



A Review in: Recent Advances and Challenges in Protozoal Vaccination Development in Veterinary Medicine

Anisha Ranabhat

Institute of Agriculture and Animal Science, Tribhuvan University, Kathmandu 44600, Nepal.

How to cite this paper: Anisha Ranabhat. (2026). A Review in: Recent Advances and Challenges in Protozoal Vaccination Development in Veterinary Medicine. *International Journal of Systems Biology and Bioinformatics*, 2(1), 1-7.
DOI: 10.26855/ijssbb.2026.06.001

Received: February 22, 2026
Accepted: March 16, 2026
Published: April 29, 2026

***Corresponding author:** Anisha Ranabhat, Institute of Agriculture and Animal Science, Tribhuvan University, Kathmandu 44600, Nepal.

Abstract

Protozoal disease remains a major formidable barrier to global livestock security, exerting profound constraint on animal welfare and agriculture economics. This review critically examine the current frontiers in veterinary protozoal immunology such as *Babesia*, *Theileria*, *Trypanosoma* and *Eimeria* alongside zoonotic threats like *Cryptosporidium* which remain a persistent foothold through sophisticated mechanisms of immune evasion, antigenic polymorphism and intricate multi stage life cycles. Despite these obstacle, we highlight the integration of immuno-informatics, reverse vaccinology and structural biology is catalyzing a shift towards rational vaccine design. Moreover, bridging the gap between these experimental breakthrough and hurdles, field ready solutions is now the imperative advances for ensuring sustainable livestock productivity and global food stability.

Keywords

Immunoprophylaxis; antiprotozoal immunity; recombinant vaccines; livestock health

1. Introduction

Protozoal disease represent a significant threat to a livestock health driving a significant economic losses through high morbidity and the silent drain of subclinical productivity declines. While chemotherapy has long been the primary line of defense, the rapid emergence of drug resistance strains in parasites such as *Babesia*, *Theileria*, *Trypanosoma*, *Eimeria*, *Toxoplasma*, has increase the necessity of strategic shift towards vaccination. Unlike the reactive nature of chemotherapeutic agents, vaccine offers a proactive framework for sustainable management, aligning with “One Health” to reduce chemical residues in the food chain.

The transition to vaccine based protection provides a sophisticated immunological landscape relying on a state of concomitant immunity, where host tolerates a low level pathogenic parasite to maintain a primed immune response. This defense is a multifaceted orchestration of cell mediated Th1 response and antibody production in order to counter the parasite's mechanism of antigenic variation. Ultimately, vaccinal protection in protozoology is a dynamic equilibrium rather than a static shield, sustained by synergy between vaccine-induced memory and continuous immunological boosting, creating a resilient and adaptive defense tailored to the realities of field conditions.

2. Current Vaccine Landscape: From Live-Attenuated to Next –Generation Platforms

Vaccination offers a long-term, sustainable strategy to mitigate the burden of protozoan diseases. However, the development of effective vaccines against protozoa remains challenging due to the complexity of parasite life cycles, high antigenic variability, and immune evasion strategies.

While most of the existing protozoal vaccines are for pathogens in the phylum Apicomplexa which are the sophisticated invaders that actively invade host cells and have both asexual and sexual reproductive cycles. Only a small number of vaccines have been produced for non-Apicomplexan protozoa and these organisms only have asexual reproduction. For example: amastigotes of *Leishmania spp.* multiply within phagocytes of the vertebrate host although they do not invade non-phagocytic cells, and the promastigote stages replicate extracellularly within arthropod vectors whereas *Giardia lamblia* and *Trichostrongylus axei* are strictly extracellular parasites [3].

To overcome the challenges of their complex life cycles and their ability to disguise themselves from the host immune system compelled the transition from risky live vaccines towards killed subunit and DNA platforms. These modern approaches aim to provide safer and more sustainable way to target the specific, stable antigens by mounting the host immune response to overcome the sophisticated evasion tactics of protozoan parasites [11].

