



International Journal of Veterinary Sciences and Animal Husbandry



ISSN Online: 2456-2912
NAAS Rating (2026): 4.61
IJVSAH 2026; 11(10): 01-08
www.veterinarypaper.com
Received: 24-07-2026
Accepted: 22-09-2026
Published: 02-10-2026

Bhesh Raj Paudel

Tribhuvan University, Institute
of Agriculture and Animal
Science, Kathmandu, Nepal

Sunil Bhattarai

Agriculture and Forestry
University, Rampur, Nepal

Subash Chhetri

Tribhuvan University, Institute
of Agriculture and Animal
Science, Kathmandu, Nepal

Sushil Neupane

Tribhuvan University, Institute
of Agriculture and Animal
Science, Kathmandu, Nepal

Bhupendra Khati

Tribhuvan University, Institute
of Agriculture and Animal
Science, Kathmandu, Nepal

Host–parasite interface in *Neospora caninum*: Intracellular survival, immune restriction, and pathogenesis

Bhesh Raj Paudel¹, Sunil Bhattarai², Subash Chhetri³, Sushil Neupane⁴, Bhupendra Khati⁵

DOI: <https://www.doi.org/10.22271/veterinary.2026.v11.i10a.3820>

ABSTRACT

Neospora caninum is an obligate intracellular apicomplexan parasite of major veterinary importance, particularly as a cause of reproductive disease and congenital infection in cattle. Successful infection depends on the parasite's ability to invade host cells, establish a specialized parasitophorous vacuole (PV), exploit host-cell resources, and evade intracellular antimicrobial mechanisms. This review synthesizes current understanding of how these parasite strategies intersect with host immunity to determine infection outcome. Microneme, rhoptry, and dense-granule proteins act sequentially to support invasion, host-cell manipulation, and remodeling of the intracellular niche, while interactions with host organelles and metabolic pathways facilitate parasite growth and persistence. In parallel, innate recognition promotes IL-12–IFN- γ -dominated immunity involving dendritic cells, macrophages, NK cells, and T cells, together with nitric oxide, nutrient deprivation, oxidative defenses, and direct targeting of the PV. Emerging evidence that immunity-associated GTPases can promote lysosomal recruitment and vacuolar acidification further identifies the PV as a critical interface between parasite survival and host restriction. *N. caninum*, however, can modulate inflammatory signaling, autophagy, mitophagy, and other host processes to favor persistence. These interactions ultimately influence dissemination, chronic infection, placental pathology, fetal infection, and vertical transmission. Integrating parasite virulence mechanisms with host cell-autonomous defense may therefore reveal new opportunities for host- and parasite-directed control of neosporosis.

Keywords: *Neospora caninum*, Neosporosis, Host–parasite interaction, Parasitophorous vacuole, Cell-autonomous immunity, Immune evasion, Vertical transmission

1. Introduction

Neospora caninum is an obligate intracellular apicomplexan parasite causing neosporosis in a broad range of domestic and wild animals (Dubey, 2003; Donahoe *et al.*, 2015) [2, 1]. The disease was first recognized as cyst-forming protozoal encephalomyelitis and myositis in dogs and was later distinguished from toxoplasmosis with recognition of *N. caninum* as a separate parasite (Bjerkås *et al.*, 1984; Dubey *et al.*, 1988) [3, 4]. Although many mammals can be infected, cattle and dogs are of greatest recognized veterinary importance: bovine neosporosis is dominated by reproductive losses, whereas canine disease commonly involves neuromuscular and neurological manifestations (Dubey *et al.*, 2007; Fisher *et al.*, 2024) [5, 6]. Detection of *Neospora*-associated infection in aborted bovine fetuses established its importance as a cause of reproductive disease (Anderson *et al.*, 1991) [7], and congenitally infected cattle have an increased risk of abortion, including in subsequent pregnancies (Thurmond *et al.*, 1997) [8]. Recent systematic evidence continues to show frequent *N. caninum* detection among aborting cattle and aborted fetuses worldwide (Nayeri *et al.*, 2022) [9]. Consequently, abortion, fetal loss, reduced reproductive performance, animal replacement, and control measures contribute to the economic importance of bovine neosporosis (Trees *et al.*, 1999; Hall *et al.*, 2005) [11, 10].

N. caninum has a heteroxenous life cycle involving canids as definitive hosts and numerous mammals as intermediate hosts (Dubey *et al.*, 2007; Goodswen *et al.*, 2013) [5, 12]. Domestic dogs shed oocysts after consuming infected tissues, while coyotes and dingoes are also definitive hosts, indicating contributions from both domestic and sylvatic canids (McAllister *et al.*, 1998; Gondim *et al.*, 2004; King *et al.*, 2010) [14, 13, 15]. Associations between infection in farm dogs and cattle support the importance of the cattle–canid interface, and dogs can shed oocysts after ingesting naturally infected bovine placental tissue (Wouda *et al.*, 1999; Dijkstra *et al.*, 2001) [17, 16]. Intermediate hosts acquire infection after ingesting sporulated oocysts;

Corresponding Author:

Bhesh Raj Paudel

Tribhuvan University,
Institute of Agriculture and
Animal Science,
Kathmandu, Nepal

rapidly proliferating tachyzoites then disseminate and may differentiate into slowly replicating bradyzoites within persistent tissue cysts (Dubey *et al.*, 2007; Goodswen *et al.*, 2013) [12]. In cattle, transplacental transmission is particularly important and can efficiently maintain infection across generations (Davison *et al.*, 1999) [18]. Transmission may result from recrudescence of persistent maternal infection during pregnancy, termed endogenous transplacental transmission, or from new infection acquired during gestation, termed exogenous transplacental transmission (Trees *et al.*, 2005; Williams *et al.*, 2009) [19, 20]. Persistent maternal infection therefore maintains the parasite within cattle lineages, whereas environmental oocyst exposure can introduce new infections (Davison *et al.*, 1999; Williams *et al.*, 2009) [18, 20].

The ability of *N. caninum* to persist, disseminate, and cause disease depends closely on parasite–host interactions (Dubey *et al.*, 2007; Goodswen *et al.*, 2013) [5, 12]. Successful infection requires host-cell invasion, intracellular replication, dissemination, and persistence, while host immune responses simultaneously restrict parasite multiplication and spread (Goodswen *et al.*, 2013) [12]. This balance is especially important during pregnancy because fetal infection does not invariably cause abortion; outcome reflects interactions among parasite transmission, persistence and replication, maternal and fetal responses, and gestational timing (Hall *et al.*, 2005; Williams *et al.*, 2009) [10, 20]. Understanding these interactions is therefore essential for explaining intracellular survival, immune evasion or resistance, persistence, neurological disease, placental infection, fetal injury, and congenital transmission (Dubey *et al.*, 2007; Goodswen *et al.*, 2013) [12]. Accordingly, this review examines host-cell invasion and intracellular survival, host immune responses and parasite immune-evasion strategies, and progression from host–parasite interaction to neosporosis pathogenesis, with emphasis on molecular determinants

of parasite survival and virulence, host restriction mechanisms, vertical transmission, disease severity, and potential host- and parasite-directed interventions.

