underutilized, tool to unravel the complex pathophysiology of neosporosis and forge new paths for intervention. By treating the buffalo not as an afterthought but as the central subject of inquiry, we can identify the immunological and molecular circuit breakers that prevent disease and translate these discoveries into actionable control strategies for cattle.

2. The Usual Suspects: A Brief Primer on the Parasite and Its Traditional Hosts

2.1. The Life Cycle

Neospora caninum is an obligate intracellular apicomplexan parasite with a heteroxenous life cycle requiring both definitive and intermediate hosts [10]. Canids—domestic dogs (*Canis lupus familiaris*), coyotes (*Canis latrans*), and gray wolves (*Canis lupus*)—serve as definitive hosts, shedding environmentally resistant oocysts in their feces [1,11,12]. Intermediate hosts, primarily cattle and other ruminants, become infected through ingestion of sporulated oocysts contaminating feed or water.

Within the intermediate host, the parasite undergoes a biphasic developmental program. Following ingestion, sporozoites excyst in the intestine, differentiate into rapidly dividing tachyzoites, and disseminate via the bloodstream throughout the host [13]. This acute phase is characterized by widespread parasite proliferation and active invasion of nucleated cells. In immunocompetent hosts, the immune response—particularly interferon-gamma (IFN-γ)-mediated mechanisms—drives tachyzoite conversion into slowly dividing bradyzoites, which form tissue cysts predominantly within neural and muscular tissues [14]. These latent cysts persist for the lifetime of the host, representing a reservoir for potential recrudescence and a vehicle for vertical transmission.

2.2. Transmission Dynamics

Neospora caninum employs two transmission strategies, each with distinct epidemiological implications. Horizontal transmission occurs when susceptible animals ingest oocysts shed by definitive hosts [15]. While this route maintains environmental contamination and introduces infection into naïve herds, its efficiency is limited by several factors: oocyst shedding by dogs is often transient, environmental oocyst survival is variable, and the infectious dose required to establish infection remains poorly characterized [16].

Vertical (transplacental) transmission represents the parasite's evolutionary masterstroke for population persistence [17]. This route occurs in two contexts. **Exogenous transplacental transmission** results from primary maternal infection acquired during pregnancy, wherein tachyzoites cross the placenta and infect the fetus [17]. **Endogenous transplacental transmission**

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arises from recrudescence of a chronic infection—pregnancy-associated immunomodulation triggers bradyzoite reactivation to tachyzoites, which then cross the placenta [18]. This endogenous route ensures perpetuation of infection across generations, with infected heifers giving birth to infected calves that maintain the parasite within the herd indefinitely. Indeed, vertical transmission efficiency can approach 95% in persistently infected cows (primarily via endogenous transmission), making neosporosis a quintessential example of a vertically maintained infection [19].

2.3. The Clinical Picture in Cattle

The pathological consequences of *N. caninum* infection in cattle are most dramatically manifested during gestation. Abortion typically occurs between 4 and 7 months of gestation, with the peak incidence at 5–6 months [20]. The pathogenesis involves multifocal placentitis with necrosis of cotyledonary villi, followed by fetal infection and systemic disease. Affected fetuses exhibit nonsuppurative encephalitis characterized by perivascular cuffing, multifocal gliosis, and necrosis—often with detectable tissue cysts in the brain [21]. Myocarditis, hepatitis, and myositis are also commonly observed [1].

The immunopathological basis of abortion lies in the delicate balance required for successful pregnancy. The maternal–fetal interface represents an immunological paradox: the semi-allogeneic fetus must be protected from maternal immune rejection while maintaining the capacity to combat pathogens. This is achieved through a Th2-biased immunological environment, characterized by anti-inflammatory cytokines including IL-4, IL-5, and IL-10 [22]. However, *N. caninum* is an intracellular pathogen requiring Th1-type immunity—particularly IFN-γ—for control [23]. The pregnancy-induced shift away from Th1 responses creates a permissive environment for tachyzoite proliferation and transplacental invasion. In essence, the very mechanisms that protect the fetus create vulnerability to the parasite.

3. The "Neospora Paradox" Deep Dive: Cattle vs. Buffalo

3.1. Epidemiological Evidence

The contrasting outcomes of *N. caninum* infection in cattle and buffaloes are well documented across diverse geographic settings. A meta-analysis of global seroprevalence studies confirms that while exposure rates are comparable between the two species, abortion risk diverges dramatically [24]. In Iran, a survey of 236 water buffaloes from one province revealed an overall seroprevalence of 17.7% using a commercial ELISA yet follow-up investigations failed to establish *N. caninum* as a significant cause of buffalo abortion [25]. Similarly, in southern Italy—a region with intensive buffalo dairy production—Guarino et al. [6] reported 29.8% seropositivity in 1547 buffalo samples, but retrospective analysis of abortion cases over a 5-year period identified *N. caninum* in less than 2% of fetal examinations.

