Artykuł przeglądowy *Review*

"One Health" approach to prevention and treatment of tick-borne babesiosis: Research advances in pharmaceuticals and vaccines

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Summary

Babesiosis, recognized as a neglected tropical disease (NTD), imposes a substantial public health burden on developing nations and represents a critical "One Health" concern. This zoonosis, caused by intraerythrocytic protozoan parasites of the genus *Babesia*, transmits primarily via tick vectors, with secondary routes including blood transfusions and transplacental transfer. Infections in mammals often lead to fetal loss or mortality, while immunocompromised individuals risk iatrogenic transmission through contaminated blood products or organ transplants, amplifying outbreak potential. Diverse drug sensitivities among *Babesia* species necessitate tailored therapeutic regimens. Moreover, prolonged subtherapeutic drug use in pastoral systems results in resistance, turning livestock into cryptic carriers. Pharmaceutical residues accumulating in ecosystems disrupt microbial communities, inadvertently fostering tick proliferation and exacerbating vector-borne disease cycles. The efficacy of the existing veterinary vaccines targeting tick vectors is limited by the incomplete understanding of immune evasion mechanisms, such as antigenic variation in *Babesia* virulence genes. Effective management of babesiosis is essential for mitigating economic losses in livestock production and safeguarding the health of companion animals amid escalating zoonotic threats. This study synthesizes empirical evidence on tick-mediated transmission dynamics and therapeutic advances, proposing a "One Health" framework to harmonize anti-parasitic treatment evaluation with translational strategies for pastoral and domestic animal management. By integrating parasitological insights with ecological and clinical data, we advance sustainable control paradigms while strengthening surveillance infrastructure to address evolving challenges under global ecological pressures.

Keywords: *Babesia*, ticks, One Health, enzyme, plant extracts, vaccine

Babesia spp. are naturally occurring protozoan pathogens with significant zoonotic potential and a complex life cycle characterized by sophisticated immune evasion mechanisms (94). Babesiosis manifests clinically with severe hemolytic anemia, fever (including low-grade presentations), pale or icteric mucous membranes, anorexia, agalactia, hyperesthesia, impaired rumination, forestomach hypomotility, dyspnea, tachycardia, and hemoglobinuria. Complications involve mainly four organ systems: cardiovascular, pulmonary, renal, and hematopoietic, with hematopoietic disorders being most prevalent. Commonly reported complications include acute respiratory distress syndrome (ARDS), congestive heart failure (CHF), disseminated intravascular coagulation (DIC), hemolytic anemia, splenic rupture, and renal failure, with significant variation in incidence and mortality (70). Rare sequelae include acute lung injury, pulmonary edema, warm antibody autoimmune hemolytic anemia (WAHA), thrombocytopenia, reactive hemophagocytic lymphohistiocytosis (HLH), and pancytopenia (4, 75, 77, 99). Babesiosis has global zoonotic importance, with over 100 identified *Babesia* species parasitizing wild and domestic mammalian hosts. Six species are human pathogens, and the most clinically significant of them are *Babesia microti* (*B. microti*), *Babesia divergens* (*B. divergens*), and *Babesia duncani* (*B. duncani*) (49). *B. microti* circulates primarily in rodent-tick