2. Host-cell invasion and intracellular survival of *Neospora caninum*

Host-cell invasion by *N. caninum* begins with attachment of extracellular tachyzoites to the host-cell surface and coordinated secretion of apical invasion proteins. *NcMIC*₁ binds sulfated host-cell glycosaminoglycans, whereas *NcMIC*₃ is secreted onto the tachyzoite surface and promotes adhesion through its EGF-like domains (Keller *et al.*, 2002; Naguleswaran *et al.*, 2001) [21, 22]. Other microneme proteins further support this process. *NcMIC*₂ is a TRAP-family protein with adhesive domains and undergoes temperature- and Ca²⁺-regulated secretion, consistent with a proposed role in attachment and invasion (Lovett *et al.*, 2000) [23]. More direct functional evidence comes from *NcMIC*₈, whose interaction with *NcMIC*₃ and antibody-mediated inhibition reduces invasion, and *NcAMA*₁, an apically localized protein against which specific antibodies inhibit host-cell entry (Wang *et al.*, 2017; Zhang *et al.*, 2007) [24, 25]. Genetic disruption of *NcMIC*₆ similarly decreases invasion and egress and reduces expression and secretion of *NcMIC*₁ and *NcMIC*₄, indicating functional co-operation among components of the microneme system (Wang *et al.*, 2021) [26]. Once entry is initiated, rhoptry proteins extend parasite manipulation beyond adhesion: *NcROP*₁₆ promotes host STAT3 phosphorylation, whereas *NcROP*₅ deletion reduces invasion and intracellular growth, showing that distinct rhoptry effectors contribute to early host manipulation and parasite fitness (Ma *et al.*, 2017; Ma *et al.*, 2017) [27, 28]. These sequential events, from initial host-cell attachment and microneme/rhoptry secretion to active invasion and formation of the parasitophorous vacuole, are summarized in Figure 1.

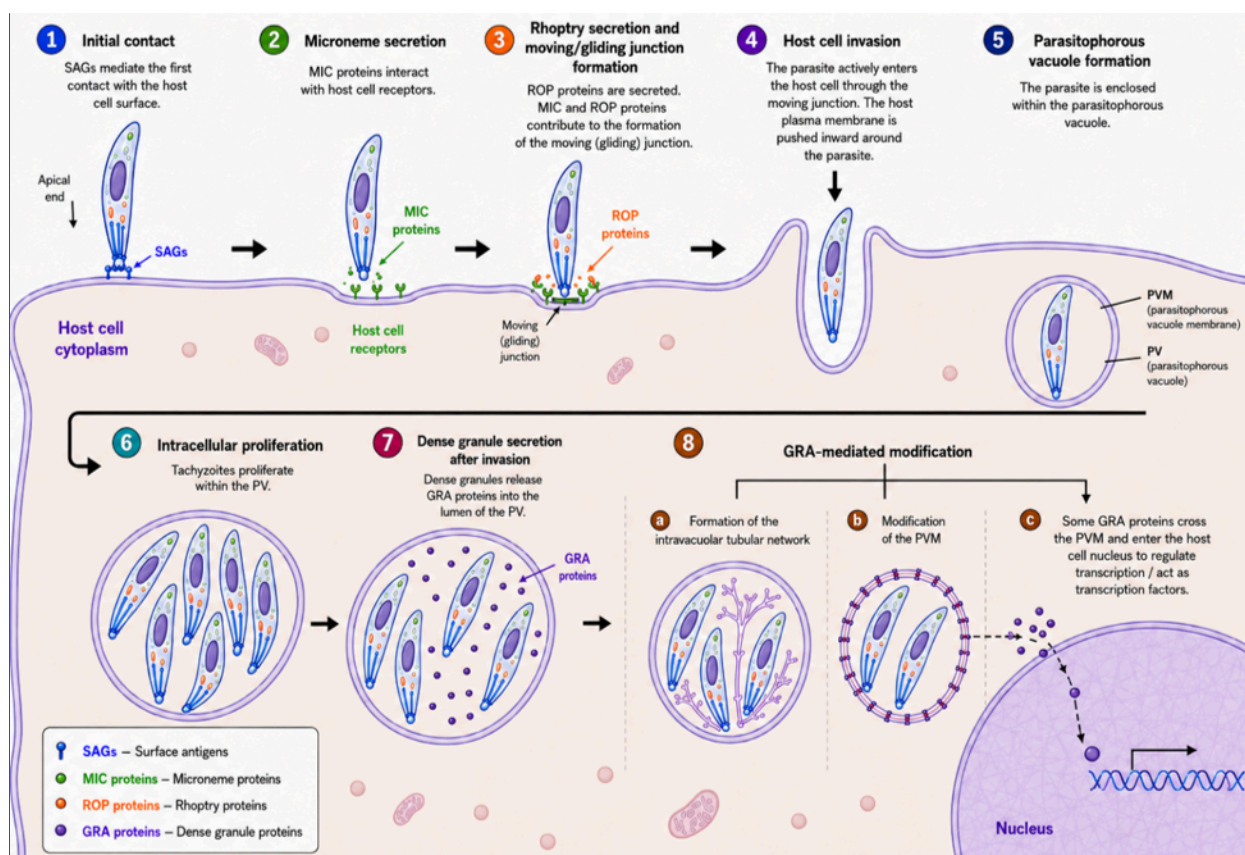


Figure 1. Sequential stages of *Neospora caninum* tachyzoite host-cell invasion, parasitophorous vacuole formation, and intracellular development

Following invasion, tachyzoites become enclosed within a specialized parasitophorous vacuole (PV), whose molecular organization is extensively shaped by dense-granule proteins. *NcGRAs* localize to the PV lumen, intravacuolar structures, and parasitophorous vacuole

membrane (PVM), demonstrating that parasite-derived proteins progressively remodel the newly formed compartment (Yang *et al.*, 2021) [29]. Several GRAs appear to support post-invasion development rather than initial entry. Disruption of *NcGRA*₆ reduces intracel

lular replication without markedly affecting invasion or egress, while *NcGRA*₂ colocalizes with *NcGRA*₆ within the intravacuolar network (IVN) and its deletion similarly slows intracellular growth (Zhao *et al.*, 2020; Dong *et al.*, 2021a) [31, 30]. *NcGRA*₁₂ is also present in soluble and membrane-associated forms within the PV and localizes to IVN membranes, further illustrating the structural complexity of the vacuolar niche, although its specific contribution to parasite survival remains unresolved (Dong *et al.*, 2021b) [32]. Dense-granule proteins can additionally influence host responses: *NcGRA*₇ modulates host signaling and cytokine expression, and its deletion reduces parasite burden and virulence in mice without substantially altering initial infection or intracellular growth in vitro (Nishikawa *et al.*, 2018) [33]. Thus, microneme, rhoptry, and dense-granule proteins function sequentially and in complementary ways, linking adhesion and invasion to host manipulation and organization of the intracellular replicative niche.