Available vaccine against protozoan diseases of livestock

2.1 Live attenuated vaccines

T263: It is a bradyzoite of live mutant *T. gondii* that does not form an oocyst. The administration of T263 leads to reduction/ prevention of oocyst shedding in cats.

S48 strain (Toxovax): It is produced by repeated passage in mice (x3000) resulting losses in ability to form bradyzoite or oocyst. It reduces abortion and neonatal mortality by 75%. Ewes are vaccinated once, at least 3 weeks before breeding.

Coccivac B: This vaccine is prepared from anticoccidial-sensitive strains of *E. acervulina*, *E. mivati*, *E. maxima* and *E. tenella* and unlike present day field oocysts, these isolates had never been subjected to selection pressure by anticoccidials resulting in resistance. Coccivac B vaccine is a valuable tool to restore the performance of existing anticoccidials.

Coccivac D: It is a live, sporulated oocyst vaccine containing different spp of *Eimeria*. In order to produce complete immunity, the original dose of coccidial oocysts must complete at least four life cycles in the flock.

Cocovax E: It is a live anti-coccidial vaccine containing sporulated oocysts of *E dispersa*, *E meleagrimitis*, *E adenoids* and *E gallopavonis*. It is administered to day old turkey poults via spray cabinet.

Eimeriavax 4m: This vaccine consists of viable oocysts of *Eimeria acervulina* strain RA, *E maxima* Strain MCK+10, *E. necatrix* Strain mednec3+8 and *E tenella* Strain Rt3+15 suspended in PBS. It promises productivity improvements.

Rhaxshavac T: It contains live schizonts grown in lymphoblast cell culture, attenuated by prolonged in-vitro passage against *Theileria spp.* This cell culture derived vaccine has an efficacy up to 95–100%.

2.2 Killed Whole-Parasite and Subunit Vaccines

Giardiavax: It is a killed culture derived trophozoite vaccine prepared for dogs and it is found effective to prevent the disease and shedding of *G. lamblia*. The vaccine is derived from *G. duodenalis* isolated from sheep. Dose rate is 1mL subcutaneously. The 1st dose is given at 8 weeks of age and 2nd dose after 2-4 weeks and then repeat annually.

Bovilis Neoguard: It is a killed tachyzoite of *Neospora caninum* with spur adjuvant which reduces abortion in cattle by more than 50%. But the drawback of the vaccine is that it may increase the early embryonic death, if used in pregnancy. It is administered in 2 doses of 5ml at one month apart. The 1st dose is given between day 75 and 90 of gestation then booster in 3- 4 weeks with 2 annual boosters 3-5 weeks apart by subcutaneous route.

Pirodog/Nobivac Piro: It is a soluble parasite antigen prepared from supernatants of in vitro culture against *Babesia spp.* It gives 80% protection and the immunity lasts for about 6 months. The 1st dose is given when the animal is 6 months old and booster dose is 3-6 weeks after the initial vaccination and repeated every 6 months by I/M route.

2.3 Recombinant Protein and DNA Vaccines

For Toxoplasma:

SAG1: 100 % protection against lethal challenge

GRA4 and ROP2: Protection against brain cyst

For Leishmania:

Canileish vaccine (Virbac, Carros, France): It is composed of 100 µg of purified secreted–excreted *L. infantum* antigens (LiESP), produced using a technology patented by the Recherche pour le Développement (IRD), combined with 60 µg of the saponin adjuvant *Quillaja saponaria* but it is officially discontinued by the European Commission

due to production issue [5].

LetiFend: This vaccine features a chimeric protein, known as the Q protein. It comprises five antigenic fragments derived from four different *L. infantum* proteins, including LiP2a, LiP2b, LiP0, and H2A, without the addition of an adjuvant.

NeoLeish: This vaccine is composed of CpG DNA islands encoding the *L. infantum* activated protein kinase C receptor analogue (LACK) gene.

For Trypanosoma:

Beta-tubulin: The beta-tubulin gene of *T.evansi* was cloned and expressed in *E.coli*. It is important for cellular structure and physical functions. Recombinant beta-tubulin was expressed as inclusion bodies in *E.coli*.

