OPEN ACCESS Freely available online
http://www.banglajol.info/index.php/BJID/index
Perspective
Bangladesh Journal of Infectious Diseases
June 2025, Volume 12, Number 1, Page 174-180
ISSN (Online) 2411-670X
ISSN (Print) 2411-4820
NLM ID: 101761093
DOI: https://doi.org/10.3329/bjid.v12i1.84187

Bioinformatics-Driven Vaccine Design for Trematodes: Advances, Challenges, and Future Directions

Fatima Noor Tanzeem[1], Md Abdullah Yusuf[2]

[1]Post Graduate Student [M.Sc. in Biotechnology], Department of Biotechnology, BRAC University, Dhaka, Bangladesh; [2]Associate Professor, Department of Microbiology, National Institute of Neurosciences and Hospital, Dhaka, Bangladesh

Abstract

Trematodes, or flukes, are parasitic flatworms that cause neglected tropical diseases such as schistosomiasis, fascioliasis, clonorchiasis, opisthorchiasis, and paragonimiasis, affecting millions worldwide and burdening public health and livestock industries. Control currently relies on chemotherapy with drugs such as praziquantel and triclabendazole, but reinfection, emerging resistance, and challenges in sustained elimination underscore the urgent need for vaccines. Vaccine development, however, remains difficult due to the parasites' complex biology, multi-stage life cycles, and immune evasion strategies. Traditional empirical vaccine approaches have achieved limited success, but bioinformatics now offers transformative opportunities. Immunoinformatics enables systematic mining of genomic and proteomic data, prediction of immune-relevant antigens, cost-effective narrowing of candidates, and even cross-species applications. Key challenges remain, including stage-specific antigen diversity, parasite immune evasion, gaps between in silico predictions and in vivo validation, host genetic variability, and limited resources in endemic regions. Future prospects include integrating multi-omics data, applying artificial intelligence for improved predictions, and advancing novel platforms such as mRNA and nanoparticle vaccines. A One Health strategy targeting both humans and livestock could further enhance impact. Harnessing these approaches may finally enable effective trematode vaccines and sustainably reduce their global disease burden. [*Bangladesh Journal of Infectious Diseases, June 2025;12(1):174-180*]

Keywords: Trematodes; neglected tropical diseases; immunoinformatics; reverse vaccinology; vaccine development; schistosomiasis

Correspondence: Fatima Noor Tanzeem, Post Graduate Student [M.Sc. in Biotechnology], Department of Biotechnology, BRAC University, Dhaka, Bangladesh; **Email:** fatima_n_tanzeem@yahoo.com; **Cell No.:** +8801834527636;
ORCID: https://orcid.org/0009-0001-7840-8554

Introduction

Trematodes, commonly known as flukes, are parasitic flatworms belonging to the class *Trematoda* within the phylum *Platyhelminthes*. They are characterized by dorsoventrally flattened bodies, bilateral symmetry, and the presence of oral and ventral suckers that aid in attachment to host tissues. The external surface of trematodes is enclosed by a multinucleated epithelial layer known as the tegument. The tegument protects the parasite and aids in immune evasion, while being associated with nutrient absorption, secretion, osmoregulation and sensory functions[1]. Digenetic trematodes have complex life cycles involving multiple hosts, typically with mollusks, especially snails, serving as

the first intermediate host. Their life stages include eggs, miracidia, sporocysts, rediae, cercariae, metacercariae, and adult worms. Humans become infected through ingestion of metacercariae present in contaminated food or water or through skin contact with cercariae in the environment. Because of their high diversity and wide distribution, digenetic trematodes affect an estimate of 200 million people worldwide. These parasites cause a variety of neglected tropical diseases that continue to pose major public health challenges[2].

Among human trematodiases, five diseases stand out for their global health importance: schistosomiasis, fascioliasis, clonorchiasis, opisthorchiasis, and paragonimiasis. Schistosomiasis, caused by blood flukes of the genus Schistosoma, continues to pose a high health burden with prevalence rates up to 22% in certain endemic regions like Ethiopia, despite ongoing control efforts[3]. Globally, the disease affects over 251 million people across 78 low and middle-income countries, resulting in an estimated 2.19 million disability-adjusted life-years (DALYs). The three main species of Schistosoma that infect humans are *Schistosoma mansoni*, *Schistosoma japonicum*, and *Schistosoma haematobium*, with *S. haematobium* classified as a Group I carcinogen by the International Agency for Research on Cancer (IARC)[4,5].