The pattern extends to South America. In Brazil, where both cattle and buffaloes are extensively raised, seroprevalence in buffaloes ranges from 22.7% to 65.5% across different states [7,26]. Yet, worldwide, seroprevalence of *N. caninum* infection in buffaloes is high, at approximately 48%—three to four times higher than that reported from the world's cattle populations (16.1% for dairy cattle and 11.5% for beef cattle)—despite clinical manifestations such as abortion being rare in buffaloes [24]. Indeed, while *N. caninum* is considered one of the major causes of abortion in cattle, the reproductive importance of neosporosis is apparently lower in buffaloes relative to cattle [27]. These contrasting patterns are not attributable to differences in management, definitive host exposure, or environmental factors—cattle and buffaloes in these studies shared pastures, water sources, and often the same farm premises.

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3.2. Beyond Seroprevalence: What Is Happening at the Placental Level?

Experimental infection studies have provided crucial insights into the mechanistic basis of the buffalo's relative resistance. Konrad et al. [28] experimentally infected pregnant buffalo heifers with *N. caninum* tachyzoites and monitored outcomes through gestation. Despite confirmed seroconversion and detection of parasite DNA in maternal blood, only 2 of 12 fetuses showed histological lesions consistent with neosporosis—and these were mild compared to the severe encephalitis typical of bovine infections. Importantly, parasite transmission to the fetuses occurred (6 of 12 fetuses were PCR-positive), but this transmission was not associated with fetal death or significant pathology.

This dissociation between parasite transmission and disease is the critical observation. In cattle, vertical transmission and fetal pathology are tightly coupled—transmission virtually guarantees the development of lesions and substantially elevates abortion risk [17]. In buffaloes, transmission can occur without triggering the inflammatory cascade that proves fatal to the fetus. This points to fundamental differences at the maternal–fetal interface—either in placental structure, local immune regulation, or the fetal inflammatory response to parasite invasion.

Ueno et al. [29] provided histological evidence supporting this hypothesis. Comparative examination of placentomes from naturally infected cattle and buffaloes revealed striking differences. Bovine placentomes exhibited extensive necrosis, mononuclear inflammatory infiltration, and edema—lesions that compromise fetal nutrient and gas exchange. Buffalo placentomes, by contrast, showed minimal inflammatory changes despite the presence of parasite DNA in adjacent tissues. The architecture of the placentome remained largely intact, suggesting that the buffalo placenta either restricts parasite invasion or mounts a regulated inflammatory response that limits collateral tissue damage.

3.3. A Question of Co-Evolution

These observations raise a compelling evolutionary hypothesis. Has *N. caninum* enjoyed a longer co-evolutionary history with *Bubalus bubalis*, leading to a more balanced host-parasite relationship? Or have buffaloes evolved unique immunological "circuit breakers" that prevent the uncontrolled tachyzoite proliferation and inflammatory pathology seen in cattle?

The water buffalo's evolutionary trajectory diverged from that of taurine cattle approximately 3–5 million years ago [30]. While both species originated in Asia, buffaloes remained confined to the Indian subcontinent and Southeast Asia until their relatively recent introduction to Europe, Africa, and the Americas [31]. *N. caninum* likely co-evolved with canid definitive hosts and wild ruminant intermediate hosts long before domestication [32]. If buffaloes served as ancient intermediate hosts in regions where the parasite was endemic, natural selection would have favored alleles conferring reduced pathology while maintaining transmission competence. This "domestication" of the parasite—the evolution of tolerance rather than resistance—would manifest as the silent infections observed today.

Cattle, by contrast, may represent a more recent host. Their expansion into parasite-endemic regions during human-mediated migrations may have exposed them to *N. caninum* without the benefit of millennia of co-adaptive evolution. The result is a maladapted host-parasite relationship characterized by excessive inflammation, tissue destruction, and reproductive failure. This evolutionary framework predicts that the buffalo's "success" lies not in superior parasite clearance—indeed, they remain infected—but in regulatory mechanisms that confine the parasite and limit immunopathology.

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4. Deconstructing the Dialogue: Molecular Mechanisms and Host–Pathogen Interactions

4.1. The Parasite's Toolkit

Understanding the buffalo's protective mechanisms requires appreciation of the parasite's sophisticated invasion machinery. *N. caninum* shares with other apicomplexans a specialized set of apical organelles—micronemes, rhoptries, and dense granules—with sequentially discharge their contents during host cell invasion [33].

Microneme proteins (MICs) initiate contact with the host cell. These adhesins, including NcMIC1, NcMIC3, and NcMIC6, mediate parasite attachment to host cell surface receptors and are essential for gliding motility [34]. Structural studies reveal that NcMIC1 contains microneme adhesive repeat (MAR) domains that bind sialic acid residues on host cell surfaces, a mechanism conserved with *T. gondii* as shown for the homologous *T. gondii* proteins [35]. Antibodies targeting MICs can inhibit invasion *in vitro*, highlighting their vulnerability as vaccine targets [36].

Rhoptry proteins (ROPs) are discharged following microneme-mediated attachment and play dual roles in invasion and modulation of host cell functions. ROP proteins are injected into the host cell cytoplasm, where they localize to the parasitophorous vacuole membrane (PVM) or traffic to the host cell nucleus [37]. NcROP40, a pseudokinase localized to the rhoptry bulbs, is more abundantly expressed in virulent isolates and has been implicated as a potential virulence factor involved in the manipulation of innate immune defense mechanisms [38,39]. NcROP16 traffics to the nucleus and modulates host gene expression, potentially creating a more permissive intracellular environment [40]. These secreted effectors represent the parasite's tools for subverting host defenses—understanding their differential activity in cattle versus buffaloes may reveal species-specific vulnerabilities.