Table 1. Summary of Protozoal Vaccine in Animal

Target Parasite	Vaccine type	Key Antigens	Host species	Administrative route	Key benefits
<i>Toxoplasma gondii</i>	T2639(Live attenuated)	Mutant bradyzoites (no oocysts)	Cats	Oral / General	Reduce oocysts shedding
	Toxovac/S48 (Live attenuated)	Mouse passaged strain (no bradyzoites)	Sheep (Pregnant ewes)	Parenteral	Reduces abortion or neonatal mortality
	DNA/Recombinant	SAG1, GRA4, ROP2	Research models	Parenteral	Brain cyst protection
<i>Trypanosoma</i>	Recombinant	Beta tubulin gene (from <i>T. evansi</i>)	Livestock (Experimental)	Parenteral	Target structural proteins
	MAP p15 (Subunit)	P15 protein	Mice (model)	Parenteral	100% protection against <i>T. brucei</i>
<i>Coccidia</i>	Coccivac B/D/E (live)	<i>Eimeria spp.</i> (sporulated oocysts)	Chickens / Turkeys	Spray/ feed/water	Restores sensitivity to anticoccidials
	Eimeriavax 4m (live)	Viable oocysts	Poultry	Oral	Productivity improvements
<i>Giardia</i>	Giardiavax (killed)	Trophozoite (from sheep)	Dogs	Subcutaneous	Prevent disease and shedding
<i>Babesia</i>	Pirodog/Nobivac Piro (subunit)	Soluble parasite antigens	Dogs	Intramuscular	80% protection provided
<i>Neospora</i>	Bovilis Neoguard (killed)	Trachyzoite with adjuvant	Cattle	Subcutaneous	Reduce abortion; not given in early pregnancy
<i>Theileria</i>	Rakshavac T (Live)	Attenuated schizonts	Cattle	Parenteral	95% efficacy
	Subunit	SPAG1, TAMS1	Cattle	Parenteral	Target sporozoite and merozoites
<i>Leishmania</i>	LentiFend (subunit)		Dogs	Parenteral	No adjuvant required
	NeoLeish (DNA)	CpG islands (LACK gene)	Dogs	Parenteral	Use gene encoding for PKC receptor analogue

3. Challenges in Vaccination Development

Vaccinating animal against the protozoal disease is a monumental challenge compared to bacterial or viral vaccine cause the protozoa are eukaryotes with complex, multicellular organism that have co-evolved with their host to disguise themselves from the host immune system.

These challenges stem from the parasites biological complexity, their ability to evade the host immune system and the practical difficulties of large-scale production of animal [4]. These biological hurdles, combined with economic

and practical constraints in veterinary medicine, make vaccine development far more challenging than for bacterial or viral diseases.

3.1 Biological Challenges

Complex life cycle or diversity in life cycle: Protozoa often have multiple developmental stages (e.g., sporozoites, merozoites, trachyzoites, and cysts), each expressing different structural and antigenic changes. So a vaccine targeting a specific one stage may not be effective against other stage.

Example: Malaria (*Plasmodium* species), the parasite moves from the mosquito (sporozoite) to the liver (ex erythrocytic stage) and then to the blood (erythrocytic stage). The RTS, S vaccine primarily targets the sporozoite stage to prevent liver infection, but in case the parasite leak into the blood stream, the vaccine protection is bypassed.

Antigenic Variation: Protozoan parasites cannot only generate diversity by genotypic variation. These parasites can also augment variability by replacing antigens which are exposed to the host immune system. This process is known as antigenic variation [1]. Continuous antigenic drift or shift necessitates frequent updates to vaccine formulations to match circulating strains since the shifting antigens can leads to B-cell exhaustion and the destruction of splenic memory, preventing the establishment of long-term protective immunity.