cycles, while *B. divergens* is a zoonotic pathogen of cattle. *Babesia bovis* (*B. bovis*), *B. bigemina*, and *Babesia caballi* (*B. caballi*) are endemic in pastoral regions, whereas *Babesia canis* (*B. canis*) and *Babesia gibsoni* (*B. gibsoni*) predominate in companion animals (44, 74). This zoonotic pathogen demonstrates broad mammalian host tropism. Ticks serve as biological vectors that transmit sporozoites to various mammalian hosts during blood-feeding activities. Such ecological interdependence between human and veterinary health paradigms epitomizes the core principles of the "One Health" framework, which emphasizes integrated surveillance of arthropod-borne pathogen transmission at human-animal-ecosystem interfaces. The molecular basis of erythrocyte invasion remains poorly characterized, primarily due to technical limitations in establishing reliable continuous in vitro cultivation systems for *Babesia* spp., which hinders mechanistic studies of host-parasite interactions. This knowledge gap persists particularly regarding tick-borne routes, vertical transmission (transplacental parasite migration in periparturient animals with untreated subclinical parasitemia), iatrogenic transmission, accidental inoculation (through erythrocyte replacement therapies in critical care settings and contaminated blood products during transfusion protocols), and vaccine breakthrough infections (pathogen dissemination via improperly inactivated vaccines or adjuvanted formulations) (40). Vertical transmission typically occurs in periparturient animals with persistent parasitemia following antenatal *Babesia* infection, where delayed antiprotozoal therapy permits transplacental parasite migration. Vaccine-derived dissemination frequently results from suboptimal vaccine production protocols, particularly inadequate pathogen attenuation or incomplete inactivation processes during antigen preparation. These multifactorial transmission dynamics generate three interconnected epidemiological consequences: (1) geographic expansion of endemic regions through anthropogenic landscape modifications and climate-driven vector migration patterns; (2) pathogen persistence in immunocompromised hosts, where species like *Babesia microti* establish chronic infections through immune evasion mechanisms (e.g., antigenic variation of VSA proteins); (3) accelerated evolution of drug resistance, observed particularly in human infections with *B. microti*, where suboptimal therapeutic practices (e.g., premature discontinuation of clindamycin-quinine regimens) create sustained selective pressures, progressively diminishing the efficacy of clinical treatment. This therapeutic challenge has driven two paradigm shifts in contemporary research: mechanistic characterization of drug resistance through standardized *in vitro* and *in vivo* evaluation platforms and exploration of innovative pharmacological targets. Phytochemical derivatives with demonstrated antipiroplasm activity, the existing ART and its derivatives (DHA, artemisine, artesunate, and artemeether) can be used to treat different species of *B. bovis*, *B. bigemina*, *B. caballi*, and *B. gibbsoni* (55). With rising worries over the side effects of medication and emerging drug resistance, there is a notable shift towards researching babesiacidal agents. Antimicrobial peptides, specifically cathelicidins known for their broad-spectrum activity and immunomodulatory functions, have emerged as potential candidates (37).

Integrated tick control strategies

As obligate hematophagous ectoparasites, ticks serve as competent vectors for numerous zoonotic pathogens. The emergence of persistent tick-borne diseases (TBDs), particularly human and veterinary babesiosis caused by *Babesia* spp., has resulted in markedly increased global incidence. The effectiveness of TBD containment depends on the disruption of the enzootic transmission cycle through preemptive elimination of vectors before they infect the host. Current evidence-based interventions include chemical control, strategic acaricide application, biological control, entomopathogenic fungi, and surveillance enhancement (GIS-based tick density mapping integrated with host movement patterns) (30, 51, 60, 70).

Conventional chemical acaricides for tick control include organochlorines, organophosphates, formamidines, pyrethroids, and macrocyclic lactones (Tab. 1). However, ecotoxicological concerns, par-

Tab. 1. Major chemosynthetic acaricides for tick control

Drug class	Representative agents	Mechanism of action	Advantages	Disadvantages	References
Organochlorines	DDT, DDD, Dicofol, Aldrin	Inhibit fertility and egg hatching via gene targeting	Effective against resistant ticks	High lipophilicity, bioaccumulation risks, banned in most regions	(22, 31, 66)
Organophosphates	Dimefox, Mipafox, Parathion	Neurotoxic effects via acetylcholinesterase inhibition	Controls organochlorine-resistant ticks	Neurotoxicity in mammals, motor system impairment	(33, 85)
Amitraz	Amitraz	Disrupts octopamine neurotransmission	Reduces oviposition in adult ticks	Carcinogenic potential, restricted use in livestock	(9, 57, 95)
Pyrethroids	Cypermethrin, Deltamethrin	Disrupt sodium channels in nerve cells	Larvicidal and ovicidal activity	Toxic to non-target organisms, environmental persistence	(24, 93)
Macrolides	Pikromycin, Tylosin	Induce muscle paralysis via neuronal inhibition	Effective against larvae and adults	Lipophilic properties, aquatic toxicity	(53, 84)
Neonicotinoids	Imidacloprid, Thiamethoxam	Genotoxic and neurotoxic effects	Suppresses female tick activity	High-dose neurotoxicity, mammalian brain atrophy risks	(18, 68, 88)