The PV is not an isolated compartment but interacts extensively with host-cell organelles and trafficking pathways. *N. caninum* reorganizes host microtubules and recruits the endoplasmic reticulum, mitochondria, Golgi-associated structures, multivesicular bodies, and endolysosomal compartments around the PV (Nolan *et al.*, 2015) [34]. These interactions are closely linked to nutrient acquisition: the parasite depends on host-derived cholesterol, accesses cholesterol associated with NPC1-containing endocytic organelles, and intercepts Rab14-positive Golgi vesicles carrying sphingolipids (Nolan *et al.*, 2015) [34]. Thus, *N. caninum* maintains selective communication with host trafficking systems to obtain metabolites required for growth while remaining within a protected intracellular compartment. Localization of *NcGRA*₂, *NcGRA*₆, and *NcGRA*₁₂ within the IVN provides a structural context for this parasite–host interface, although direct roles of individual GRAs in specific nutrient-acquisition pathways remain to be established (Dong *et al.*, 2021a, 2021b; Zhao *et al.*, 2020) [30, 32]. Successful intracellular replication is also associated with modification of host metabolism, signaling, apoptosis, and autophagy. Infection enhances host glycolysis through a JNK–HIF-1 α –PFKFB3-associated pathway, and inhibition or knockdown of PFKFB3 reduces parasite propagation, demonstrating that host metabolic reprogramming can favor intracellular growth (Tao *et al.*, 2025) [35]. Secreted effectors provide additional routes for host manipulation: *NcROP*₁₆ activates STAT3, whereas *NcGRA*₇ alters signaling and cytokine responses (Ma *et al.*, 2017; Nishikawa *et al.*, 2018) [27, 33]. Effects on apoptosis appear context dependent. Infection can inhibit death-receptor-mediated apoptosis and caspase-3 activation, potentially prolonging host-cell viability, whereas *NcROP*₁₆ has been associated with increased expression of apoptosis-related factors under different experimental conditions (Herman *et al.*, 2007; Ma *et al.*, 2017) [36, 27]. Autophagy shows similar context dependence. In macrophage-based models, infection is associated with accumulation of autophagic structures, impaired autophagic flux, and inefficient autophagosome–lysosome fusion through TLR2- and mTOR-associated signaling (Wang *et al.*, 2021) [37]. In contrast, infection of caprine endometrial epithelial cells enhances autophagic flux through mTOR inhibition, and experimentally promoting autophagy increases tachyzoite propagation (Zhao *et al.*, 2022) [38]. *N. caninum* also induces mitophagy in macrophages; enhanced mitophagy reduces ROS and proinflammatory responses and is associated with increased parasite burdens, suggesting another mechanism by which host homeostatic pathways can be redirected in favor of parasite persistence (Zhang *et al.*, 2022) [39].

Maintenance of this intracellular niche ultimately depends on preserving a vacuolar environment that limits destructive lysosomal activity while retaining selective interactions with host organelles. The *N. caninum* PV is generally considered largely non-fusogenic and resistant to conventional lysosomal degradation (Yang *et al.*, 2021) [29], although lysosomal and endosomal structures can accumulate around the vacuole as part of broader host-organelle rearrangement (Nolan *et al.*, 2015) [34]. This distinction suggests that intracellular survival depends not on complete exclusion of the endolysosomal system but on preventing productive fusion and acidification that would destroy the parasite. Supporting this interpretation, upregulation of GIMAP4, GIMAP5, or GIMAP6 promotes LAMP1 accumulation at the PVM, PV acidification, and reduced parasite proliferation in rat and bovine cells (Azam *et al.*, 2025) [40]. Together with the reduced post-invasion growth observed after disruption of *NcROP*₅, *NcGRA*₂, or *NcGRA*₆ (Ma *et al.*, 2017; Dong *et al.*, 2021a; Zhao *et al.*, 2020) [28, 30, 31], these findings indicate that *N. caninum* intracellular survival is multifactorial, integrating formation of a specialized PV, secretion of parasite effectors, exploitation of host nutrients, modulation of metabolic and degradative pathways, and protection of the vacuolar niche from destructive lysosomal activity.

3. Host immune responses and parasite immune-evasion strategies

Recognition of *N. caninum* by the innate immune system initiates a predominantly type 1 inflammatory response involving macrophages, dendritic cells, and other innate effector populations. TLR signaling contributes substantially to this recognition: TLR2 promotes antigen-presenting-cell activation and subsequent Th1 polarization, whereas TLR3 recognizes parasite-derived RNA through TRIF-dependent signaling that promotes IL-12, TNF- α , type I interferons, and other inflammatory mediators (Mineo *et al.*, 2010; Miranda *et al.*, 2019) [41, 42]. Consistently, MyD88-deficient mice show impaired dendritic-cell IL-12 production, delayed IFN- γ responses, and increased susceptibility, linking innate sensing directly to protective cytokine responses (Mineo *et al.*, 2009) [43]. Intracellular recognition also involves NOD2, whose deficiency reduces IL-6 and TNF- α production and impairs parasite control, although NOD2-driven inflammation may additionally contribute to immunopathology (Davoli-Ferreira *et al.*, 2016) [44]. In murine macrophages, TLR11-dependent MEK–ERK signaling promotes *IL-12*_{p40} production, providing another connection between recognition and type 1 immunity, although this pathway should be interpreted specifically within the murine model (Jin *et al.*, 2017) [45]. Both conventional and plasmacytoid dendritic cells produce IL-12 early after infection, linking parasite sensing with IFN- γ -mediated immunity (Teixeira *et al.*, 2010) [46]. Macrophages likewise have a dual role as parasite host cells and immune effectors: tachyzoites can invade these cells, yet macrophage recruitment and activation contribute substantially to protection, as their depletion markedly increases susceptibility to infection (Dion *et al.*, 2011; Abe *et al.*, 2014) [48, 47]. In cattle, NK cells provide an additional early defense by producing IFN- γ in response to *N. caninum* and killing infected fibroblasts predominantly through perforin-dependent mechanisms, although NK cells themselves can also be invaded by the parasite (Boysen *et al.*, 2006) [49]. Collectively, these findings indicate that multiple recognition pathways converge on antigen-presenting-cell activation, IL-12 production, and early IFN- γ responses. The major innate, adaptive, and cell-autonomous host immune mechanisms involved in the control of *N. caninum* infection are summarized in Table 1.

Table 1. Major host immune responses involved in protection against *Neospora caninum* infection

Major immune component	Main response during <i>N. caninum</i> infection	Protective significance	Key reference(s)
Pattern-recognition receptor signaling (TLR2, TLR3, TLR11, MyD88)	Detects parasite-derived molecules and promote inflammatory signaling, antigen-presenting-cell activation, and IL-12 production	Initiates type 1 immunity and links parasite recognition to downstream IFN- γ responses	Mineo <i>et al.</i> , 2009, 2010; Miranda <i>et al.</i> , 2019; Jin <i>et al.</i> , 2017

(continued on next page)