Examples:

- *Babesia bovis*, a major cause of bovine babesiosis, uses the **Variant erythrocyte Surface Antigen 1 (VSEA1)** to survive within the host through the rapid **segmental gene conversion**, creating a nearly infinite structural diversity. This **VESA1** surface protein promotes cytoadhesion and microvascular sequestration, leading to severe cerebral babesiosis [15].
- *Anaplasma marginale* is a rickettsial pathogen causing bovine anaplasmosis, with **Major Surface Protein 2 (MSP2)** which undergoes recombination of pseudo genes into a functional expression site. The antigenic shift in **MSP2** results in super infection, where animals can be infected by multiple strains simultaneously [14].

Immune Invasion: The ability of pathogens to evade the immune response is a major concern in vaccine development, as vaccines rely on the immune system's ability to recognize and eliminate the pathogen. Protozoa have evolved multiple strategies to avoid recognition and destruction by the host immune system, making it difficult for vaccines to induce lasting protection.

For example: *Trypanosoma brucei*: Switches its **Variant Surface Glycoproteins (VSGs)** regularly, making antibody-based vaccines ineffective.

Toxoplasma gondii: Survives inside host macrophages by preventing fusion of lysosomes with its parasitophorous vacuole, avoiding destruction and actively block the cell's natural defense mechanisms (like IFN- γ signaling) that would normally trigger cell death.

Species Diversity: Due to diversity in livestock species, each with unique immune response the vaccine often fails or unsafe due to different physiological system and immune response of various livestock species.

Example: *Eimeria* (Coccidiosis) in chicken. There are seven main species affecting chickens so a vaccine targeting only *Eimeria tenella* will leave the flock completely vulnerable to *E. maxima* or *E. acervulina*. Moreover, creating a 7 way vaccine is not economical friendly and is also complex than a single strain version.

3.2 Technical and Research Barriers

Lack of correlates of protection: For many protozoal disease scientist often fail to understand the host immune response due to diversity in livestock species. Each species with unique immune response causes the vaccine to often fail or unsafe due to different physiological system and immune response of various livestock species.

Example: In case of Bovine Neosporiasis (caused by *Neospora caninum*), for a vaccine to be effective, it must prevent trachyzoite multiplication and also prevent vertical transmission. But vaccine development face hurdles because we are not fully able to understand which specific immune markers (cytokines, antibody titers, or T-cell types) guarantee the vaccine effectiveness.

- **“Th1 vs. Th2” imbalance:** In most of the protozoal infection Th1 response (characterized by IFN- γ) is necessary to kill the parasite but pregnancy is naturally a Th2 biased state. So if a vaccine triggers a Th1 response strong enough to kill the parasite but might inadvertently cause inflammation at the placental interface, leading to fetal death.

Limited adjuvants and delivery system: Adjuvants are immuno-enhancing materials which increase the response to a vaccine, while not having any specific antigenic effect. Vaccine adjuvants straddle a fine line between tissue toxicity and efficacy. Adjuvant like Aluminum salts or oil-in-water emulsions can cause sterile abscesses or fibrous scarring which on during slaughter required carcass trimming, leading to economic loss. Beyond the injection site, potent adjuvants can cause lethargy and fever, which halts weight gain and milk production [8].

Resistance to chemotherapy: The overuse of chemotherapeutics has led to increased drug residues in animal products, posing risks to public health. For instance, *Plasmodium falciparum* has developed resistance to nearly all antimalarial drugs, including chloroquine and artemisinin derivatives [4]. Similarly, *Eimeria* has developed widespread resistance to almost all classes of coccidiostats (Ionophore and chemicals). If a bird is on a specific coccidiostat, it might kill the live-attenuated oocysts in the vaccine before they can trigger an immune response, rendering the vaccination useless.

4. Recent Advances in Protozoal Vaccine Development

4.1 Recombinant (Subunit vaccine)

Even though live attenuated vaccine has been a successful approach to vaccination against several protozoan diseases however, there are still several drawbacks to using live parasites like, the limited shelf-life of the vaccine, the possibility of causing morbidity and mortality in vaccinates, and the risk of attenuated organisms reverting to a more pathogenic state. So subunit vaccines, derived from native antigens of the parasite or as recombinant proteins from cloned DNA, may overcome these difficulties [12]. For e.g.:

Cryptosporidiosis: First maternal vaccine

- Recently in 2023/2024 MSD Animal Health received approval (UK/EU) for *Bovilis cryptium*, as a first subunit vaccine containing the recombinant **gp40 protein** to protect pregnant cattle against the parasite *Cryptosporidium parvum* in order to raise antibodies in colostrum against Gp40 of *C. parvum*. This novel vaccine offers preventive neonatal protection, which can help preserve cattle well-being from the earliest days of life, as well as help contribute to global food production and safety.