Dense granule proteins (GRAs) are secreted throughout intracellular development and contribute to modification of the parasitophorous vacuole. NcGRA6 and NcGRA7 localize to the PVM and vacuolar lumen, where they participate in nutrient acquisition and immune evasion [41]. NcGRA7 is particularly immunogenic and has been exploited for diagnostic applications [42]. Notably, GRA proteins from *N. caninum* and *T. gondii* exhibit only 40–60% amino acid identity, suggesting species-specific adaptations to host environments.

4.2. CRISPR-Cas9 and Virulence

The application of CRISPR-Cas9 gene editing to apicomplexan parasites has revolutionized our understanding of *N. caninum* virulence factors. Research has applied CRISPR-Cas9 to dissect the function of specific *N. caninum* genes. For instance, NcROP5 has been identified as a key virulence factor, as its deletion significantly attenuates virulence in a murine model [43]. Similarly, the generation of knockout mutants for NcGRA7 and NcROP40 has revealed their involvement in parasite virulence, with NcGRA7 deletion considerably impairing pathogenicity in pregnant mice [44]. The application of CRISPR-Cas9 in *N. caninum* has been further advanced by the development of TaqMan-quantitative PCR assays to reliably assess mutagenesis efficiency [45].

Furthermore, using this approach, Yang et al. [46] identified the dense granule protein NcGRA17 as an important regulator of parasitophorous vacuole (PV) morphology and pathogenicity, with knockout strains exhibiting aberrant PV formation and loss of virulence. Similarly, Du et al. [47] generated NcMYR1 knockout strains, demonstrating that NcMYR1 is a virulence factor critical for processes such as invasion and replication. These tools now enable direct testing of whether polymorphisms or differential expression of such effectors contribute to species-specific outcomes. For example, one could compare the transcriptomes of *N. caninum* isolated from cattle versus buffaloes to investigate if strains adapt to their host species through selection of particular effector alleles, or conversely, if host factors in buffaloes render these parasite effectors less effective.

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4.3. The Host's Response: A Tale of Two Cytokines

The host immune response to *N. caninum* represents a double-edged sword, particularly during pregnancy. The **Th1/Th2 paradigm** provides the conceptual framework: successful pregnancy requires a Th2-biased uterine environment dominated by IL-4, IL-5, and IL-10, which suppress cell-mediated immunity and promote maternal-fetal tolerance [48]. However, control of intracellular pathogens like *N. caninum* depends on Th1 immunity, particularly IFN-γ production by NK cells and CD4+ T lymphocytes [49]. IFN-γ activates macrophages to kill intracellular parasites, induces nitric oxide production, and promotes Th1 polarization.

This immunological conflict creates vulnerability. During mid-gestation—coinciding with peak *N. caninum* abortion risk—placental IFN-γ expression is naturally downregulated to protect the fetus [18]. This creates a window of opportunity for tachyzoite recrudescence and transplacental invasion. Experimental studies confirm that neutralization of IFN-γ in pregnant cattle dramatically increases vertical transmission and fetal pathology [50]. Conversely, excessive IFN-γ production at the placenta can itself be embryotoxic, highlighting the narrow therapeutic window.

Recrudescence represents the practical manifestation of this immunological conflict. Latent bradyzoite cysts persist within neural tissues, continuously monitored by the immune system. Pregnancy-induced immunomodulation—particularly reduced IFN-γ bioavailability—can trigger bradyzoite-to-tachyzoite conversion [51]. Released tachyzoites then disseminate, reaching the placenta and fetus. This endogenous transplacental transmission explains how chronically infected cows can abort multiple times or produce infected offspring across successive pregnancies.

4.3.1. Epigenetic Regulation of Stage Conversion

Beyond cytokine signaling, epigenetic mechanisms are increasingly recognized as key regulators of the bradyzoite-tachyzoite switch. In *T. gondii*, histone acetyltransferases (e.g., GCN5) and deacetylases (e.g., HDAC3) control the expression of stage-specific genes, and similar chromatin-modifying machinery is conserved in *N. caninum* [52]. Host-mediated epigenetic modifications—such as DNA methylation of parasite promoters or changes in host cell chromatin accessibility during pregnancy—could influence recrudescence efficiency. Comparative epigenetic profiling of placentomes from infected cattle versus buffaloes might reveal species-specific differences in histone marks or DNA methylation patterns that explain why bradyzoite reactivation leads to severe pathology in one host but not the other. This unexplored avenue offers promising opportunities for future research.

4.4. The Buffalo Difference: Hypotheses

The buffalo's resistance to *N. caninum*-induced abortion likely reflects modifications to this immunological balancing act. Several non-mutually exclusive hypotheses warrant investigation.

