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BEYOND ANTIGENIC VARIATION: A REVIEW OF INNOVATIVE IMMUNO-EPIDEMIOLOGIC APPROACHES TO TRYPANOSOMIASIS VACCINE DESIGN

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ABSTRACT

Trypanosomiasis remains a major neglected tropical disease, largely uncontrolled due to rising drug resistance and the absence of an effective vaccine. Decades of vaccine failure are primarily linked to the parasite's immune-evasion strategy of Antigenic Variation (AV), driven by continual switching of the Variant Surface Glycoprotein (VSG) coat. This review proposes an integrated immuno-epidemiologic framework that moves beyond VSG-focused strategies by targeting conserved functional vulnerabilities and incorporating population-level transmission dynamics. The approach redirects vaccine development toward invariant, indispensable antigens such as the Transferrin Receptor (TfR), Invariant Surface Glycoproteins (ISGs), and Paraflagellar Rod (PFR) components. Antibodies against these molecules can induce conserved killing mechanisms – including iron starvation or disruption of essential membrane functions – regardless of VSG switching. Advances in reverse vaccinology enable multi-epitope and mRNA platforms to encode these structurally constrained targets, while complementary transmission-blocking and vector-focused strategies aim to interrupt parasite development within tsetse flies. A central argument is that partially protective vaccines (PPVs) can produce substantial epidemiologic benefits by lowering parasitemia, shortening infectious duration, and reducing the probability of vector infection. Such effects can meaningfully suppress transmission without requiring sterilizing immunity. A One Health approach, coupled with realistic population-based efficacy metrics, is essential for future vaccine development. By exploiting conserved molecular constraints and integrating immunologic, ecological, and epidemiologic insights, this framework outlines a practical pathway toward effective trypanosomiasis vaccination.

Keywords: *One Health, vaccines, antigenic variation, immuno-epidemiology, trypanosomiasis*

INTRODUCTION

Trypanosomiasis is a major neglected tropical disease caused by several species of *Trypanosoma*, affecting both humans, through Human African Trypanosomiasis (HAT, or sleeping sickness), which threatens millions with a fatal neurological disease, and animals through Animal African Trypanosomiasis (AAT, or Nagana), which is responsible for massive losses in livestock productivity and remains a primary obstacle to agricultural development in subsaharan Africa (1). These protozoan parasites are primarily transmitted by tsetse flies (*Glossina* sp.), although mechanical transmission by biting flies also contributes to disease spread (2). Two subspecies of *Trypanosoma brucei* cause HAT: *T. b. gambiense* and *T. b. rhodesiense* (2,3). *T. b. gambiense* predominates in West and Central Africa, causing a chronic, slow-progressing infection that often involves the central nervous system. In contrast, *T. b. rhodesiense*, found in East and Southern Africa, is a zoonotic parasite that leads to an acute, rapidly progressing disease with early systemic involvement (3). AAT, on the other hand, is caused by multiple species – *T. congolense, T. vivax, T. evansi, T. equiperdum, T. simiae, T. suis, T. theileri,* and *T. brucei* – and is responsible for significant losses in livestock productivity across sub-Saharan Africa, with wildlife often serving as reservoir hosts (4,5). The coexistence of competent vectors, abundant animal reservoirs, and human populations in endemic regions ensures the continued circulation of trypanosomes, sustaining both the public health and socioeconomic burden of the disease (6,7).

Current control strategies rely on chemotherapy and vector control, but these approaches are increasingly undermined by drug toxicity, resistance, limited efficacy, and a stagnant drug development pipeline (6,7). Vaccination remains the most promising long-term solution in both animals and humans; the One Health strategy for trypanosomiasis centers on the idea that vaccinating livestock serves as a "human shield" by reducing the animal reservoirs that lead to zoonotic human infection, however, despite extensive research, no trypanosomiasis vaccine has reached practical deployment. The central obstacle is the parasite's capacity for immune evasion through antigenic variation – its ability to continually switch its Variant Surface Glycoprotein (VSG) coat, and escape antibody recognition (8). While antigenic variation is a formidable challenge, decades of vaccine failures also reflect conceptual limitations: many approaches focus narrowly on molecular immune evasion without considering the broader host-parasite-population dynamics that shape infection and transmission (9).

This manuscript therefore reviews emerging immuno-epidemiologic frameworks for both human and animal trypanosomiasis, bridging molecular immunology with population-

level epidemiology. By integrating insights across these domains, we aim to redefine vaccine target selection, evaluation criteria, and design strategies for trypanosomiasis and other antigenically variable pathogens.