4.2 DNA Vaccine

DNA vaccines induce a complete immune response against the encoded antigen. They are extremely safe as they do not contain any pathogenic organism that may revert in virulence and also Anti DNA antibodies or autoimmunity have also been addressed in a growing number of preclinical and clinical studies, [2] which confirmed the high safety of these vaccines.

Canine Leishmaniasis: Market Shifts

- **NeoLeish (New candidate):** Neoleish is a third generation DNA vaccine based on the *L. infantum* LACK gene encoding the 36 kDa protein, analogue of the receptor of the activated protein kinase C (LACK/p36) included in the antibiotic resistance-free plasmid pPAL. Once safety and efficacy of this intranasally delivered vaccine was confirmed in the preclinical phase, this randomized double-blind field trial was performed to assess safety and efficacy of the Neoleish vaccine. It showed 3-fold lower risk (~72.7%) reduction in clinical disease and significant reduction in parasite load, positioning it as a strong future competitor [7].
- **Neosporum caninum** (cattle abortion): Recent trial with the **NcIs491** strain (live-frozen tachyzoites) was vaccinated on days 120–140 of pregnant heifers have shown promise in preventing abortion in naturally infected herds [6]. A frozen live vaccine is desired for use in the field, as it survives longer, permitting large-scale production, conservation, and availability.

4.3 Technological Innovations

Reverse Vaccinology and Bioinformatics

Reverse vaccinology is emerging as a rapid, cost-effective approach for antigen discovery, especially for bovine protozoal parasites like *Babesia*, though most studies remain in silico or early-stage [3]. Instead of growing the actual pathogen in a lab, scientist often use bioinformatics to screen the parasite's entire genome to identify hidden proteins that the immune system is likely to attack.

Nano-carrier and Oral Vaccine Systems

Nanoparticle-based oral vaccines can be developed by selecting a suitable carrier into which vaccines can be incorporated that can provoke a better immune response. Liposomal nanocarriers and oral vaccine formulations are being explored to enhance immunogenicity and ease of administration, with promising immune modulatory effects in preclinical studies [9]. This method provokes a stronger, more targeted immune response and allows for easier mass administration through feed or water.

Multivalent antiprotozoal vaccines

Multivalent vaccines aim to incorporate epitopes conserved across multiple protozoan species or strains, offering a cost-effective and scalable strategy for simultaneous protection against several pathogens. For example: the SAG1 and MIC antigens of *Toxoplasma gondii* show homology with surface antigens of *Neospora caninum*, suggesting the feasibility of dual-species vaccines [4]. This approach is highly scalable and reduces the cost of livestock management by providing simultaneous protection against several pathogens at once.

Conclusion

Vaccination offers significant promise in overcoming the challenges associated with developing effective vaccines for neglected protozoan diseases. Novel vaccine platforms, such as mRNA and viral vector vaccines, aim to induce broad immune responses against conserved pathogen epitopes, circumventing antigenic variation. Furthermore, advances in adjuvant technology and delivery systems enhance vaccine immunogenicity, promoting durable immunity against diverse pathogens. These innovations facilitate more efficient target identification, enhance vaccine design, and considerably shorten development timelines, establishing a new benchmark for combating protozoan infections. Ongoing research and investment in this field are crucial to fully leverage these powerful tools, ultimately advancing global health and reducing the impact of protozoan diseases worldwide.

By uniting computational tools, immuno-informatics, and experimental science, the global health community can transform the fight against protozoan diseases [4]. This evolution in vaccine architecture- supported by targeted investment-is the key to reducing the global burden of protozoan infections and delivering precision-medicine solutions to neglected regions. This approach not only addresses current challenges but also paves the way for innovative, accessible, and effective vaccines tailored to the unique needs of diverse populations worldwide.