Hypothesis 1. ***Placentomal structural differences.*** *The buffalo placenta exhibits distinct morphological features compared to cattle, including non-stalked placentomes with long, slender, and moderately branched villi, in contrast to the stalked, mushroom-shaped placentomes with broad, conical, and complexly branched villi observed in cattle [53]. Additionally, the fetal vasculature of the water buffalo placentome undergoes progressive development throughout pregnancy, with conical villous trees changing from wide to slender shapes as gestation advances [54]. These structural differences might physically impede tachyzoite invasion or restrict dissemination within placental tissues. The observation of intact buffalo placentome architecture despite parasite presence [29] supports this mechanical barrier hypothesis.*

Hypothesis 2. ***Enhanced local immune regulation.*** *Buffaloes may possess more abundant or functionally enhanced regulatory T cell (Treg) populations at the maternal–fetal interface. Tregs suppress effector T cell responses through IL-10 and TGF-β production, potentially limiting both anti-parasite immunity and inflammatory pathology [55]. If buffalo decidual Tregs are more numerous or functionally superior, they might*

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restrict parasite-induced inflammation while maintaining sufficient parasite control to prevent an overwhelming infection.

Hypothesis 3. ***More stringent control of parasite recrudescence.*** *The balance between bradyzoite latency and their reactivation is influenced by host immune status and parasite-intrinsic factors. Buffaloes might maintain higher baseline IFN-γ levels in neural tissues where cysts reside, preventing recrudescence. Alternatively, buffalo bradyzoites might exhibit intrinsically lower reactivation rates due to host-imposed epigenetic modifications.*

Hypothesis 4. ***Distinct cytokine response profiles.*** *Comparative transcriptomic analyses could reveal species-specific differences in the kinetics or magnitude of cytokine responses. Perhaps buffaloes mount rapid, localized IFN-γ responses sufficient to control tachyzoites without systemic Th1 polarization that threatens pregnancy. This "Goldilocks" response—defined as an immune state that is neither too weak (allowing uncontrolled parasite growth) nor too strong (causing immunopathology)—would represent the ideal equilibrium.*

Hypothesis 5. ***Genetic polymorphisms in innate immune receptors.*** *Toll-like receptors (TLRs) and MHC class I/II molecules exhibit species-specific polymorphisms that shape pathogen recognition and antigen presentation. Genome-wide association studies (GWAS) comparing infected cattle and buffaloes might identify buffalo alleles associated with protection. Candidate genes include TLR2 and TLR4, which recognize parasite glycosylphosphatidylinositol anchors [56], and MHC class II genes that determine immunodominant epitope presentation.*

5. New Horizons in Diagnostics and Control: Learning from the Resistant Host

5.1. Diagnostics

Accurate diagnosis of *N. caninum* infection underpins both clinical management and epidemiological surveillance. Traditional serological methods—indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assays (ELISA)—remain widely used but face limitations including cross-reactivity with *T. gondii* and variable performance across laboratories [57].

Recent advances focus on recombinant and chimeric antigens to improve specificity. NcSRS2, the immunodominant surface antigen, forms the basis of several commercial ELISAs and shows 95–98% sensitivity in cattle [58]. NcSAG1 and NcGRA7 have been incorporated into multi-antigen platforms to enhance detection of both acute and chronic infections [42]. Chimeric antigens—engineered proteins combining multiple immunogenic epitopes—offer promise. Using a genome-scale immunoinformatics approach, Pereira et al. [59] designed a novel chimeric antigen and evaluated it in an ELISA format. The assay demonstrated a sensitivity of 96.6% (±3.4) and a specificity of 97.0% (±2.9) when tested against a panel of sera from cattle naturally infected with *N. caninum* and healthy controls, with minimal cross-reactivity against other abortive pathogens. Point-of-care immunochromatographic tests (ICTs) now enable rapid field diagnosis without laboratory infrastructure. Du et al. [60] developed an ICT using recombinant NcSAG1 that detects anti-*N. caninum* antibodies in 15 minutes with 94.3% sensitivity and 97.6% specificity compared to IFAT. Such tools facilitate on-farm screening and timely intervention during abortion outbreaks.

5.2. The Vaccine Conundrum

The quest for an effective *N. caninum* vaccine has spanned three decades with limited success. Traditional approaches—killed whole parasites, live-attenuated strains, and subunit vaccines—have each encountered obstacles [61].

Killed vaccines (e.g., Bovilis® Neoguard) showed initial promise in reducing abortion risk but provided incomplete protection and required frequent boosting [62].

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Live-attenuated vaccines generated through prolonged *in vitro* passage induce stronger cellular immunity but raise safety concerns regarding reversion to virulence and residual pathogenicity in immunocompromised animals [63]. The Nc-Nowra strain, isolated from a naturally infected calf, showed reduced virulence and protected against homologous challenge in sheep but has not progressed to commercial development [64].

Subunit vaccines targeting key antigens—NcSRS2, NcGRA7, NcMIC proteins—have been evaluated with variable results. Immunization with recombinant NcSRS2 adjuvanted with Quil A reduced cerebral parasite burden in mice but failed to prevent vertical transmission in cattle [65]. The major challenge lies in inducing durable Th1 immunity at mucosal and placental surfaces—precisely where protection is needed.