Major immune component	Main response during <i>N. caninum</i> infection	Protective significance	Key reference(s)
NOD2-mediated intracellular recognition	Promotes IL-6 and TNF- α production following intracellular parasite sensing	Supports parasite control, although excessive signaling may contribute to immunopathology	Davoli-Ferreira <i>et al.</i> , 2016
Dendritic cells	Produce IL-12 early after infection and activate downstream cellular immune responses	Connect innate recognition with development of IFN- γ -dominated immunity	Teixeira <i>et al.</i> , 2010
Macrophages	Function as both parasite host cells and antimicrobial effector cells	Activated macrophages contribute substantially to parasite restriction; their depletion increases susceptibility	Dion <i>et al.</i> , 2011; Abe <i>et al.</i> , 2014
NK cells	Produce early IFN- γ and kill infected cells through predominantly perforin-dependent mechanisms	Provide rapid innate protection before full adaptive immunity develops	Boysen <i>et al.</i> , 2006
IL-12–IFN- γ axis	IL-12 stimulates IFN- γ responses, while IFN- γ activates multiple intracellular antimicrobial pathways	Central coordinating mechanism of protective type 1 immunity	Baszler <i>et al.</i> , 1999; Lundén <i>et al.</i> , 1998
Cell-autonomous metabolic and oxidative defenses	Includes iNOS/NO production, IDO-mediated tryptophan depletion, ROS, and inflammasome-associated responses	Directly restricts intracellular parasite replication through toxic, metabolic, and inflammatory mechanisms	Tanaka <i>et al.</i> , 2000b; Spekter <i>et al.</i> , 2009; Mota <i>et al.</i> , 2020
CD4 ⁺ T cells	Support IFN- γ mediated cellular immunity and parasite-specific antibody responses	Coordinate protective adaptive immunity and contribute to survival during infection	Tanaka <i>et al.</i> , 2000a
CD8 ⁺ T cells	Restrict parasite burden predominantly through IFN- γ -dependent mechanisms	Provide additional adaptive cellular protection	Correia <i>et al.</i> , 2015
Direct parasitophorous-vacuole targeting: IRGs and GIMAPs	IRGs accumulate on the PVM in murine cells, while GIMAP4/5/6 promote LAMP1 recruitment, vacuolar acidification, and parasite restriction	Extends host defense from cytokine signaling to direct intracellular targeting of the parasite-containing vacuole	Reid <i>et al.</i> , 2012; Azam <i>et al.</i> , 2025

The IL-12–IFN- γ axis is central to parasite control, but its protection is mediated through several complementary mechanisms. Neutralization of IFN- γ markedly increases parasite burden, tissue damage, morbidity, and mortality, whereas IL-12 enhances resistance largely through an IFN- γ -dependent mechanism (Baszler *et al.*, 1999) [50]. This type 1 response is also evident in cattle, where experimental infection induces rapid parasite-specific lymphocyte proliferation accompanied by strong IFN- γ production (Lundén *et al.*, 1998) [51]. Downstream of IFN- γ , macrophages restrict intracellular replication through inducible nitric oxide synthase and nitric oxide, since interference with this pathway diminishes parasite control (Tanaka *et al.*, 2000b) [52]. IFN- γ also induces indoleamine 2,3-dioxygenase in bovine and human cells, causing tryptophan depletion and inhibition of parasite growth; reversal by tryptophan supplementation or IDO inhibition confirms nutrient deprivation as another cell-autonomous mechanism (Spekter *et al.*, 2009) [53]. Oxidative and inflammasome-dependent defenses provide an additional layer, as *N. caninum* activates inflammasome components together with ROS-associated responses, and disruption of these pathways compromises restriction of intracellular replication (Mota *et al.*, 2020) [54].

Adaptive immunity reinforces these mechanisms. CD4⁺ T-cell depletion increases mortality and reduces parasite-specific antibody responses, whereas high antibody titers cannot compensate for loss of IFN- γ during acute infection, indicating that humoral immunity functions most effectively alongside cellular responses (Tanaka *et al.*, 2000a) [55]. CD8⁺ T cells also contribute to protection: CD8-deficient mice carry higher parasite burdens, and adoptive-transfer experiments indicate that their protective activity depends predominantly on IFN- γ rather than perforin-mediated cytotoxicity (Correia *et al.*, 2015) [56]. TLR2-dependent innate activation influences both CD4⁺ and CD8⁺ responses by supporting parasite-specific proliferation and an appropriate IFN- γ /IL-10 balance (Mineo *et al.*, 2010) [41]. In murine cells, IFN- γ additionally induces immunity-related GTPases such as *Irga6* that accumulate on the *N. caninum* PVM; unlike virulent *Toxoplasma*

gondii, *N. caninum* lacks a fully functional ROP18-mediated mechanism for neutralizing this response, illustrating direct IFN- γ -dependent targeting of the parasite vacuole in this model (Reid *et al.*, 2012) [57]. Thus, IFN- γ integrates systemic immunity with nitric oxide production, nutrient restriction, oxidative responses, T-cell effector functions, and, in mice, PVM-directed intracellular defense.

Despite these protective responses, *N. caninum* can modify host signaling and homeostatic pathways in ways that favor persistence. Parasite-induced p38 MAPK activation in macrophages appears to dampen protective activation, because p38 inhibition increases IL-12_{p40} production and MHC/B7 expression and, in vivo, is associated with stronger inflammatory responses, reduced parasite burden, and improved survival (Mota *et al.*, 2016) [58]. Infection-induced mitophagy provides another mechanism: enhanced mitochondrial removal reduces ROS and suppresses ERK-, p38-, and NLRP3-associated inflammatory responses, whereas experimental promotion of mitophagy increases parasite burden (Zhang *et al.*, 2022) [39]. This contrasts with the protective ROS–inflammasome interaction observed during infection and illustrates how parasite manipulation of host homeostatic pathways can shift the balance away from intracellular restriction (Mota *et al.*, 2020; Zhang *et al.*, 2022) [54, 39]. Host cells nevertheless possess mechanisms capable of directly overcoming the protected PV. Upregulation of GIMAP4, GIMAP5, or GIMAP6 promotes LAMP1 accumulation at the PVM, vacuolar acidification, and restriction of intracellular *N. caninum* proliferation in rat macrophages, while corresponding bovine GIMAP proteins also restrict parasite growth in bovine cells (Azam *et al.*, 2025) [40]. Together with murine IRG-mediated PVM targeting (Reid *et al.*, 2012) [57], these findings expand *anti-Neospora* immunity beyond cytokine production to direct intracellular trafficking and vacuolar targeting. Overall, resistance reflects coordinated innate recognition, IL-12–IFN- γ -driven cellular immunity, metabolic and oxidative restriction, adaptive responses, and direct targeting of the PV, whereas parasite persistence is favored when *N. caninum* redirects signaling or cel

lular processes such as p38 activation and mitophagy to attenuate antimicrobial defenses (Mota *et al.*, 2016; Zhang *et al.*, 2022) [58, 39].

4. From host–parasite interaction to neosporosis pathogenesis

The clinical outcome of *N. caninum* infection reflects the cumulative balance between parasite replication and the host-restriction mechanisms described above. After establishment of the intracellular niche, tachyzoites depend on host resources and organelle interactions to sustain growth. Recruitment of endoplasmic reticulum, mitochondria, Golgi-associated vesicles, lysosomes, and other endomembrane compartments toward the PV, together with acquisition of host-derived cholesterol and sphingolipids, supports parasite replication (Nolan *et al.*, 2015) [34]. At the same time, GIMAP4-, GIMAP5-, and GIMAP6-associated recruitment of LAMP1 and PV acidification can render this niche less permissive to growth (Azam *et al.*, 2025) [40]. Disease progression therefore depends not simply on successful invasion but on whether parasite-mediated maintenance of the PV and nutrient acquisition can overcome host mechanisms that destabilize the intracellular compartment. Parasite-intrinsic factors further shape this balance: deletion of *NcROP5* reduces invasion, intracellular replication, cerebral parasite burden, and virulence, whereas loss of *NcSAG1* attenuates disease and decreases brain parasite burdens in offspring (Ma *et al.*, 2017; Abdelbaky *et al.*, 2024) [28, 59]. Likewise, deletion of *NcGRA7* or *NcROP40* attenuates disease and reduces maternal brain parasite burdens, with a stronger effect following *NcGRA7* disruption, while loss of *NcROP24* reduces virulence and congenital transmission and impairs parasite growth in bovine macrophages in a host-cell-dependent manner (Rico-San Román *et al.*, 2022; Amieva *et al.*, 2026) [61, 60]. Collectively, these findings show that pathogenicity emerges from interactions between host-cell permissiveness, host restriction, and multiple parasite effectors rather than from a single virulence determinant.