Fascioliasis, caused by the liver flukes *Fasciola hepatica* and *Fasciola gigantica*, is an emerging zoonotic disease with a global prevalence of *approximately* 4.5%, disproportionately affecting regions of South America, Africa, and parts of Asia, with hotspots in Bolivia, Peru, and Egypt[6]. Beyond its public health significance, fascioliasis has a major economic impact on the livestock industry, with infection rates of 10–80% reported in cattle, leading to reduced meat, milk, and wool production. In humans, the infection can range from asymptomatic to symptomatic disease, manifesting as abdominal pain, anorexia, or gastrointestinal disturbances. Children are particularly vulnerable, with chronic infection contributing to anemia, malnutrition, and impaired growth and cognitive development. Globally, fascioliasis accounts for an estimated 90,000 DALYs, reflecting both acute and chronic morbidity. Long-term complications include hepatic fibrosis, biliary obstruction, and liver abscesses, sometimes necessitating surgical intervention[7].

Fish-borne liver flukes represent a critical public health challenge. Clonorchiasis, caused by *Clonorchis sinensis*, remains endemic in East Asia, affecting 15–20 million people globally and contributing to an estimated loss of 275,370 DALYs. The infection is associated with severe hepatobiliary complications, and in 2009 the International Agency for Research on Cancer (IARC) classified *C. sinensis* as a Group I biological carcinogen linked to cholangiocarcinoma[8,9]. Opisthorchiasis, caused by *Opisthorchis viverrini* and *Opisthorchis felineus*, is primarily endemic to Southeast Asia and parts of Eastern Europe. Both *C. sinensis* and *O. viverrini* are recognized Group I carcinogens. Chronic opisthorchiasis can result in hepatobiliary morbidity, cirrhosis, gallstone disease, and bile duct cancer[10–12]. Despite decades of control efforts, the disease remains endemic with frequent exacerbations, long-term progression, and diagnostic challenges.

Paragonimiasis, the lung fluke infection caused by species of *Paragonimus*, is distributed widely across Africa, Asia, and the Americas[13]. Its clinical manifestations often mimic tuberculosis, pneumonia, or even lung cancer, complicating diagnosis and delaying treatment. Severe or ectopic cases, particularly cerebral paragonimiasis, can be devastating if untreated[14,15].

Taken together, these diseases exemplify the dual threat of trematodiases to public health and economic stability. Schistosome and foodborne trematode infections affect more than 294 million people globally, leading to an annual loss of approximately 3.9 million DALYs. These trematodiases are prevalent in 78 countries and predominantly burden impoverished communities[5]. Transmission of FBTs occurs primarily through ingestion of metacercariae in raw or undercooked freshwater fish, crustaceans, or aquatic vegetation, while schistosome infections occur via skin penetration by cercariae in contaminated freshwater. Trematodes disproportionately affect populations with poor sanitation and limited public health awareness. Their diverse species, wide range of clinical presentations, lack of sensitive diagnostics, and weak surveillance systems contribute to underestimation and underreporting of the true burden. Cultural dietary habits and differences in host susceptibility further complicate accurate assessment of prevalence. This pattern makes reinfection common and complicates elimination efforts, even in regions with mass drug administration programs[12].

Current Therapeutic Strategies and Limitations

Despite decades of research and mass drug administration (MDA), the management of human trematodiases remains heavily dependent on a small number of drugs, particularly praziquantel and triclabendazole (TCBZ). For fascioliasis, TCBZ is

the only consistently effective treatment for both acute and chronic stages, but its availability is limited to a few endemic countries such as Peru, Egypt, and Ecuador, and treatment failures have been reported even under recommended regimens. Other drugs, including praziquantel, nitazoxanide, albendazole, metronidazole, and artemether, have shown poor or inconsistent efficacy, while oxfendazole has demonstrated effectiveness only in livestock. The extensive use of TCBZ in veterinary medicine has also driven the emergence of resistant *Fasciola hepatica* populations, raising concerns for zoonotic transmission[16].

Paragonimiasis is managed primarily with praziquantel, and although community-based programs that combine case detection, treatment, and education have shown impact, reinfection remains frequent due to persistent high-risk dietary practices, underdosing, and limited clinician awareness[12]. Clonorchiasis and opisthorchiasis are likewise treated with praziquantel, typically delivered through MDA alongside health education, but cultural traditions of raw fish consumption, poor compliance with multi-dose regimens, and the presence of untreated animal reservoirs such as dogs, cats, and pigs sustain high transmission. In opisthorchiasis, repeated cycles of treatment and reinfection not only risk drug resistance but may also exacerbate the long-term risk of cholangiocarcinoma[17].