5.3. Therapeutic Prospects

Pharmacological intervention for neosporosis remains limited, particularly in food animals where tissue residue concerns restrict drug options. Repurposed compounds with *in vitro* activity against *N. caninum* include:

- **Monensin**, an ionophore antibiotic widely used as a ruminant feed additive, inhibits N. caninum proliferation in cell culture at micromolar concentrations. Feeding monensin to pregnant cattle reduced vertical transmission risk by 50–75% in some studies but showed inconsistent results in others [66]. The mechanism likely involves disruption of parasite intracellular ion homeostasis but achieving effective concentrations at placental and fetal tissues remains challenging.
- **Pyrimethamine and sulfadiazine**, synergistic inhibitors of folate metabolism, effectively control acute toxoplasmosis in humans and have demonstrated activity against N. caninum in vitro [67]. However, their use in cattle is precluded by long withdrawal times for milk and meat, and concerns about teratogenicity during pregnancy.
- **Toltrazuril**, a triazine derivative used for coccidiosis control, reduces N. caninum replication in cell culture and showed partial efficacy in experimentally infected calves [68]. However, its high cost and regulatory status limit practical application.

Recently, **niclosamide** has emerged as a promising therapeutic candidate against *N. caninum* infection, acting through NLRP3 inflammasome activation and direct antiparasitic effects that reduce invasion, intracellular proliferation, and mitochondrial function in tachyzoites, while improving survival and reducing tissue damage in infected mice [69]. This drug has yet to be evaluated in cattle and buffalo.

5.4. The Innovative Idea: "Buffalo-Derived" Biomarkers

The central innovation of this review lies in proposing that the resistant buffalo host can guide identification of protective biomarkers and therapeutic targets. Rather than continuing the empirical screening of vaccine candidates and drugs in susceptible cattle, we advocate for a reverse translational approach: decipher the mechanisms of natural resistance in buffaloes, then engineer those mechanisms into cattle.

Cellular signatures represent one promising avenue. Comparative analysis of immune cell populations in infected cattle versus buffaloes might reveal protective cell subsets enriched in the resistant host. For example, regulatory T cells (Tregs) expressing high levels of Forkhead Box P3 (FoxP3) proteins and Cytotoxic T lymphocyte antigen-4 (CTLA-4) might be more abundant in buffalo decidua, limiting inflammatory damage while permitting parasite control [70]. Alternatively, buffaloes might harbor unique dendritic cell subsets with superior capacity to present parasite antigens without inducing pathogenic Th1 responses.

Genetic markers identified through genome-wide association studies (GWAS) could pinpoint protective alleles. Advances in buffalo genomics—including the recent publication of the water buffalo reference genome [71]—now enable large-scale association studies. Candidate genes warranting investigation include:

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- **Toll-like receptors (TLR1, TLR2, TLR4, TLR9):** These pattern recognition receptors initiate innate immune responses to parasite ligands. Polymorphisms affecting ligand binding or signaling efficiency could shape infection outcomes.
- **MHC class I and II genes:** These highly polymorphic loci determine which parasite epitopes are presented to T lymphocytes. Certain MHC haplotypes might favor recognition of conserved epitopes that elicit protective rather than pathogenic responses.
- **Cytokine genes (IFNG, IL10, TGFB1):** Polymorphisms in regulatory regions could alter cytokine production kinetics, influencing the Th1/Th2 balance.
- **Genes encoding structural placental proteins:** Variants affecting trophoblast integrity or interhaemal barrier function might physically impede parasite transmigration.

The "Goldilocks" immune response—the conceptual goal—would be an immune state characterized by: (1) rapid recognition of invading tachyzoites through pattern recognition receptors; (2) controlled IFN-γ production sufficient to activate parasite killing mechanisms; (3) localized immune responses restricted to sites of infection without systemic Th1 polarization; (4) effective regulatory mechanisms (IL-10, TGF-β, Tregs) to limit inflammatory damage; and (5) memory responses that accelerate control upon re-exposure. If buffaloes naturally achieve this Goldilocks state, then identifying the molecular and cellular components that enable it could revolutionize neosporosis control.

6. Beyond Ruminants: The Broader Implications of the Neospora–Toxoplasma Connection

The central comparison between cattle and buffalo can be expanded to explore the parasite's host range on a much wider scale, with *T. gondii* serving as the ultimate "control" organism. This comparative lens reveals fundamental principles governing host–pathogen compatibility and immune recognition.

6.1. The Zoonotic Enigma: Does N. caninum Infect Humans?

The question of whether *N. caninum* infects humans carries profound public health implications and has been investigated for over two decades.

The serological puzzle: Numerous studies have reported antibodies reactive with *N. caninum* antigens in human sera, suggesting possible exposure. A systematic review compiling data from 1998–2009 found seropositivity rates ranging from 0.9% in healthy blood donors to as high as 38% in HIV-positive patients [1,72]. These findings generated speculation about a possible zoonotic role for *N. caninum* and raised concerns about its potential to cause human neurological disease.

The cross-reactivity problem: The central technical challenge lies in the close phylogenetic relationship between *N. caninum* and *T. gondii*. These parasites share extensive antigenic similarity, and most serological tests cannot reliably distinguish antibodies generated against one parasite from those generated against the other [73]. Antibodies against *T. gondii*—which infects an estimated one-third of the global human population—can cross-react with *N. caninum* antigens in ELISAs and IFATs, producing false-positive results.

A definitive study from Spain addressed this limitation using a two-step approach. Fernández-Escobar et al. [74] analyzed 600 human clinical samples from patients with suspected toxoplasmosis (lymphadenopathy, chorioretinitis, encephalitis) who had tested negative for *T. gondii*. Samples were analyzed by *N. caninum*-specific PCR targeting the Nc5 region and by peptide-based serology using antigens selected for minimal cross-reactivity. No *N. caninum* DNA was detected in any sample, and only 2 of 600 samples showed borderline seroreactivity that could not be confirmed by Western blot.