ticularly bioaccumulation risks and toxicity for non-target organisms, have led to regulatory restrictions on most classes. In intensive livestock systems, precise acaricide delivery is achieved through immersion systems and aerial dispersal (73). Phytochemical alternatives demonstrate multi-stage anti-tick efficacy (*Asteraceae*, *Fabaceae*, *Pinaceae*, *Meliaceous*, *Solanaceae*, *Verbenaceae*) (8, 15). These botanical extracts produce multi-stage inhibitory effects on tick development, targeting distinct lifecycle phases (larval, nymphal, and adult stages) and reproductive processes (vitellogenesis and oviposition). Their stage-specific efficacy and eco-compatibility position plant-derived formulations as critical components in next-generation integrated strategies of tick management, offering sustainable alternatives to synthetic chemical interventions (98). Numerous naturally occurring pathogens and predators demonstrate acaricidal potential against ixodid ticks. Specific viral strains, bacterial species, entomopathogenic fungi, predatory insects, and avian species exhibit measurable tick suppression efficacy (19, 45, 86). Anti-tick vaccine research currently prioritizes disruption of the pathogen-tick biological interface by targeting candidate protective antigens. These antigens are identified mostly through functional characterization of proteins critical to vector feeding behavior, reproductive physiology, developmental biology, and immunomodulatory processes (62). Current anti-tick vaccine candidates target three main functional categories: salivary gland antigens, midgut membrane antigens, and olfactory chemosensory antigens. Major vaccine modalities under development include protein-based subunit vaccines, DNA plasmid vaccines, mRNA lipid nanoparticle (LNP) vaccines, and viral vector platforms (23, 34, 62, 67). Among genome-editing tools, CRISPR-Cas9 has emerged as the most versatile and cost-effective platform for precise genetic manipulation in tick models (92). This technology can specifically control the development of pest populations and vector-borne diseases, altering the landscape of the genetic research of arthropods through gene editing and thus making it an operable reality (58).

Therapeutic interventions for babesiosis

Among hematoparasitic diseases affecting mammals, *Babesia* spp. infections rank second in global prevalence only to *Trypanosoma* spp. infections. Babesiosis outbreaks demonstrate marked geographic specificity (32, 50). Outbreaks of the disease are highly regional and may cause losses in dairy and meat production, the death of affected animals, and a negative impact on international livestock trade. Although chemical drugs and attenuated parasite vaccines have been approved for use in some countries, control of this disease is still poor (5). Based on the "One Health" concept, we investigated the significant progress in babesiosis-related drug treatment and vaccine R&D, including cases in both livestock and companion animals.

Previous chemosynthetic drug regimens for babesiosis

For decades, the therapeutic arsenal against babesiosis has been limited to antiprotozoal agents, including diminazene aceturate (DA), imidocarb dipropionate (ID), atovaquone (ATO), azithromycin (AZI), clindamycin, quinine, and clofazimine (CF). However, the emergence of drug-resistant *Babesia* strains has been an increasingly documented problem, compounded by the prolonged elimination half-lives and dose-dependent toxicities of the antiprotozoal agents. Human babesiosis, first identified in 1957 in a European asplenic patient, shares clinical hallmarks with *Plasmodium* infections (e.g., cyclical fevers and hemolytic anemia), with mortality rates exceeding 20% in immunocompromised and geriatric populations (97). The human-to-human transmission of babesiosis in healthy humans is usually caused by the transfusion of red blood cells and whole blood-derived platelet concentrates (25). It is characterized by non-immune hemolytic anemia, which can resolve after antibacterial and parasitemia clearance treatment. The Clindamycin-Quinine combination served as the 20th-century therapeutic standard, but caused frequent adverse effects. Contemporary protocols favor the Atovaquone-Azithromycin (ATO-AZI) regimen, demonstrating a comparable efficacy with reduced toxicity (48). The persistent chemotherapeutic rotation and evolving parasite resistance have led to a progressive decline in therapeutic efficacy, making it necessary to explore innovative intervention strategies. Current research prioritizes synergistic combination therapies (90).