METHODS

This manuscript is structured as a narrative review synthesizing recent advances in trypanosome immunology, molecular biology, structural biology, and epidemiology. Literature was identified through targeted searches of PubMed, Web of Science, and Google Scholar using terms related to *Trypanosoma brucei*, antigenic variation, VSG structure, invariant antigens, and vaccine development. Priority was given to studies published between 2015-2025, with seminal older studies included where necessary to clarify foundational concepts. The goal was not to conduct a systematic evidence appraisal, but to integrate mechanistic insights and epidemiologic frameworks into a cohesive conceptual model for vaccine innovation.

BEYOND THE VSG BARRIER: MECHANISTIC CONSTRAINTS AND OPPORTUNITIES

Recent advances in structural biology, including high-resolution X-ray crystallography coupled with AlphaFold predictions, have deepened understanding of the Variant Surface Glycoprotein (VSG) coat, the central component of *Trypanosoma brucei* immune evasion (11). The VSG is a ~60 kDa homodimer composed of an elongated N-terminal domain and a compact C-terminal domain anchored to the membrane by a glycosylphosphatidylinositol (GPI) moiety (10). Although the parasite possesses an extensive genomic archive of VSG genes, stringent allelic exclusion ensures that only one VSG is expressed at a time (10,11), this single-expression system, coupled with access to a vast VSG repertoire, enables the parasite to generate an almost inexhaustible series of antigenically distinct coats during infection (10-13).

This architecture underlies two major evasion strategies: antigenic switching, which enables the parasite to stay ahead of host antibody responses by continually replacing the dominant VSG coat, and active suppression of humoral immunity, including destruction of B-cell memory in the spleen, which prevents the establishment of long-term protective immunity (12,13). In addition, the densely packed VSG layer facilitates rapid lateral movement and endocytosis of antibody-VSG complexes, thereby clearing bound immunoglobulins and complement factors before they can trigger lysis (13). Together, these interconnected mechanisms have historically rendered VSG-directed vaccination ineffective (11).

Although VSG antigenic variation imposes significant constraints, recent data suggest these barriers can be circumvented by shifting vaccine development toward invariant or semi-

invariant antigens essential to parasite survival (12), these molecules either reside beneath the VSG coat or are functionally critical enough that their structure and sequence remain highly conserved. One promising class includes the Invariant Surface Glycoproteins, particularly ISG65 and ISG75 (12,13). These proteins are stable across strains and implicated in nutrient uptake and surface protein turnover. Antibodies against ISG65/ISG75 may penetrate or map through the VSG layer sufficiently to disrupt membrane processes, induce opsonization, or potentiate phagocytic clearance mechanisms that do not depend on VSG identity (8,12,13).

The transferrin receptor (TfR) complex represents another compelling target. TfR mediates high-affinity iron acquisition, a process indispensable for parasite survival. Antibody-mediated inhibition of TfR would effectively starve the parasite of iron, impairing growth irrespective of the expressed VSG variant (14-16). Because iron uptake is fundamental to parasite metabolism, TfR is also less prone to diversifying selection, making it an attractive vaccine candidate.

Finally, elements of the paraflagellar rod (PFR), a trypanosome-specific cytoskeletal organelle required for motility, offer an internal but highly conserved target (17,18). Disruption of PFR function compromises parasite movement and survival within both mammalian hosts and the tsetse vector. Although accessibility remains a challenge, PFR-derived peptides incorporated into next-generation vaccine platforms could stimulate T-cell–mediated clearance. Collectively, these approaches signal a conceptual shift: rather than confronting the VSG barrier directly, vaccine strategies can exploit the parasite's conserved structural and functional dependencies, offering a rational pathway toward VSG-independent immunity.

IMMUNO-EPIDEMIOLOGIC RATIONALE FOR NOVEL VACCINES

Vaccine performance reflects interactions among pathogen biology, host genetics, and population-level factors (19). Individual variation in immune-related genes, including HLA alleles, cytokine profiles, and Toll-like receptors, modulates antigen processing and T/B-cell responses, underscoring the need for vaccine designs that extend beyond strain matching to incorporate host diversity, pathogen evolution, and ecological context (19).

Trypanosome infections produce dynamic immune disruptions – such as B-cell exhaustion and fluctuating parasitaemia – that influence infectiousness over time. An immuno-epidemiologic framework can link these within-host processes to transmission potential, prioritizing antigens that reduce peak parasitaemia, shorten the infectious period, or lower the probability of onward transmission (8).