The current consensus: Research from the Center for Diseases Control (CDC) and other reference laboratories has established that **there is currently no confirmed evidence of *N. caninum* infection in humans** [73,74]. Despite its ability to infect a wide range of animal species

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experimentally—including non-human primates, in which transplacental transmission has been demonstrated [75]—*N. caninum* is not considered a zoonotic agent. This starkly contrasts with *T. gondii*, underscoring the remarkable, and still mysterious, host specificity of *N. caninum*.

6.2. A Tale of Two Parasites: Genetic Kin with Opposite Strategies

Comparing *N. caninum* to its well-known relative *T. gondii* provides a powerful framework for understanding its unique biology. Table 1 summarizes key differences.

Table 1. Comparative features of *Neospora caninum* and *Toxoplasma gondii*.

Feature	*Neospora caninum*	*Toxoplasma gondii*
Definitive hosts	*Canids (dogs, wolves, coyotes) [11,12]*	*Felids (cats) [76]*
Intermediate hosts	*Narrow range: primarily ruminants (cattle, buffalo, sheep) and canids [1]*	*Extremely broad: virtually all warm-blooded animals, including humans [76]*
Human infection	*No confirmed cases; not considered zoonotic [73,74]*	*Yes; major zoonotic pathogen infecting ~1/3 of global population [77]*
Murine model	*Mice are highly resistant; infection rapidly controlled [80]*	*Mice are natural intermediate host; infection can be lethal [78]*
Vertical transmission	*Highly efficient; primary route of persistence in herds [17]*	*Less efficient in immunocompetent hosts; more important in primary infection [79]*
Clinical outcome in pregnancy	*Abortion storms in cattle; minimal disease in buffaloes*	*Congenital toxoplasmosis: chorioretinitis, hydrocephalus, intracranial calcifications [81]*

6.2.1. Human Infection as a Point of Contrast

Does host range define parasite success? The comparison between *N. caninum* and *T. gondii* becomes particularly informative when extended to human infection, where *T. gondii* represents one of the most successful zoonotic apicomplexan parasites. Unlike *N. caninum*, which has negligible zoonotic potential despite extensive investigation [73,74], *T. gondii* infects a wide range of intermediate hosts, including humans, in whom it typically establishes life-long chronic infection [76,78].

Human infection reveals key differences: In immunocompetent individuals, infection is usually asymptomatic, with parasite persistence maintained through the formation of intracellular bradyzoite cysts within neural and muscular tissues. However, clinically significant diseases arise in two key contexts: congenital infection following primary maternal infection and reactivation during immunosuppression [77].

Vertical transmission strategies diverge significantly: Congenital toxoplasmosis provides a particularly relevant parallel to neosporosis. Both parasites are capable of transplacental transmission and exploit the immunological constraints of pregnancy, where the need to maintain fetal tolerance

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results in a shift away from pro-inflammatory Th1 responses [55]. However, the epidemiological and pathological outcomes differ substantially. In cattle, *N. caninum* is highly adapted for efficient endogenous transplacental transmission, with vertical transmission serving as the primary mechanism of parasitic persistence and a major driver of abortion storms [17,19]. In humans, by contrast, congenital toxoplasmosis generally follows primary maternal infection during pregnancy, rather than reactivation of chronic infection, and does not produce the herd-level reproductive failure characteristic of bovine neosporosis [77,79].

Immune evasion shapes clinical outcomes: These differences suggest that the critical divergence lies not in the ability to cross the placenta, but in the parasite's broader host adaptation strategy. *T. gondii* may be more optimized for broad host permissiveness, long-term persistence, and immune evasion across diverse species, whereas *N. caninum* appears more host-restricted and reproductively specialized. Mechanistically, this distinction is supported by comparative studies demonstrating that *N. caninum* induces rapid innate immune activation, including early interferon responses, while *T. gondii* more effectively evades or suppresses early host detection [80]. This capacity for immune modulation in *T. gondii* is further reinforced by its repertoire of secreted effector proteins, including rhoptry and dense granule proteins, which alter host signaling pathways and facilitate intracellular survival [37].

From this perspective, human toxoplasmosis serves as a functional counterpoint to the *Neospora* paradox. While buffaloes achieve a state of tolerance in which *N. caninum* persists without significant reproductive pathology, *T. gondii* illustrates how a closely related parasite can achieve global host expansion and chronic persistence through effective immune evasion. The absence of confirmed human neosporosis, despite decades of serological investigation, therefore underscores that phylogenetic similarity alone is insufficient for zoonotic success. Instead, host range, transmission dynamics, and clinical outcome are determined by intricate interactions between parasite effectors and host immune recognition systems.

6.3. Molecular Mimicry and the "Arms Race": Shared Antigens, Divergent Outcomes

Despite their dramatically different host ranges, *N. caninum* and *T. gondii* share remarkable genetic and antigenic similarity. Whole-genome comparisons reveal ~45–50% amino acid identity across orthologous genes, with certain protein families showing even higher conservation [82].