In dogs, babesiosis is caused by several *Babesia* species, including *Babesia rossi* (*B. rossi*), *B. canis*, *B. gibsoni*, *Babesia conradae* (*B. conradae*), and *Babesia vogeli* (*B. vogeli*), and the most clinically significant infections are caused by *B. canis* and *B. gibsoni* (52). With regard to transmission blocking, experimental oral administration of afoxolaner (2.5 mg/kg) plus milbemycin oxime (0.5 mg/kg) (NexGard Spectra®), calculated based on body weight, completely prevented *B. canis* transmission. Treated dogs exhibited no clinical signs after a tick challenge, with all diagnostic tests yielding negative results (96). Similarly, a single oral dose of sarolaner, moxidectin, and pyrantel (Simparica Trio®), simulating the minimum recommended clinical dose, achieved 100% transmission blocking efficacy against *B. canis*. Dogs in the treatment group showed no clinical signs after a challenge at 21 or 28 days post-administration. Polymerase chain reaction (PCR) and immunofluorescence antibody (IFA) tests remained consistently negative, and parasitic ticks were rapidly eliminated (17). For therapeutic management of *B. gibsoni* infection in lactating bitches, a 10-day course of atovaquone, azithromycin, and artemether (Malarone® + artemether) cleared the infection. Partial clearance in puppies occurred via drug transfer in milk. Puppies not

cleared via milk required an extended 28-day course of the same drug combination for successful clearance. Oral tafenoquine (10-20 mg/kg), administered twice (day 0 and day 7), reduced clinical signs in *B. gibsoni*-infected dogs, although some recurrence was noted (46). Traditional treatments for canine babesiosis include imidocarb dipropionate (7.2 mg/kg, SC/IM) combined with diminazene aceturate (3.5 mg/kg, IM), often supplemented with supportive drugs, such as prednisolone (0.1 mg/kg), maropitant (anti-emetic, 1 mg/kg), and hepatoprotectants. Protocols for *B. gibsoni* typically involve atovaquone plus azithromycin, clindamycin, or proguanil. However, these traditional approaches are frequently associated with issues, such as drug resistance, high relapse rates, and the necessity for repeated administration (39).

In equids, *B. caballi* infection causes severe acute disease with high mortality in non-endemic areas. ID is a preferred treatment, inhibiting parasite energy metabolism by blocking inositol uptake (38, 59). The recommended regimen is 2.2 mg/kg intramuscularly (IM), though cholinergic side effects may necessitate anticholinergic therapy. It should be used with caution in pregnant and lactating mares (10). Buparvaquone (BPQ), which disrupts mitochondrial electron transport, is administered at 2.5-5 mg/kg IM with repeat dosing every 48-72 hours and produces mild adverse effects (21). Diminazene aceturate (5-7 mg/kg IM) inhibits nucleic acid synthesis. Novel agents, such as tafenoquine, show efficacy against canine babesiosis, but require equine validation. Although nitazoxanide derivatives demonstrate *in vitro* activity, they remain investigational. Combination therapies (e.g., imidocarb + oxytetracycline; BPQ + nitazoxanide) may enhance parasite clearance and reduce resistance development (59).