A partially protective vaccine (PPV) that reduces parasite density and compresses the infectious window can generate substantial population-level benefits without requiring sterilizing immunity (19,20). As seen with the RTS, S malaria vaccine, reductions in infectivity alone can significantly lower incidence and confer herd effects (20,21). Incorporating PPV effects into transmission models, as continuous reductions in infectiousness rather than binary protection, helps identify ecological contexts (e.g., reservoir composition, vector density) where modest immunologic effects translate to large epidemiologic gains (21).

Decades of unsuccessful VSG-based and classical subunit vaccines highlight that future strategies must engage with both the parasite's immune-evasion machinery and the broader transmission system (9). A forward-looking framework integrates multi-epitope or mRNA platforms with targets that exploit conserved functional vulnerabilities and complements these with transmission-blocking or vector-directed approaches. Lessons from malaria, HIV, and influenza vaccine development provide useful parallels for designing interventions against antigenically variable pathogens.

TARGETING FUNCTIONAL VULNERABILITIES BEYOND VSG

Effective vaccine design must prioritize functionally constrained antigens, targets the parasite cannot readily modify without major fitness costs, rather than focusing solely on "invariant" surface markers. Although a diverse sub-VSG compartment exists, vaccinability requires demonstrable structural accessibility, essential function, and favorable endocytic kinetics (22). For example, ISG75 is immunogenic but likely fails due to rapid internalization and limited functional leverage (22,23). Priority targets therefore include nutrient-acquisition receptors, metabolic chokepoints (e.g., bloodstream-form glycolytic enzymes), and vector-stage molecules with reduced antigenic shielding.

Next-Generation Vaccine Platforms for Trypanosomiasis. Modern strategies for a trypanosomiasis vaccine are shifting from traditional methods toward a sophisticated array of molecular platforms designed to bypass the parasite's complex immune evasion. Recombinant protein vaccines have identified high-potential candidates like the invariant glycoprotein IFX and structural proteins such as β-tubulin and actin, though results remain mixed as antigens like ISG75 fail to provide protection despite their immunogenicity (24). To overcome these limitations, mRNA and self-amplifying RNA (saRNA) platforms – often paired with lipid nanoparticles – alongside viral vector-based systems are being developed to enhance T-cell activation and allow for the rapid, cost-effective production of multivalent vaccines suitable for field deployment (25). These are increasingly supplemented by immuno-informatics to design

virus-like particles and multi-epitope vaccines that focus the immune response on the most protective fragments of the parasite. Perhaps most promisingly, CRISPR-attenuated vaccines utilize precise gene editing to knock out multigene virulence families, such as trans-sialidases in *T. cruzi* or oligopeptidase B in *T. congolense*, creating safe, non-pathogenic live parasites that elicit a robust, broad-spectrum immunity far superior to single-subunit approaches (26).

Transmission-Blocking and Vector-Focused Strategies. A complementary approach is to target transmission rather than sterilizing immunity (27). Even modest reductions in parasitemia or vector infection efficiency can suppress population-level spread.

Key strategies include:

- Reducing reservoir competence: Livestock vaccination that lowers parasitemia decreases tsetse infection probability and can push transmission below sustainable thresholds.
- Targeting parasite stages in the tsetse fly**:** Antibodies against vector-stage epitopes may disrupt parasite development during midgut or salivary-gland transit.
- Anti-vector targets: Vaccines directed at essential tsetse midgut or salivary proteins may reduce feeding efficiency or vector competence.

Together, these host-, parasite-, and vector-focused strategies highlight that meaningful transmission reduction is achievable without sterilizing immunity and should be integral to next-generation vaccine design (27).

CROSS-DISEASE ANALOGIES AND TRANSLATABLE PRINCIPLES

Insights from other antigenically variable pathogens offer clear guidance for trypanosome vaccine design. Influenza “universal” vaccine efforts redirect immunity toward conserved structural elements (e.g., M2e, HA stem), HIV strategies target functionally constrained envelope domains, and malaria transmission-blocking vaccines show that population-level protection is achievable without sterilizing host immunity (21,29). Together, these examples reinforce the value of focusing on structurally constrained epitopes and integrating transmission-aware strategies into next-generation African trypanosomiasis vaccines.

CONCLUSION AND RECOMMENDATIONS

Strategic Path Forward – A Framework for Action. Advancing trypanosomiasis vaccine development requires an integrated framework linking molecular immunology, field epidemiology, and computational modeling. At the molecular level, priority should be given to conserved, functionally indispensable antigens, such as invariant surface proteins, nutrient-

uptake complexes, and essential enzymes, evaluated through standardized pipelines that assess structural accessibility, immunogenicity, and evolutionary stability.