References

- [1] Cornelissen AWCA, Schettters TPM. Vaccines against protozoal diseases of veterinary importance. *FEMS Immunol Med Microbiol*. 1996;15(2-3):61-72. doi:10.1111/j.1574-695X.1996.tb00055.x.
- [2] Dumonteil E. DNA Vaccines against Protozoan Parasites: Advances and Challenges. *J Biomed Biotechnol*. 2007;2007:90520. doi:10.1155/2007/90520.
- [3] Goodswen SJ, Kennedy PJ, Ellis JT. A state-of-the-art methodology for high-throughput in silico vaccine discovery against protozoan parasites and exemplified with discovered candidates for *Toxoplasma gondii*. *Sci Rep*. 2023;13(1):8243. doi:10.1038/s41598-023-34863-9.
- [4] Hashim O, Dimier-Poisson I. Computational vaccine development against protozoa. *Comput Struct Biotechnol J*. 2025;27:2386-2393. doi:10.1016/j.csbj.2025.06.011.
- [5] Martiniano De Pádua JA, Ribeiro D, De Aguiar VFF, et al. Overview of Commercial Vaccines Against Canine Visceral Leishmaniasis: Current Landscape and Future Directions. *Pathogens*. 2025;14(10):970. doi:10.3390/pathogens14100970.
- [6] Mazuz ML, Leibovitz B, Savitsky I, et al. The Effect of Vaccination with *Neospora caninum* Live-Frozen Tachyzoites on Abortion Rates of Naturally Infected Pregnant Cows. *Vaccines*. 2021;9(4):401. doi:10.3390/vaccines9040401.
- [7] Páez L, Parra A, Sotelo E, et al. Large-scale randomized double-blind field clinical trial for safety and efficacy assessment of the DNA vaccine Neoleish against canine leishmaniasis. *PLoS Negl Trop Dis*. 2025;19(11):e0012707. doi:10.1371/journal.pntd.0012707.
- [8] Wilson-Welder JH, Torres MP, Kipper MJ, Mallapragada SK, Wannemuehler MJ, Narasimhan B. Vaccine adjuvants: Current challenges and future approaches. *J Pharm Sci*. 2009;98(4):1278-1316. doi:10.1002/jps.21523.
- [9] Zafar A, Arshad R, Ur Rehman A, Ahmed N, Akhtar H. Recent Developments in Oral Delivery of Vaccines Using

Nanocarriers. *Vaccines*. 2023;11(2):490. doi:10.3390/vaccines11020490.

- [10] Patra G, Kumar A, Ghosh S, Lalnunpuia C, Bachan M, Saikia B, et al. Vaccines against protozoan parasites of veterinary importance: A review. *J Entomol Zool Stud*. 2017;5(6):1016-1021.
- [11] Wright IG, editor. *Veterinary protozoan and hemoparasite vaccines*. Boca Raton: CRC Press; 1989.
- [12] Jenkins MC. Advances and prospects for subunit vaccines against protozoa of veterinary importance. *Vet Parasitol*. 2001;101(3-4):291-310. doi:10.1016/S0304-4017(01)00557-X.
- [13] McAllister MM. Successful vaccines for naturally occurring protozoal diseases of animals should guide human vaccine research. A review of protozoal vaccines and their designs. *Parasitology*. 2014;141(5):624-640. doi:10.1017/S0031182013002060.
- [14] Pereira SH, Alves FP, Teixeira SMR. Animal Trypanosomiasis: Challenges and Prospects for New Vaccination Strategies. *Microorganisms*. 2024;12(12):2575. doi:10.3390/microorganisms12122575.
- [15] Xiao YP, Al-Khedery B, Allred DR. The *Babesia bovis* VESA1 virulence factor subunit 1b is encoded by the 1 β branch of the ves multigene family. *Mol Biochem Parasitol*. 2010;171(2):81-8. doi:10.1016/j.molbiopara.2010.03.001.