Evaluation of novel chemotherapeutic agents for babesiosis treatment

The screening pipeline for babesiosis therapeutics involves sequential *in vitro* growth inhibition assays of antimalarial/antiprotozoal compounds, followed by *in vivo* efficacy evaluation in murine infection models. To assess clinical improvement, therapeutic outcomes are monitored through parasitemia quantification (Giemsa-stained blood smears) and hematocrit restoration analyses. Promising candidates identified through this paradigm include naphthoquine phosphate (NQP), artesunate, ellagic acid microspheres (EA), and sitamaquine (SQ) (16, 35, 43, 56). NQP exhibits *in vitro* inhibitory activity against *B. gibsoni*, with an IC_{50} of 3.3 ± 0.5 μM. Artesunate demonstrates dose-dependent growth inhibition of *B. bovis* and *B. gibsoni in vitro* at concentrations ≥ 2.6 μM. *In vivo*, artesunate effectively treats *B. microti* infection in murine models, with doses $\geq$ 10 mg/kg body weight achieving significant parasite clearance. EA and its nanoparticle formulations (β-CD-EA, APSP-EA) show efficacy against multiple species: *B. bovis*, *B. bigemina*, *B. divergens*, and *B. caballi in vitro* (IC50 range: 0.92-9.58 μM), while *B. microti* was evaluated in mice *in vivo*, showing a peak parasite suppression rate of 68.1%. SQ inhibits *B. gibsoni* growth *in vitro*. Most of the antimalarial drugs have good inhibitory effects against *babesiosis*, proving their efficacy and potential as drug candidates. Current combinatorial strategies emphasize heat shock protein 90 (Hsp90) inhibitors, such as 17-DMAG, which paired with diminazene aceturate (DA) or atovaquone (AV), achieves a 3.2 × synergistic index (CI = 0.31) against *B. bovis in vitro*, with half-dose 17-DMAG (25 nM) combined with subtherapeutic DA concentrations yielding 89% growth inhibition (36). Rizk et al. pioneered atom pair fingerprint (APfp)-guided drug repositioning, revealing structural congruence (MSS = 0.87) between DA and imidocarb that enables low-dose synergy (6.25 mg/kg DA + 8.5 mg/kg ID enhancing *B. bovis* inhibition by 32%), while norfloxacin-luteolin-pyronaridine tetraphosphate (PYR) combinations (MSS = 0.79) demonstrate dose-dependent inhibition, reduced host cytotoxicity, and 68% suppression of *B. bigemina* at subtoxic concentrations. Both fluroquinolones significantly inhibited ($P < 0.05$) the *in vitro* growth of *B. bovis*, *B. bigemina*, and *B. caballi* in a dose-dependent manner. These structurally mimetic regimens mitigate resistance risks and toxicity associated with conventional monotherapies (79, 80, 82). These new combination drugs are very similar to the original antiparasitic drugs in structure and can be used as replacement drugs to overcome the potential problems of *Babesia* resistance and host toxicity induced by full dose DA, ID, and fluoroquinolones.

Emerging therapeutic targets in *Babesia* inhibition

Proteolytic enzymes regulating critical cellular processes – including proliferation, apoptosis, and differentiation (3, 6, 7, 91) – have emerged as promising therapeutic targets, with *aspartic proteases* implicated in the asexual replication cycle of *Babesia* spp. (63). Aderanti et al. identified five potent *in vitro* growth inhibitors of *Babesia duncani* (*B. duncani*) and *B. microti*, among which ixazomib demonstrated superior potency (IC = 12 nM) and therapeutic index (TI = 28), positioning it as a lead candidate for preclinical evaluation (2). Furthermore, phosphatidylinositol 4-kinase (PI4K), a conserved eukaryotic lipid kinase governing intracellular signaling, is targeted by the inhibitor MMV390048, which exhibits nanomolar efficacy against atovaquone-resistant *Babesia* strains (EC = 8.3 nM) and sustains parasite suppression in immunocompromised murine models through intermittent dosing (41, 42). Targeting specific enzymes in *Babesia* spp. has also been the subject of numerous studies, such as the parasite calcium-dependent protein kinase (CDPK) with specificity for the Bump Kinase Inhibitor (BKI), which has been shown to reduce

Tab. 2. Phytotherapeutic regimens for babesiosis

Plant extract	Combination therapy	Target parasite	Dosage (mg/kg)	Inhibition rate (%)	References
Resveratrol (RVT)	Imidocarb dipropionate (ID)	*B. microti*	5 RVT + 8.5 ID	81.55 (Day 10)	(26)
Artemisia herba-alba	Diminazene aceturate (DA)	*B. microti*	100 *A. herba-alba* + 15 DA	51.26 (Day 12)	(28)
Moringa oleifera (MOL)	Diminazene aceturate (DA)	*B. bovis*	80 MOL + 12.5 DA	78.57 (Day 10)	(83)
Pomegranate peel	Diminazene aceturate (DA)	*B. microti*	50 *Punica granatum* + 15 DA	66.00 (Day 12)	(29)
Ginger rhizome (ZOR)	Diminazene aceturate (DA)	*B. microti*	100 ZOR + 12.5 DA	76.77 (Day 6)	(81)
Artemisia herba-alba	Diminazene aceturate (DA)	*B. microti*	100 *A. herba-alba* + 15 DA	51.26 (Day 12)	(28)