Field epidemiology provides the ecological context needed to test and deploy these targets. Heterogeneity in host species, vector density, and transmission settings necessitates grounding antigen selection in real-world exposure patterns and reinfection dynamics, improving predictions of vaccine performance under natural pressures.

Computational modeling connects these domains by estimating how varying levels of partial immunity, coverage, and host-vector interactions influence transmission thresholds (30). Such models help identify the most impactful targets and guide optimal delivery strategies. Together, these three components form a continuous feedback loop that aligns laboratory discovery with ecological realities and predictive insights.

One Health Design and Realistic Efficacy Metrics. A sustainable vaccine strategy must adopt a One Health perspective, recognizing the interconnected roles of humans, livestock, wildlife reservoirs, and tsetse vectors. Vaccines should be evaluated for their cross-species benefits, for example, reducing livestock parasitemia can directly lower human exposure, while vector-stage antigens can block parasite development before it reaches mammalian hosts. This requires coordinated work across veterinary, public health, ecological, and molecular research sectors.

Equally important is redefining vaccine success, because sterilizing immunity is unlikely for pathogens with strong antigenic variation and immune-manipulation strategies. Instead, metrics should emphasize reductions in parasitemia, infectiousness, and disease progression, impacts that can substantially decrease transmission even without complete protection. Framing efficacy in terms of population-level impact broadens viable vaccine options and aligns with approaches used for other complex vector-borne diseases.

Antigenic variation, rather than an absolute barrier, should be viewed as a constraint that can be strategically exploited. By targeting conserved vulnerabilities and focusing on transmission reduction, vaccine design can shift from chasing antigenic diversity to influencing disease ecology. A roadmap grounded in molecular innovation, ecological insight, predictive modeling, and One Health collaboration provides the most realistic and impactful path toward trypanosomiasis control, and a blueprint for other antigenically variable pathogens.

REFERENCES

1. Papagni R, Novara R, Minardi ML, Frallonardo L, Panico GG, Pallara E, et al. Human African Trypanosomiasis (sleeping sickness): Current knowledge and future challenges. Front Trop Dis. 2023;4.
2. Franco J, Simarro P, Diarra A, Jannin J. Epidemiology of human African trypanosomiasis. Clin Epidemiol. 2014;6:257.
3. Steverding D. The history of African trypanosomiasis. Parasites Vectors. 2008;1(1):3.
4. Desquesnes M, Gonzatti M, Sazmand A, Thévenon S, Bossard G, Boulangé A, et al. A review on the diagnosis of animal trypanosomoses. Parasites Vectors. 2022;15(1).
5. Fèvre EM, Wissmann Bv, Welburn SC, Lutumba P. The burden of human African trypanosomiasis. PLoS Negl Trop Dis. 2008;2(12):e333.
6. Adewuyi O, Sijuwola E, Agbajelola VI. African animal trypanosomosis: Insight into host-parasite interactions and management strategies. World Sci News. 2025;199:75–89.
7. Venturelli A, Tagliazucchi L, Lima C, Venuti F, Malpezzi G, Magoulas GE, et al. Current treatments to control African Trypanosomiasis and one health perspective. Microorganisms. 2022;10(7):1298.
8. Magez S, Li Z, Nguyen HTT, Pinto Torres JE, Van Wielendaele P, Radwanska M, et al. The history of anti-trypanosome vaccine development shows that highly immunogenic and exposed pathogen-derived antigens are not necessarily good target candidates: Enolase and ISG75 as examples. Pathogens. 2021;10(8):1050.
9. Đaković S, Zeelen JP, Gkeka A, Chandra M, van Straaten M, Foti K, et al. A structural classification of the variant surface glycoproteins of the African trypanosome. PLoS Negl Trop Dis. 2023;17(9):e0011621–e0011621.
10. Zeelen J, van Straaten M, Verdi J, Hempelmann A, Hashemi H, Perez K, et al. Structure of trypanosome coat protein VSGsur and function in suramin resistance. Nat Microbiol. 2021;6(3):392–400.
11. Aresta-Branco F, Sanches-Vaz M, Bento F, Rodrigues JA, Figueiredo LM. African trypanosomes expressing multiple VSGs are rapidly eliminated by the host immune system. Proc Natl Acad Sci U S A. 2019;116(41):20725–35.
12. Banerjee S, Minshall N, Webb H, Carrington M. How are Trypanosoma brucei receptors protected from host antibody-mediated attack? BioEssays. 2024;46(7).