Following acute dissemination, host restriction alters parasite distribution and favors establishment of chronic infection. In experimentally infected mice, early infection is characterized by parasitemia and widespread parasite detection in the spleen, liver, and lungs, whereas later infection becomes increasingly concentrated in the brain as peripheral parasite burdens decline (Collantes-Fernández *et al.*, 2006) [62]. A similar CNS tropism is evident in cattle, where later infection is associated predominantly with parasite DNA in neural tissues together with focal necrosis, glial activation, and perivascular inflammation (Nishimura *et al.*, 2013) [63]. Persistence is facilitated by differentiation of rapidly multiplying tachyzoites into slowly replicating bradyzoites associated with tissue cysts. Experimental stress induces bradyzoite-associated markers and cyst-like structures *in vitro*, although this does not fully reproduce mature tissue-cyst formation (Weiss *et al.*, 1999) [64]. Direct evidence from naturally infected cattle and dogs confirms BAG-positive bradyzoites within tissue cysts, including cysts in skeletal muscle, showing that persistent stages are not confined to the CNS (Peters *et al.*, 2001) [65]. Stage conversion itself may influence virulence: *NcBPK1* deletion increases tachyzoite replication while impairing expression of the bradyzoite marker *NcSAG4* under differentiation conditions, suggesting that differentiation mechanisms help regulate the balance between acute proliferation and chronic persistence (Amieva *et al.*, 2025) [66]. This persistence has major reproductive consequences because chronically infected cows can transmit infection during pregnancy; increased maternal parasite-specific antibody associated with transmission is consistent with pregnancy-associated recrudescence of established infection rather than necessarily requiring a new exogenous exposure (Guy *et al.*, 2001) [67]. Persistent infection therefore provides a reservoir from which recrudescence, renewed dissemination, and endogenous vertical transmission can arise.

Pregnancy most clearly demonstrates how parasite replication, tissue tropism, host immunity, and gestational stage converge to determine

pathogenesis. During mid-gestation infection, *N. caninum* reaches the placenta and fetus, producing necrosis and inflammation within maternal caruncular septa and fetal villi and later focal inflammatory and necrotic lesions in the fetal CNS (Maley *et al.*, 2003) [68]. Placental pathology therefore represents both a route of transmission and a major site where parasite replication and host inflammatory responses interact. Early-gestation infection associated with fetal death produces extensive placental necrosis, inflammatory-cell infiltration, and increased expression of IFN- γ , *IL-12*_{p40}, TNF- α , IL-18, IL-2, IL-4, and IL-10, although these responses correlate with rather than establish a direct causal mechanism for abortion (Rosbottom *et al.*, 2008) [69]. Consistently, fetal death after early infection is associated with more extensive parasite dissemination and parasite-associated necrosis in placental and fetal tissues, whereas later infection shows more restricted fetal parasite distribution, particularly toward neural tissues (Gibney *et al.*, 2008) [70]. In contrast, infection at day 210 can produce efficient vertical transmission without abortion, with viable fetuses showing comparatively limited pathology and evidence of improving maternal and fetal control (Benavides *et al.*, 2012) [71].

Parasite genotype further modifies these gestational outcomes. Nc-Spain7 disseminates to placental and fetal tissues more efficiently and is associated with earlier fetal mortality than less virulent Nc-Spain8 (Regidor-Cerrillo *et al.*, 2014) [72], while low-virulence Nc-Spain1H causes less parasitemia and placental/fetal involvement and did not induce the fetopathy observed with Nc-1 under comparable early-gestation conditions (Rojo-Montejo *et al.*, 2009) [73]. Early infection-dynamics studies similarly show rapid placental colonization, extensive parasite detection, and efficient vertical transmission following Nc-Spain7 challenge, whereas Nc-Spain1H remains markedly more restricted (Jiménez-Pelayo *et al.*, 2019) [74]. Fetal outcome therefore depends not on transplacental infection alone but on the combined effects of gestational age, maternal and fetal immune competence, parasite replication and dissemination, placental injury, and intrinsic parasite virulence.

These observations also define major knowledge gaps and potential intervention points. The mechanisms governing progression from intracellular replication to dissemination, stage conversion, chronic persistence, neurological disease, and congenital transmission remain incompletely understood. Functional studies of *NcROP5*, *NcSAG1*, *NcGRA7*, *NcROP40*, *NcROP24*, and *NcBPK1* show that parasite proteins can differentially influence invasion, replication, host-cell interaction, tissue burden, stage conversion, and congenital disease, reinforcing the view that virulence is multifactorial (Ma, J. Liu, *et al.*, 2017; Abdelbaky *et al.*, 2024 [59]; Rico-San Román *et al.*, 2022 [61]; Amieva *et al.*, 2025 [66], 2026). Similarly, although PV–host organelle interactions support lipid acquisition and intracellular replication, it remains unclear which trafficking pathways can be manipulated safely *in vivo* (Nolan *et al.*, 2015) [34]. Host-directed approaches are attractive because GIMAP-mediated lysosomal recruitment and PV acidification demonstrate that the protected vacuole can be rendered less permissive to parasite growth, although therapeutic manipulation of this response has not been established (Azam *et al.*, 2025) [40]. Parasite-directed approaches are also moving toward functional validation. The CDPK1-targeting bumped kinase inhibitor BKI-1748 inhibits tachyzoite proliferation and disrupts replication and egress *in vitro*; in pregnant mice, treatment improves offspring survival and markedly reduces vertical transmission, although maternal cerebral parasite burden is not significantly reduced and efficacy in cattle remains unknown (Imhof *et al.*, 2021) [75]. Future studies should therefore integrate parasite genetics, stage-conversion biology, PV–host interactions, placental immunopathology, and host cell-autonomous restriction to identify the mechanisms that determine whether infection progresses toward controlled persistence, neurological disease, abortion, or congenital transmission and to define parasite- or host-directed interventions capable of limiting disease without exacerbating tissue damage.