infections by a variety of blood parasites (72). The calcium-dependent protein kinase (CDPK) family, particularly *CDPK4*, represents another critical vulnerability, with bumped kinase inhibitors (BKIs) inducing marked phenotypic alterations in *B. bovis* merogony by impairing schizont segmentation. Chemogenetic validation has identified *ASP2*, *ASP3*, and *PKG* as druggable targets essential for parasite invasion and differentiation, where *ASP3* and *PKG* coordinate glide some-mediated motility, while *CDPK4* regulates signal transduction cascades. Comparative genomic analyses reveal species-specific protease arsenals – *B. bovis* encodes 82 catalytically active proteases, *B. microti* 64, with 39 conserved orthologs – highlighting opportunities for selective inhibition of lineage-critical enzymes (27).

Phytotherapeutic strategies against drug-resistant *Babesia*

Medicinal plants have emerged as valuable sources of novel chemotherapeutic agents for refractory parasitic infections, offering enhanced therapeutic indices and reduced off-target effects compared to those of their synthetic counterparts. Bioactive phytoconstituents – including flavonoids, polyphenols, alkaloids, and sesquiterpenes – demonstrate potent anti-*Babesia* activity through multimodal mechanisms. Notably, synergistic combinations of flavonoid derivatives (trans-chalcone (TC) and chalcone 4-hydrate (CH)) with diminazene aceturate (DA) or clofazimine (CF) achieve suppression of *B. microti* parasitemia, outperforming monotherapies (14). *Cinnamomum verum* extracts AECV and EAECV contain large amounts of polyphenols, and the combination of the two inhibited *B. microti* at an effective concentration of 150 mg/kg (13). Polyphenol-rich extracts from the bark of *Cinnamomum verum* (AECV and EAECV) inhibit *B. microti* at 150 mg/kg through redox cycling disruption. The indoloquinoline alkaloid cryptolepine (CRY), when combined with DA (2.5 mg/kg CRY + 12.5 mg/kg DA) or atovaquone (AQ) (2.5 mg/kg CRY + 10 mg/kg AQ), reduces *B. microti* burdens by 92% within 12 days (12). Furthermore, the sesquiterpene nerolidol (100 mg/kg), isolated from *Citrus aurantium* essential oils, produces a broad-spectrum inhibition of *B. bovis*, *B. bigemina*, *B. ovata*, and *B. caballi* by destabilizing parasitic membranes (1). While single phytochemicals show species-specific efficacy, combinatorial regimens make it possible to reduce the dose (to 1/4-1/7 of monotherapy thresholds) while circumventing drug resistance. Current evidence underscores the need for standardized phytochemical formulations to balance therapeutic potency and safety profiles in veterinary applications (Tab. 2).

Advances in vaccine development against babesiosis

Babesia has different growth and reproduction stages in the host, and its antigen structure is complex. Current immunization strategies involve primarily live-attenuated vaccines derived from serial passaging in splenectomized bovines, recombinant protein vaccines, and viral vector-based platforms. While these vaccines induce moderate protection – particularly in yearling calves – their efficacy remains incomplete, and commercialization is hindered by safety concerns and regulatory constraints on food-producing animals (61). Under the "One Health" paradigm, understanding the dynamic interplay between *Babesia* spp., host immune responses, and transmission ecology is critical for developing next-generation vaccines that simultaneously mitigate acute disease and block zoonotic transmission. Recent studies leverage transcriptomic profiling to identify virulence-associated genes and differentially expressed transcripts across the developmental stages of parasites. Integrated Gene Ontology (GO) analyses further elucidate strain-specific variations in cellular components (e.g., apical complex proteins), biological processes (e.g., erythrocyte invasion pathways), and molecular functions (e.g., proteolytic activity), revealing molecular signatures linked to attenuation or pathogenicity, The data generated make it possible to identify and target specific genes, leading to the development of precise intervention strategies, such as new vaccines (87).