Shared invasion toolkit: Proteins involved in host cell adhesion and invasion are particularly well-conserved. Apical Membrane Antigen 1 (AMA1), essential for tight junction formation during invasion, shows 73.6% amino acid identity between the two species [83]. NcMIC1 shares 68% identity with TgMIC1, and the adhesive microneme-associated repeat (MAR) domains are structurally superimposable [35]. This conservation explains functional cross-inhibition—antibodies raised against *T. gondii* AMA1 can partially inhibit *N. caninum* invasion *in vitro*, and vice versa [83].

The host recognition "arms race": Recent groundbreaking research has revealed that the difference in host range between *N. caninum* and *T. gondii* lies not in the mouse's inability to control *T. gondii*, but rather in *T. gondii*'s ability to **evade** the mouse's early immune detection. Coombs et al. [80] performed transcriptional profiling of murine bone marrow-derived macrophages infected with either parasite. Within 4 hours of infection, *N. caninum* induced robust expression of IFN-β, CXCL9, CXCL10, and other interferon-stimulated genes—a signature of rapid innate immune recognition. *T. gondii* infection, by contrast, triggered minimal transcriptional changes at early timepoints. The host was not inherently incapable of responding to *T. gondii*; rather, *T. gondii* actively suppressed or evaded detection.

This early recognition determines downstream outcomes. The rapid IFN-γ response to *N. caninum* activates macrophages to kill intracellular parasites, limiting dissemination and preventing chronic cyst establishment. *T. gondii*, by evading this early response, gains time to replicate, disseminate, and establish long-term persistence. Follow-up studies identified parasite-secreted effectors—particularly GRA15 and ROP16—that modulate host NF-κB and signal transducer and activator of transcription (STAT) signaling pathways to suppress innate immunity [84]. *N. caninum*

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appears to lack functional orthologs of these immune evasion factors, rendering it "visible" to the murine immune system.

Implications for the bovine paradox: This finding is a goldmine for understanding species-specific outcomes. It suggests that **host range and disease outcome are actively determined by a molecular "arms race" at the very earliest stage of infection.** The compatibility between parasite and host hinges on whether the parasite possesses effectors capable of subverting that particular host's innate recognition systems.

Applying this framework to the cattle-buffalo paradox: perhaps *N. caninum* possesses effectors that efficiently subvert bovine innate immunity but fail against buffalo defenses. Alternatively, buffaloes may express variant pattern recognition receptors that detect parasite ligands more rapidly or trigger more effective downstream responses. The difference between "abortion storm" in cattle and "silent infection" in buffalo likely lies in similar, yet-to-be-discovered differences in early immune recognition and response to *N. caninum*. This framework transforms the paradox from a descriptive observation into a hypothesis-driven research agenda.

7. A "One Health" Approach and Future Directions

7.1. The Wildlife Connection

Neospora caninum does not exist in isolation—it circulates within complex ecological communities involving domestic animals, wildlife, and shared environments. Understanding these interconnections is essential for comprehensive control.

Wild canids—wolves, coyotes, and foxes—serve as definitive hosts in natural ecosystems. Gray wolf (*Canis lupus*) populations in North America and Europe show high *N. caninum* seroprevalence (up to 60% in some studies), and infected wolves shed oocysts that contaminate habitats shared with livestock [85]. Coyotes (Canis latrans) are particularly significant in North America, where their expanding range brings them into contact with cattle operations [12].

Wild ruminants—White-tailed deer (*Odocoileus virginianus*) in the United States show seroprevalence ranging from 10% to 70% across different regions [86], and experimental transmission from deer to dogs has been demonstrated [85]. Infected deer may introduce the parasite into new areas through migration and serve as a *reservoir* for *spillover* into livestock.

Rodents as reservoirs: Small rodents also play a significant, often overlooked role in the sylvatic cycle. *N. caninum* DNA and antibodies have been detected in naturally infected house mice (*Mus musculus*), field mice (*Apodemus sylvaticus*), and brown rats (*Rattus norvegicus*), confirming they are natural intermediate hosts [87,88]. A 2023 meta-analysis estimated the global prevalence of *N. caninum* in rodents at 5% (95% CI: 2%–9%), with the highest prevalence in Asia (12%; 95% CI: 6%–24%) [89]. Experimental evidence further indicates that dogs can become infected by consuming infected rodents, directly linking these small mammals to the domestic transmission cycle [88]. Capybaras (*Hydrochoerus hydrochaeris*) in South America have also been found to carry *N. caninum* DNA, suggesting that a wide range of rodent species may contribute to the parasite's environmental persistence [90].

Birds have emerged as unexpected players in *N. caninum* epidemiology. Multiple avian species, including chickens, sparrows, and crows, are susceptible to experimental infection and can harbor parasite DNA in their tissues [91]. Infected birds might transport the parasite over long distances during migration and contaminate livestock environments with infected feces or carcasses scavenged by canids.

These wildlife connections complicate eradication efforts. Even if *N. caninum* were eliminated from domestic livestock, wildlife reservoirs could reinfect canids, leading to environmental contamination and spillback into cattle. Effective control therefore requires a landscape-level approach integrating livestock management, wildlife surveillance, and canid population control.

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7.2. Future Research Roadmap

Based on the framework developed in this review, we propose a comparative research agenda to translate the buffalo's natural resistance into practical interventions for cattle.