References

- Donahoe SL, Lindsay SA, Krockenberger M, Phalen D, Šlapeta J. A review of neosporosis and pathologic findings of *Neospora caninum* infection in wildlife. *International Journal for Parasitology: Parasites and Wildlife*. 2015;4(2):216–238.
- Dubey JP. Review of *Neospora caninum* and neosporosis in animals. *The Korean Journal of Parasitology*. 2003;41(1):1–16.
- Bjerkås I, Mohn SF, Presthus J. Unidentified cyst-forming sporozoan causing encephalomyelitis and myositis in dogs. *Zeitschrift für Parasitenkunde*. 1984;70(2):271–274.
- Dubey JP, Carpenter JL, Speer CA, Topper MJ, Uggla A. Newly recognized fatal protozoan disease of dogs. *Journal of the American Veterinary Medical Association*. 1988;192(9):1269–1285.
- Dubey JP, Schares G, Ortega-Mora LM. Epidemiology and control of neosporosis and *Neospora caninum*. *Clinical Microbiology Reviews*. 2007;20(2):323–367.
- Fisher C, Seferidis N, Zilli J, Roberts T, Harcourt-Brown T. Insights into the clinical presentation, diagnostics and outcome in dogs presenting with neurological signs secondary to infection with *Neospora caninum*: 41 cases (2014–2023). *Journal of Small Animal Practice*. 2024;65(7):582–588.
- Anderson ML, Blanchard PC, Barr BC, Dubey JP, Hoffman RL, Conrad PA. Neospora-like protozoan infection as a major cause of abortion in California dairy cattle. *Journal of the American Veterinary Medical Association*. 1991;198(2):241–244.
- Thurmond MC, Hietala SK. Effect of congenitally acquired *Neospora caninum* infection on risk of abortion and subsequent abortions in dairy cattle. *American Journal of Veterinary Research*. 1997;58(12):1381–1385.
- Nayeri T, Moosazadeh M, Sarvi S, Daryani A. *Neospora caninum* infection in aborting bovines and lost fetuses: A systematic review and meta-analysis. *PLOS ONE*. 2022;17(5):e0268903.
- Hall CA, Reichel MP, Ellis JT. *Neospora* abortions in dairy cattle: Diagnosis, mode of transmission and control. *Veterinary Parasitology*. 2005;128(3–4):231–241.
- Trees AJ, Davison HC, Innes EA, Wastling JM. Towards evaluating the economic impact of bovine neosporosis. *International Journal for Parasitology*. 1999;29(8):1195–1200.
- Goodswen SJ, Kennedy PJ, Ellis JT. A review of the infection, genetics, and evolution of *Neospora caninum*: From the past to the present. *Infection, Genetics and Evolution*. 2013;13:133–150.
- Gondim LFP, McAllister MM, Pitt WC, Zemlicka DE. Coyotes (*Canis latrans*) are definitive hosts of *Neospora caninum*. *International Journal for Parasitology*. 2004;34(2):159–161.
- McAllister MM, Dubey JP, Lindsay DS, Jolley WR, Wills RA, McGuire AM. Dogs are definitive hosts of *Neospora caninum*. *International Journal for Parasitology*. 1998;28(9):1473–1478.
- King JS, Šlapeta J, Jenkins DJ, Al-Qassab SE, Ellis JT, Windsor PA. Australian dingoes are definitive hosts of *Neospora caninum*. *International Journal for Parasitology*. 2010;40(8):945–950.
- Dijkstra T, Eysker M, Schares G, Conraths FJ, Wouda W, Barkema HW. Dogs shed *Neospora caninum* oocysts after ingestion of naturally infected bovine placenta but not after ingestion of colostrum spiked with *Neospora caninum* tachyzoites. *International Journal for Parasitology*. 2001;31(8):747–752.
- Wouda W, Dijkstra T, Kramer AMH, van Maanen C, Brinkhof JMA. Seroepidemiological evidence for a relationship between *Neospora caninum* infections in dogs and cattle. *International Journal for Parasitology*. 1999;29(10):1677–1682.
- Davison HC, Otter A, Trees AJ. Estimation of vertical and horizontal transmission parameters of *Neospora caninum* infections in dairy cattle. *International Journal for Parasitology*. 1999;29(10):1683–1689.
- Trees AJ, Williams DJL. Endogenous and exogenous transplacental infection in *Neospora caninum* and *Toxoplasma gondii*. *Trends in Parasitology*. 2005;21(12):558–561.
- Williams DJL, Hartley CS, Björkman C, Trees AJ. Endogenous and exogenous transplacental transmission of *Neospora caninum*—How the route of transmission impacts on epidemiology and control of disease. *Parasitology*. 2009;136(14):1895–1900.
- Keller N, Naguleswaran A, Cannas A, Vonlaufen N, Bienz M, Björkman C, Bohne W, Hemphill A. Identification of a *Neospora caninum* microneme protein (NcMIC1) which interacts with sulfated host cell surface glycosaminoglycans. *Infection and Immunity*. 2002;70(6):3187–3198.
- Naguleswaran A, Cannas A, Keller N, Vonlaufen N, Schares G, Conraths FJ, Björkman C, Hemphill A. *Neospora caninum* microneme protein NcMIC3: Secretion, subcellular localization, and functional involvement in host cell interaction. *Infection and Immunity*. 2001;69(10):6483–6494.
- Lovett JL, Howe DK, Sibley LD. Molecular characterization of a thrombospondin-related anonymous protein homologue in *Neospora caninum*. *Molecular and Biochemical Parasitology*. 2000;107(1):33–43.
- Wang J, Tang D, Li W, Xu J, Liu Q, Liu J. A new microneme protein of *Neospora caninum*, NcMIC8 is involved in host cell invasion. *Experimental Parasitology*. 2017;175:21–27.
- Zhang H, Compaore MKA, Lee EG, Liao M, Zhang G, Sugimoto C, Fujisaki K, Nishikawa Y, Xuan X. Apical membrane antigen 1 is a cross-reactive antigen between *Neospora caninum* and *Toxoplasma gondii*, and the anti-NcAMA1 antibody inhibits host cell invasion by both parasites. *Molecular and Biochemical Parasitology*. 2007;151(2):205–212.
- Wang X, Tang D, Wang F, Jin G, Wang L, Liu Q, Liu J. Microneme protein 6 is involved in invasion and egress by *Neospora caninum*. *Pathogens*. 2021;10(2):201.
- Ma L, Liu G, Liu J, Li M, Zhang H, Tang D, Liu Q. *Neospora caninum* ROP16 play an important role in the pathogenicity by phosphorylating host cell STAT3. *Veterinary Parasitology*. 2017;243:135–147.
- Ma L, Liu J, Li M, Fu Y, Zhang X, Liu Q. Rhoptry protein 5 (ROP5) is a key virulence factor in *Neospora caninum*. *Frontiers in Microbiology*. 2017;8:370.
- Yang C, Wang C, Liu J, Liu Q. Biotinylation of the *Neospora caninum* parasitophorous vacuole reveals novel dense granule proteins. *Parasites & Vectors*. 2021;14:521.
- Dong J, Zhang N, Zhao P, Li J, Cao L, Wang X, Li X, Yang J, Zhang X, Gong P. Disruption of dense granular protein 2 (GRA2) decreases the virulence of *Neospora caninum*. *Frontiers in Veterinary Science*. 2021;8:634612.
- Zhao P, Zhang N, Dong J, Li J, Wang X, Li X, Li X, Yang J, Gong P, Zhang X. Effects of dense granular protein 6 (GRA6) disruption on *Neospora caninum* virulence. *Frontiers in Veterinary Science*. 2020;7:562730.
- Dong J, Zhang N, Zhao P, Li J, Wang X, Li X, Gong P, Zhang X. GRA12, a novel dense granule protein from *Neospora caninum*. *Parasitology International*. 2021;81:102268.
- Nishikawa Y, Shimoda N, Fereig RM, Moritaka T, Umeda K, Nishimura M, Ihara F, Kobayashi K, Himori Y, Suzuki Y, Furuoka H. *Neospora caninum* dense granule protein 7 regulates the pathogenesis of neosporosis by modulating host immune response. *Applied and Environmental Microbiology*. 2018;84(18):e01350-18.
- Nolan SJ, Romano JD, Luechtefeld T, Coppens I. *Neospora caninum* recruits host cell structures to its parasitophorous vacuole and salvages lipids from organelles. *Eukaryotic Cell*. 2015;14(5):454–473.