Live-attenuated vaccines

Conventional live-attenuated vaccines against babesiosis involve serial passaging of *Babesia*-infected blood from splenectomized donor animals into immunocompetent hosts, progressively attenuating parasite virulence until inoculated animals remain asymptomatic while retaining immunogenicity. Although this method is capable of broad immunization of suscep-

tible populations, it carries inherent risks: prolonged development timelines (> 5 years for strain stabilization) and potential transmission of occult pathogens (e.g., bovine leukemia virus or foot-and-mouth disease virus). Rauf et al. (76) developed a subcutaneous live-attenuated *B. bovis* vaccine that induces robust humoral immunity and cellular responses (CD4 and CD8 T-cell proliferation), achieving 89% parasite clearance in vaccinated calves. Current attenuation protocols, derived mostly from mid-20th-century methodologies, face critical limitations: (1) genomic divergence between field strains and attenuated lines, (2) vaccine-induced acute babesiosis in immunologically naive or genetically susceptible breeds, and (3) lack of validated molecular signatures correlating with stable virulence loss. Recent advancements address these challenges through non-tick-transmitted attenuated strains. The *B. bovis* Att-S74-T3Bo strain has been used as a candidate live attenuated vaccine for babesiosis in the experimental stage. Highly susceptible adult cattle are safe after vaccination with the Att-S74-T3Bo vaccine and can induce protection from virulence attack by homologous *B. bigemina* (11).

Recombinant subunit vaccines

Recombinant subunit vaccines, developed through the monoclonal antibody-based identification of native parasite antigens, elicit robust immune responses against *Babesia* infections. These vaccines, composed of non-living subunit antigens, eliminate the risk of vaccine-induced disease and exhibit enhanced stability, making them particularly suitable for immunocompromised individuals. Promising candidate antigens include apical membrane antigen 1 (AMA-1), CBA protein, heat shock protein 70 (HSP70), thrombospondin-related adhesive protein (TRAP), Bm2D41, and BgP50, all of which demonstrate significant inhibitory effects on *Babesia* growth *in vitro* and *in vivo* (54, 78, 100). The recombinant glycosylphosphatidylinositol-anchored protein (GPI-AP) vaccine has demonstrated efficacy in cattle and dogs. *Babesia divergens* recombinant Bd37 (a 37 kDa GPI-AP retaining its hydrophobic C-terminal sequence) maintains a membrane-bound conformation and induces broad-spectrum protection in hamsters. This vaccine confers heterologous protection, significantly reduces sepsis, and alleviates clinical signs. For *Babesia canis*, recombinant Bc28.1 (a 40 kDa GPI-AP) mimics the natural membrane-binding conformation. Vaccinated dogs develop high-titer antibodies that confer complete protection against virulent challenge and markedly reduce infection-associated pathology (65). Bivalent vaccines containing soluble parasite antigen (SPA) from *B. canis* and *B. rossi* culture supernatants induced clinical protection against *B. canis* challenge in vaccinated dogs. While these vaccines did not significantly mitigate parasitemia-associated anemia, they reduced plasma SPA levels. The vaccine-induced immune response varies across target species, suggesting divergent protective mechanisms (89). However, no commercial subunit vaccines are currently available, primarily because of two major challenges. First, recombinant protein antigens produced in *Escherichia coli* often lack the native conformational structures required for effective antigen presentation, limiting their immunogenicity. Second, the vast genomic diversity of *Babesia* spp. necessitates the discovery of additional protective antigens, as the current repertoire remains insufficient to address the parasite's antigenic complexity. *Babesia* parasites exhibit stage-specific antigen expression during their life cycle, complicating vaccine design. Single-antigen subunit vaccines may fail to provide comprehensive protection due to the dynamic nature of parasitic antigen presentation. To overcome this limitation, researchers are exploring multi-antigen formulations that combine targets from distinct developmental stages, such as erythrocytic merozoites and tick-phase sporozoites. This synergistic approach enhances immune efficacy by broadening antigenic coverage, prolonging protective immunity, and reducing transmission potential. For example, co-administration of AMA-1 (critical for erythrocyte invasion) and BgP50 (expressed during tick midgut development) has shown additive inhibitory effects in preclinical trials, underscoring the value of multi-stage antigen strategies. Further advancements in antigen selection, structural optimization, and delivery systems are essential to translate these findings into clinically viable vaccines.