Schistosomiasis control also relies almost entirely on praziquantel, which is effective against adult worms but not juvenile schistosomula, meaning that individuals treated early can still develop active disease and contribute to ongoing transmission. Standard regimens achieve 63–85% cure rates and significant reductions in egg excretion, and while artemisinin derivatives such as artemether and artesunate have shown promise against larval stages, their optimal use in combination with praziquantel requires further investigation[18].

Current chemotherapy-based control efforts face major limitations. Reinfection remains a pervasive challenge, drug availability is uneven, efficacy is often stage- or species-specific, and environmental and animal reservoirs sustain transmission cycles. Over-reliance on a small number of anthelmintics, particularly praziquantel and triclabendazole, further heightens the risk of emerging resistance. These constraints underscore the urgent need for novel, sustainable interventions. In this review, we acknowledge the profound severity of trematodiasis and the lack of effective interventions, and we highlight the potential of vaccines, especially those developed through bioinformatics, focusing on computational approaches that enable rational antigen discovery and design, as a promising solution by targeting multiple parasite stages, reducing reinfection, and complementing existing drug-based strategies for long-term control.

Rationale for Bioinformatics in Trematode Vaccine Design

While early vaccine studies using irradiated parasites provided valuable insights into protective immunity and key antigen candidates, translating these approaches into safe and effective human vaccines remains challenging. The complexity of trematode biology, multi-stage life cycles, and immune evasion strategies make empirical vaccine development slow and resource intensive[19]. In this context, bioinformatics offers a transformative approach to trematode vaccine design. Through immunoinformatics, researchers can rapidly mine genomic, transcriptomic, and proteomic data to identify antigens most likely to elicit protective immunity. Computational tools enable prediction of surface-exposed and secreted proteins, modeling of antigen–antibody interactions, and evaluation of epitope binding across diverse HLA alleles. This in silico-driven strategy reduces experimental burden, accelerates candidate selection, and allows cross-species applicability, opening the possibility for broad-spectrum vaccines effective against multiple trematode species.

Bioinformatics Approaches in Trematode Vaccine Design

A typical workflow starts with selecting vaccine targets from transcriptomic and proteomic datasets, prioritizing proteins highly expressed in infective or adult parasite stages and involved in essential processes such as metabolism, host invasion, and immune evasion. Immunoinformatics tools are used to predict B-cell, helper T-cell, and cytotoxic T-cell epitopes, with evaluation of antigenicity, allergenicity, toxicity, and population coverage to ensure a safe and focused immune response. Selected epitopes are assembled into multi-epitope constructs, incorporating peptide linkers to maintain structural integrity, adjuvants to enhance immunogenicity, and expression tags to improve protein production and stability, all optimized computationally prior to wet-lab testing. Structural bioinformatics and docking studies assess tertiary conformation and interactions with host immune receptors, such as toll-like receptors, while molecular dynamics simulations evaluate stability and flexibility. Codon optimization and in silico immune simulations further predict the

magnitude and quality of antibody and cellular responses. This integrative, computational approach enables rational vaccine design, reduces experimental burden, and can be applied across trematode species to target multiple life stages and conserved antigens, offering a promising path toward effective vaccines against neglected tropical diseases[20,21].

Current Advances Using Bioinformatics in Trematode Vaccines

Bioinformatics and immunoinformatics have significantly accelerated vaccine research for trematodiases. For schistosomiasis, reverse vaccinology and epitope prediction have prioritized surface and secreted proteins such as tetraspanins (Sm-TSP-2) and fatty acid–binding proteins (Sm14). Multi-epitope constructs derived from *S. mansoni* transmembrane proteins demonstrated stable interactions with TLR4 and predicted IFN-γ–mediated responses[22]. Proteomic profiling of *S. japonicum* schistosomula further revealed over 700 excretory-secretory proteins, including heat shock proteins, calpain, and tetraspanins, many with high immunogenic potential[23]. Translational progress is highlighted by SchistoShield®, an Sm-p80–based vaccine that has moved from bioinformatics-driven antigen selection into Phase 1 clinical trials, showing both safety and cross-stage efficacy[24].

In fascioliasis, immunoinformatics-guided screening of Fasciola hepatica proteomes has identified cathepsins (FhCL1–FhCL5), glutathione S-transferases, and leucine aminopeptidase as promising targets[23,25]. Epitope-based constructs derived from these proteins demonstrated favorable antigenicity, stability, and strong predicted immune activation in docking and molecular dynamics simulations. Proteomic studies of excretory-secretory products have broadened the antigen repertoire, while extracellular vesicles from *F. gigantica* have been shown to carry immune-evasive proteins, suggesting vesicle proteins as innovative vaccine targets[23].