13. Umaer K, Aresta-Branco F, Chandra M, van Straaten M, Zeelen J, Lapouge K, et al. Dynamic, variable oligomerization and the trafficking of variant surface glycoproteins of Trypanosoma brucei. Traffic. 2021;22(8):274–83.
14. Pinger J, Chowdhury S, Papavasiliou FN. Variant surface glycoprotein density defines an immune evasion threshold for African trypanosomes undergoing antigenic variation. Nat Commun. 2017;8(1).
15. Sülzen H, Volkov AN, Geens R, Zahedifard F, Stijlemans B, Zoltner M, et al. Beyond the VSG layer: Exploring the role of intrinsic disorder in the invariant surface glycoproteins of African trypanosomes. PLoS Pathog. 2024;20(4):e1012186–e1012186.
16. Kariuki CK, Stijlemans B, Magez S. The trypanosomal transferrin receptor of Trypanosoma brucei—A review. Trop Med Infect Dis. 2019;4(4):126.
17. Dick CF, Alcântara CL, Carvalho-Kelly LF, Lacerda-Abreu MA, Cunha-e-Silva NL, Meyer-Fernandes JR, et al. Iron uptake controls Trypanosoma cruzi metabolic shift and cell proliferation. Antioxidants. 2023;12(5):984–984.
18. Portman N, Gull K. The paraflagellar rod of kinetoplastid parasites: From structure to components and function. Int J Parasitol. 2010;40(2):135–48.
19. van Dorst MM, Pyuza JJ, Nkurunungi G, Kullaya VI, Smits HH, Hogendoorn PC, et al. Immunological factors linked to geographical variation in vaccine responses. Nat Rev Immunol. 2024;24(4):250–63.
20. Akter J. Exploring mechanisms of host control and parasite growth during experimental blood-stage malaria in vivo [dissertation]. 2019.
21. Penny MA, Camponovo F, Chitnis N, Smith TA, Tanner M. Future use-cases of vaccines in malaria control and elimination. Parasite Epidemiol Control. 2020;10:e00145.
22. Oli AN, Obialor WO, Aneke CJ, Okoyeh JN, Emechebe GO, Aguiyi JC, et al. Immunoinformatics and immunogenomics: Novel solutions to emerging infectious diseases. ImmunoTargets Ther. 2020;9:11–27.
23. Danazumi AU, Gital SI, Idris S, Dibba LBS, Balogun EO, Górna MW. Immunoinformatic design of a putative multi-epitope vaccine candidate against Trypanosoma brucei gambiense. Comput Struct Biotechnol J. 2022;20:5574–85.
24. Pereira SH, Alves FP, Ribeiro M. Animal Trypanosomiasis: Challenges and Prospects for New Vaccination Strategies. Microorganisms. 2024;12(12):2575.
25. Poveda C, Leão AC, Mancino C, Taraballi F, Chen YL, Adhikari R, et al. Heterologous mRNA-protein vaccination with Tc24 induces a robust cellular immune response

against Trypanosoma cruzi, characterized by an increased level of polyfunctional CD8+ T-cells. Curr Res Immunol. 2023;4:100066.

26. Burle-Caldas GA, Dos Santos NSA, de Castro JT, Mugge FLB, Grazielle-Silva V, Oliveira AER, et al. Disruption of Active Trans-Sialidase Genes Impairs Egress from Mammalian Host Cells and Generates Highly Attenuated Trypanosoma cruzi Parasites. mBio. 2022;13(1):e03478-21.
27. Magez S, Li Z, Nguyen HTT, Pinto Torres JE, Van Wielendaele P, Radwanska M, Began J, Zoll S, Sterckx YG-J. The history of anti-trypanosome vaccine development shows that highly immunogenic and exposed pathogen-derived antigens are not necessarily good target candidates: Enolase and ISG75 as examples. Pathogens. 2020;9(8):618.
28. Helikumi M, Mushayabasa S. Mathematical modeling of trypanosomiasis control strategies in communities where human, cattle and wildlife interact. Anim Dis. 2023;3:25.
29. Tan MP, Tan WS, Alitheen NBM, Yap WB. M2e-based influenza vaccines with nucleoprotein: A review. Vaccines. 2021;9(7):739.
30. Michel-Todó L, Bigey P, Reche PA, Pinazo MJ, Gascón J, Alonso-Padilla J. Design of an epitope-based vaccine ensemble for animal trypanosomiasis by computational methods. Vaccines. 2020;8(1):130.

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