Viral vector vaccines

Viral vector vaccines utilize non-pathogenic viral vectors as delivery platforms to enhance both humoral and cellular immune responses. Vaccination strategies employing DNA plasmids and poxvirus vectors (e.g., recombinant vaccinia virus or RVV) have demonstrated efficacy against *Babesia* infections in canine (*B. canis*) and bovine (*B. bovis*) hosts (20, 40). The modified vaccinia Ankara (MVA) strain, a highly attenuated non-replicating vector, elicits strong immunogenicity by expressing high levels of recombinant proteins in eukaryotic systems. For instance, vaccines encoding *B. canis* gametocyte antigen-related protein (BgGARP) via recombinant MVA plasmids induce significant reductions in infection rates and relapse frequencies when administered through prime-boost regimens. These viral vector platforms stimulate robust immune activation, including high-titer IgG antibodies, CD4 and CD8 T-cell proliferation, and elevated TNF-α/IFN-γ production, collectively enhancing host defenses against *B. bovis* challenges.

Challenges and prospects of babesiosis control

Ticks, as hematophagous arthropod vectors capable of transmitting multiple pathogens, pose escalating threats to livestock and human populations in agri-

cultural and pastoral regions. Controlling *Babesia* transmission remains challenging due to the ecological resilience of ticks, their broad geographic distribution across shrublands and mountainous terrains, and practical limitations in implementing acaricide applications. Current obstacles include insufficient integrated tick management programs, rising acaricide resistance, and inconsistent surveillance systems that fail to detect early outbreaks. In some endemic areas, reduced prevention efforts and inadequate protective measures among herders have led to resurgence events, with human and animal exposure remaining prevalent through occupational contact with infected livestock. Advancing babesiosis control requires optimized diagnostic technologies and deeper insights into parasite pathogenesis. Studies using animal models reveal species-specific virulence mechanisms – for instance, vascular occlusion dominates in infections by *B. bovis*, but not necessarily in those by *B. divergens*. Developing 3D organoid cultures could supplement traditional models while addressing ethical concerns (47). Seasonal and geographic patterns of tick activity dictate disease epidemiology, often complicating clinical management through co-infections with other tick-borne pathogens. Internationally, vector control has proven effective against malaria and dengue, which suggests that similar strategies could reduce *Babesia* transmission. However, overreliance on chemical acaricides accelerates resistance, necessitating novel approaches, such as anti-tick vaccines targeting salivary proteins. Such vaccines may induce localized immune responses that inhibit pathogen transmission during tick feeding (64, 69). Babesiosis control remains a multifaceted challenge requiring integrated approaches across parasitology, veterinary medicine, and public health. Current strategies, including live-attenuated vaccines, recombinant subunit formulations, and viral vector platforms, show promise but face limitations in efficacy, safety, and scalability. The ecological resilience of tick vectors, coupled with rising acaricide resistance and incomplete surveillance systems, further complicates disease management. Advances in multi-omics technologies and CRISPR-based gene editing have deepened our understanding of *Babesia* pathogenesis and revealed novel therapeutic targets, but translating these discoveries into field-applicable solutions demands sustained innovation. Future progress hinges on several key priorities. First, next-generation vaccines must address antigenic complexity through multi-stage antigen cocktails or pan-*Babesia* epitopes, leveraging structural vaccinology to enhance immunogenicity. Second, vector control strategies should integrate anti-tick vaccines targeting salivary proteins with environmentally sustainable acaricide rotation programs to mitigate resistance. Third, advanced surveillance systems incorporating meteorological data and machine learning algorithms can be used for real-time prediction of tick activity and outbreak risks. Finally, the development of 3D organoid models and multi-species infection platforms will refine our understanding of host-parasite interactions while reducing reliance on animal testing. The "One Health" paradigm remains central to these efforts, emphasizing the interconnectedness of human, animal, and environmental health. By fostering cross-disciplinary collaboration – linking genomic insights with ecological monitoring and clinical practice – researchers can design holistic interventions that curb *Babesia* transmission while preserving ecosystem integrity. As climate change and global trade reshape disease landscapes, proactive investments in vaccine development, vector control, and public health infrastructure will be critical to safeguarding both livestock economies and vulnerable human populations. Building on these advances, the scientific community is poised to transform babesiosis from a persistent threat into a manageable disease within the next decade.

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