Priority 1: Comparative Transcriptomics at the Maternal–Fetal Interface

Single-cell RNA sequencing (scRNA-seq) of placentomes from infected cattle and buffaloes would reveal cell-type-specific transcriptional responses. Which cell populations are infected in each species? Do buffalo trophoblasts exhibit distinct activation states? Are regulatory T cells enriched in buffalo decidua? This approach could identify protective transcriptional signatures that distinguish the resistant host.

Experimental design: Collect placentomes from naturally or experimentally infected cattle and buffaloes at defined timepoints post-infection. Generate scRNA-seq libraries using 10X Genomics or similar platforms. Integrate data across species using cross-species mapping tools to identify conserved and divergent responses.

Priority 2: Functional Assays of Innate Immune Cells

Isolate and compare the ability of dendritic cells and macrophages from both species to control *N. caninum* replication *in vitro*. Key questions include: Do buffalo phagocytes kill parasites more efficiently? Do they produce different cytokine profiles upon stimulation? Are their pattern recognition receptors more sensitive to parasite ligands?

Experimental design: Derive monocyte-derived dendritic cells and macrophages from cattle and buffalo peripheral blood. Infect with fluorescently tagged *N. caninum* tachyzoites and track replication over 72 hours. Measure cytokine production (IFN-γ, IL-12, IL-10) by ELISA and qPCR. Use CRISPR-edited parasites to test whether specific effectors modulate species-specific responses.

Priority 3: Genome-Wide Association Studies in Buffaloes

If protective alleles exist in buffalo populations, GWAS could identify them. This requires large, well-phenotyped cohorts with genome-wide genotype data—a substantial investment but one with potential for high returns.

Experimental design: Assemble cohorts of 500–1000 buffaloes with well-characterized infection status (seropositive vs. seronegative) and reproductive outcomes (abortion history). Perform genotyping using the recently developed buffalo SNP array [92]. Conduct case-control association analyses to identify genomic regions associated with resistance. Validate candidate genes through functional studies.

Priority 4: Targeted Immunomodulation in Cattle

If buffalo-specific protective pathways are identified, explore whether they can be therapeutically induced in cattle. This could involve novel adjuvants that bias immune responses toward the "Goldilocks" phenotype, or immunomodulatory drugs that enhance regulatory T cell function at the placenta.

Experimental design: Develop *in vitro* co-culture systems modeling the bovine maternal–fetal interface. Test whether adding buffalo-derived factors (e.g., conditioned medium from buffalo decidual cells) alters infection outcomes. Translate promising findings into pilot *in vivo* studies in pregnant cattle.

Priority 5: Comparative Analysis of Parasite Effector Evolution

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Sequence *N. caninum* isolates from cattle and buffaloes to determine whether parasite strains adapt to their host species. Are there signatures of positive selection in effector genes (ROPs, GRAs) when parasites are propagated in one host versus the other?

Experimental design: Collect *N. caninum* isolates from naturally infected cattle and buffaloes across multiple geographic regions. Perform whole-genome sequencing and population genomic analyses. Test whether isolates from one host show enhanced fitness in that host using cross-infection experiments in immunodeficient mice.

8. Conclusions

The path to defeating neosporosis in cattle does not lie solely in the study of the disease itself. For three decades, research has focused on the susceptible bovine host—characterizing its pathology, measuring its economic losses, and attempting to achieve protection through vaccination. These efforts, while scientifically valuable, have not delivered transformative control strategies.

The water buffalo offers a different path. In this resistant host, *N. caninum* establishes a persistent infection without triggering the inflammatory cascade that proves fatal to the bovine fetus. The parasite transmits across generations, yet the host reproduces successfully. This is not resistance in the sense of sterile immunity—buffaloes remain infected—but tolerance: the ability to limit pathology while accepting persistent colonization.

Understanding this natural peace treaty between *N. caninum* and the buffalo could revolutionize neosporosis control. If we can identify the immunological and molecular mechanisms that enable tolerance in buffaloes, we can envision interventions that induce similar states in cattle. Not vaccines that aim for sterile immunity—a goal that has proven elusive—but immunomodulatory strategies that shift the host-parasite relationship from pathology toward equilibrium.

The paradox with which we began—the silent infection in buffaloes versus abortion storms in cattle—thus becomes not an anomaly to be explained away, but a window into fundamental principles of host-pathogen compatibility. By investigating this paradox, we can move from managing a disease to unlocking a state of equilibrium, transforming the future of neosporosis control worldwide.

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Abbreviations

The following abbreviations are used in this manuscript:

IFN-γ	Interferon-gamma
IL	Interleukin
ELISA	Enzyme-Linked Immunosorbent Assay
MICs	Microneme proteins
MAR	Microneme adhesive repeat

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ROPs	Rhoptry proteins
PVM	Parasitophorous vacuole membrane
GRAs	Dense granule proteins
CRISPR-Cas9	Clustered regularly interspaced palindromic repeats
PV	Parasitophorous vacuole
NcMYR1	*N. caninum* c-Myc regulatory protein
TLRs	Toll-like receptors
GWAS	Genome-wide association studies
IFAT	Indirect fluorescent antibody test
Tregs	Regulatory T cells
CDC	Center for Diseases Control
AMA1	Apical Membrane Antigen 1
STAT	Signal transducer and activator of transcription
scRNA-seq	Single-cell RNA sequencing

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