35. Tao DL, Chen JM, Wu JP, Zhao SS, Qi BF, Yang X, Fan YY, Song JK, Zhao GH. *Neospora caninum* hijacks host PFKFB3-driven glycolysis to facilitate intracellular propagation of parasites. *Veterinary Research*. 2025;56:94.
36. Herman RK, Molestina RE, Sinai AP, Howe DK. The apicomplexan pathogen *Neospora caninum* inhibits host cell apoptosis in the absence of discernible NF- κ B activation. *Infection and Immunity*. 2007;75(9):4255–4262.
37. Wang J, Wang X, Gong P, Ren F, Li X, Zhang N, Zhang X, Zhang X, Li J. The protective role of TLR2 mediates impaired autophagic flux by activating the mTOR pathway during *Neospora caninum* infection in mice. *Frontiers in Cellular and Infection Microbiology*. 2021;11:788340.
38. Zhao SS, Tao DL, Chen JM, Chen X, Geng XL, Wang JW, Yang X, Song JK, Liu Q, Zhao GH. *Neospora caninum* infection activated autophagy of caprine endometrial epithelial cells via mTOR signaling. *Veterinary Parasitology*. 2022;304:109685.
39. Zhang X, Wang Y, Gong P, Wang X, Zhang N, Chen M, Wei R, Zhang X, Li X, Li J. *Neospora caninum* evades immunity via inducing host cell mitophagy to inhibit production of proinflammatory cytokines in a ROS-dependent manner. *Frontiers in Immunology*. 2022;13:827004.
40. Azam AR, Mia MM, Bedwani J, Witola WH. Upregulated small GTPase immunity-associated proteins confer resistance to *Neospora caninum* in rat and bovine cells. *Frontiers in Cellular and Infection Microbiology*. 2025;15:1674380.
41. Mineo TWP, Oliveira CJF, Gutierrez FRS, Silva JS. Recognition by Toll-like receptor 2 induces antigen-presenting cell activation and Th1 programming during infection by *Neospora caninum*. *Immunology and Cell Biology*. 2010;88(8):825–833.
42. Miranda V dos S, França FBF, da Costa MS, Silva VRS, Mota CM, Barros P da SC, Parreira KS, Santiago FM, Mineo JR, Mineo TWP. Toll-like receptor 3–TRIF pathway activation by *Neospora caninum* RNA enhances infection control in mice. *Infection and Immunity*. 2019;87(4):e00739-18.
43. Mineo TWP, Benevides L, Silva NM, Silva JS. Myeloid differentiation factor 88 is required for resistance to *Neospora caninum* infection. *Veterinary Research*. 2009;40(4):32.
44. Davoli-Ferreira M, Fonseca DM, Mota CM, Dias MS, Lima-Junior DS, da Silva MV, Quirino GFS, Zamboni DS, Silva JS, Mineo TWP. Nucleotide-binding oligomerization domain-containing protein 2 prompts potent inflammatory stimuli during *Neospora caninum* infection. *Scientific Reports*. 2016;6:29289.
45. Jin X, Gong P, Zhang X, Li G, Zhu T, Zhang M, Li J. Activation of ERK signaling via TLR11 induces IL-12p40 production in peritoneal macrophages challenged by *Neospora caninum*. *Frontiers in Microbiology*. 2017;8:1393.
46. Teixeira L, Botelho AS, Mesquita SD, Correia A, Cerca F, Costa R, Sampaio P, Castro AG, Vilanova M. Plasmacytoid and conventional dendritic cells are early producers of IL-12 in *Neospora caninum*-infected mice. *Immunology and Cell Biology*. 2010;88(1):79–86.
47. Abe C, Tanaka S, Ihara F, Nishikawa Y. Macrophage depletion prior to *Neospora caninum* infection results in severe neosporosis in mice. *Clinical and Vaccine Immunology*. 2014;21(8):1185–1188.
48. Dion S, Germon S, Guiton R, Ducournau C, Dimier-Poisson I. Functional activation of T cells by dendritic cells and macrophages exposed to the intracellular parasite *Neospora caninum*. *International Journal for Parasitology*. 2011;41(6):685–695.
49. Boysen P, Klevar S, Olsen I, Storset AK. The protozoan *Neospora caninum* directly triggers bovine NK cells to produce gamma interferon and to kill infected fibroblasts. *Infection and Immunity*. 2006;74(2):953–960.
50. Baszler TV, Long MT, McElwain TF, Mathison BA. Interferon-gamma and interleukin-12 mediate protection to acute *Neospora caninum* infection in BALB/c mice. *International Journal for Parasitology*. 1999;29(10):1635–1646.
51. Lundén A, Marks J, Maley SW, Innes EA. Cellular immune responses in cattle experimentally infected with *Neospora caninum*. *Parasite Immunology*. 1998;20(11):519–526.
52. Tanaka T, Nagasawa H, Fujisaki K, Suzuki N, Mikami T. Growth-inhibitory effects of interferon-gamma on *Neospora caninum* in murine macrophages by a nitric oxide mechanism. *Parasitology Research*. 2000;86(9):768–771.
53. Spekter K, Czesla M, Ince V, Heseler K, Schmidt SK, Schares G, Däubener W. Indoleamine 2,3-dioxygenase is involved in defense against *Neospora caninum* in human and bovine cells. *Infection and Immunity*. 2009;77(10):4496–4501.
54. Mota CM, Lima-Junior D de S, França FBF, Torres JDA, Barros P da SC, Santiago FM, Silva JS, Mineo JR, Zamboni DS, Mineo TWP. Interplay between reactive oxygen species and the inflammasome are crucial for restriction of *Neospora caninum* replication. *Frontiers in Cellular and Infection Microbiology*. 2020;10:243.
55. Tanaka T, Hamada T, Inoue N, Nagasawa H, Fujisaki K, Suzuki N, Mikami T. The role of CD4+ or CD8+ T cells in the protective immune response of BALB/c mice to *Neospora caninum* infection. *Veterinary Parasitology*. 2000;90(3):183–191.
56. Correia A, Ferreira P, Botelho S, Belinha A, Leitão C, Caramalho Í, Teixeira L, González-Fernández Á, Appelberg R, Vilanova M. Predominant role of interferon- γ in the host protective effect of CD8+ T cells against *Neospora caninum* infection. *Scientific Reports*. 2015;5:14913.
57. Reid AJ, Vermont SJ, Cotton JA, Harris D, Hill-Cawthorne GA, Könen-Waisman S, Latham SM, Mourier T, Norton R, Quail MA, Sanders M, Shanmugam D, Sohal A, Wasmuth JD, Brunk B, Grigg ME, Howard JC, Parkinson J, Roos DS, Wastling JM. Comparative genomics of the apicomplexan parasites *Toxoplasma gondii* and *Neospora caninum*: Coccidia differing in host range and transmission strategy. *PLoS Pathogens*. 2012;8(3):e1002567.
58. Mota CM, Oliveira ACM, Davoli-Ferreira M, Silva MV, Santiago FM, Nadipuram SM, Vashisht AA, Wohlschlegel JA, Bradley PJ, Silva JS, Mineo JR, Mineo TWP. *Neospora caninum* activates p38 MAPK as an evasion mechanism against innate immunity. *Frontiers in Microbiology*. 2016;7:1456.
59. Abdelbaky HH, Rahman MM, Shimoda N, Chen Y, Hasan T, Ushio N, Nishikawa Y. *Neospora caninum* surface antigen 1 is a major determinant of the pathogenesis of neosporosis in nonpregnant and pregnant mice. *Frontiers in Microbiology*. 2024;14:1334447.
60. Amieva R, Rico-San Román L, Coronado M, Powell J, Hassan MA, Hemphill A, Boubaker G, Pfarrer C, Ortega-Mora LM, Collantes-Fernández E, Horcajo P. NrOP24 loss attenuates *Neospora caninum* virulence and alters rophry organization. *International Journal for Parasitology*. 2026;56(7):104770.
61. Rico-San Román L, Amieva R, Regidor-Cerrillo J, García-Sánchez M, Collantes-Fernández E, Pastor-Fernández I, Saeij JPJ, Ortega-Mora LM, Horcajo P. NrGRA7 and NrOP40 play a role in the virulence of *Neospora caninum* in a pregnant mouse model. *Pathogens*. 2022;11(9):998.
62. Collantes-Fernández E, López-Pérez I, Álvarez-García G, Ortega-Mora LM. Temporal distribution and parasite load kinetics in blood and tissues during *Neospora caninum* infection in mice. *Infection and Immunity*. 2006;74(4):2491–2494.
63. Nishimura M, Kohara J, Hiasa J, Muroi Y, Yokoyama N, Kida K, Xuan X, Furuoka H, Nishikawa Y. Tissue distribution of *Neospora caninum* in experimentally infected cattle. *Clinical and Vaccine Immunology*. 2013;20(2):309–312.

64. Weiss LM, Ma YF, Halonen S, McAllister MM, Zhang YW. The in vitro development of *Neospora caninum* bradyzoites. *International Journal for Parasitology*. 1999;29(10):1713–1723.
65. Peters M, Lütkefells E, Heckerroth AR, Schares G. Immunohistochemical and ultrastructural evidence for *Neospora caninum* tissue cysts in skeletal muscles of naturally infected dogs and cattle. *International Journal for Parasitology*. 2001;31(10):1144–1148.
66. Amieva R, Rico-San Román L, Pastor-Fernández I, Hemphill A, Boubaker G, Collantes-Fernández E, Ortega-Mora LM, Horcajo P. Loss of NcBPK1 impairs bradyzoite differentiation and enhances virulence in *Neospora caninum*. *Parasites & Vectors*. 2025;18:422.
67. Guy CS, Williams DJL, Kelly DF, McGarry JW, Guy F, Björkman C, Smith RF, Trees AJ. *Neospora caninum* in persistently infected, pregnant cows: Spontaneous transplacental infection is associated with an acute increase in maternal antibody. *Veterinary Record*. 2001;149(15):443–449.
68. Maley SW, Buxton D, Rae AG, Wright SE, Schock A, Bartley PM, Esteban-Redondo I, Swales C, Hamilton CM, Sales J, Innes EA. The pathogenesis of neosporosis in pregnant cattle: Inoculation at mid-gestation. *Journal of Comparative Pathology*. 2003;129(2–3):186–195.
69. Rosbottom A, Gibney EH, Guy CS, Kipar A, Smith RF, Kaiser P, Trees AJ, Williams DJL. Upregulation of cytokines is detected in the placentas of cattle infected with *Neospora caninum* and is more marked early in gestation when fetal death is observed. *Infection and Immunity*. 2008;76(6):2352–2361.
70. Gibney EH, Kipar A, Rosbottom A, Guy CS, Smith RF, Hetzel U, Trees AJ, Williams DJL. The extent of parasite-associated necrosis in the placenta and foetal tissues of cattle following *Neospora caninum* infection in early and late gestation correlates with foetal death. *International Journal for Parasitology*. 2008;38(5):579–588.
71. Benavides J, Katzer F, Maley SW, Bartley PM, Cantón G, Palarea-Albaladejo J, Purslow CA, Pang Y, Rocchi MS, Chianini F, Buxton D, Innes EA. High rate of transplacental infection and transmission of *Neospora caninum* following experimental challenge of cattle at day 210 of gestation. *Veterinary Research*. 2012;43:83.
72. Regidor-Cerrillo J, Arranz-Solis D, Benavides J, Gómez-Bautista M, Castro-Hermida JA, Mezo M, Pérez V, Ortega-Mora LM, González-Warleta M. *Neospora caninum* infection during early pregnancy in cattle: How the isolate influences infection dynamics, clinical outcome and peripheral and local immune responses. *Veterinary Research*. 2014;45:10.
73. Rojo-Montejo S, Collantes-Fernández E, Blanco-Murcia J, Rodríguez-Bertos A, Risco-Castillo V, Ortega-Mora LM. Experimental infection with a low virulence isolate of *Neospora caninum* at 70 days gestation in cattle did not result in foetopathy. *Veterinary Research*. 2009;40(5):49.
74. Jiménez-Pelayo L, García-Sánchez M, Vázquez P, Regidor-Cerrillo J, Horcajo P, Collantes-Fernández E, Blanco-Murcia J, Gutiérrez-Expósito D, Román-Trufero A, Osoro K, Benavides J, Ortega-Mora LM. Early *Neospora caninum* infection dynamics in cattle after inoculation at mid-gestation with high (Nc-Spain7)- or low (Nc-Spain1H)-virulence isolates. *Veterinary Research*. 2019;50:72.
75. Imhof D, Anghel N, Winzer P, Balmer V, Ramseier J, Hänggeli K, Choi R, Hulverson MA, Whitman GR, Arnold SLM, Ojo KK, Van Voorhis WC, Doggett JS, Ortega-Mora LM, Hemphill A. In vitro activity, safety and in vivo efficacy of the novel bumped kinase inhibitor BKI-1748 in non-pregnant and pregnant mice experimentally infected with *Neospora caninum* tachyzoites and *Toxoplasma gondii* oocysts. *International Journal for Parasitology: Drugs and Drug Resistance*. 2021;16:90–101.

How to cite this article: Paudel BR, Bhattarai S, Chhetri S, Neupane S, Khati B. Host–parasite interface in *Neospora caninum*: Intracellular survival, immune restriction, and pathogenesis. *International Journal of Veterinary Sciences and Animal Husbandry*. 2026;11(10):01-08. doi: <https://www.doi.org/10.22271/veterinary.2026.v11.i10a.3820>