For clonorchiasis, bioinformatics analyses have prioritized cysteine proteases (CsCP1–3) of *Clonorchis sinensis*. These proteins display stage-specific expression and have been validated in murine models, where recombinant protein immunization reduced hepatic pathology and induced strong mixed Th1/Th2 responses[26]. Such findings demonstrate the translational relevance of bioinformatics-prioritized proteases in guiding vaccine design against this carcinogenic liver fluke.

In opisthorchiasis, integration of genomic, transcriptomic, and proteomic datasets with immunoinformatics has identified tegumental proteins, antioxidant enzymes, and excretory-secretory products of *Opisthorchis viverrini* as vaccine candidates. Machine learning–based epitope prediction and docking studies suggest that these antigens could stimulate both humoral and cellular responses, offering a rational and targeted pipeline for vaccine development against opisthorchiasis[27].

Research on paragonimiasis is less advanced but increasingly supported by multi-omics resources. Comparative genomics of four *Paragonimus* species identified 256 conserved gene families enriched in immune evasion functions, providing a foundation for antigen discovery[28]. Proteomic investigations have highlighted cysteine proteases and heat shock proteins as immunogenic molecules[23]. Recent immunoinformatics studies targeting *Paragonimus westermani* cysteine proteases constructed a multi-epitope vaccine by linking antigenic epitopes with GPGPG linkers and a β-defensin adjuvant. The construct was predicted to be antigenic, nonallergenic, and water-soluble, with strong docking affinity to immune receptors and robust simulated activation of both B- and T-cell responses[29].

Collectively, bioinformatics and proteomics are transforming trematode vaccine development. Genome mining, epitope prediction, structural modeling, and immune simulations now allow rapid prioritization of antigens before expensive wet-lab testing. While most candidates remain at the computational or preclinical stage, the advance of SchistoShield® demonstrates real translational potential. Proteomic insights, including stage-specific secretory proteins, extracellular vesicle components, and conserved cross-species antigens, further support the design of broad and cross-protective vaccines.

Challenges and Limitations of Bioinformatics in Trematode Vaccine Design

Although bioinformatics has transformed antigen discovery, several challenges limit its effective application in trematode vaccine development. The first and most fundamental issue lies in the complex biology and antigenic diversity of trematodes. These parasites have intricated multi-stage life cycles, with each developmental stage—cercariae, schistosomula, and adult worms—expressing distinct proteomic profiles. Consequently, antigens predicted to be immunogenic at one stage may not confer protection at another. Moreover, trematodes

employ immune evasion strategies such as tegument shedding and secretion of immunomodulatory proteins, which complicates computational prediction of consistently protective targets[27].

A second major limitation is the gap between in silico predictions and in vivo validation. Many epitopes identified through immunoinformatics score highly for antigenicity and MHC binding but fail to elicit protective responses in animal models. Recent reviews emphasize that multi-epitope vaccines (MEVs), though rationally designed, often show inconsistent outcomes, with reported protection rates ranging from as little as 12.0% to nearly 100.0% across different trials[30]. This validation gap highlights the difficulty of translating computational promise into biological efficacy.

In addition, limitations within current bioinformatics pipelines constrain progress. Many tools were originally designed for bacterial or viral pathogens and require adaptation for multicellular parasites. Homology-based filters can mistakenly exclude potential antigens that share sequence similarity with host proteins, even when immunogenic domains are distinct. Similarly, essentiality predictions are often based on unicellular knockout models, which do not capture the functional redundancy and complexity of trematode biology. As emphasized in proteomics and vaccinology reviews, more sophisticated network-based approaches and improved annotation are necessary to overcome these shortcomings[31].

Another key challenge is genetic and population variability. Trematode species exhibit substantial strain-level diversity across endemic regions, and host populations differ markedly in their HLA allele frequencies. As a result, epitopes optimized for one geographic or genetic background may be less effective in another. This limits the universality of computationally designed vaccine candidates and complicates population-wide coverage[32].

The problem is compounded by incomplete functional annotation and database gaps. Although the number of sequenced trematode genomes and proteomes is growing, many proteins remain poorly characterized, and few have confirmed roles in virulence or host interaction. Without reliable experimental annotation, subtractive proteomics and epitope prediction are constrained, often yielding candidate lists that lack strong biological justification[31].

Finally, there are concerns regarding immunogenicity, safety, and resource availability. Epitope-based constructs, despite careful filtering for allergenicity and toxicity, may still induce weak immune responses or unintended cross-reactivity in vivo[32]. Moreover, since trematodiases predominantly affect low- and middle-income countries, the infrastructure required for high-throughput sequencing, structural modeling, and validation studies is often lacking, slowing the translation of computational predictions into viable vaccines[27].

These limitations show that while bioinformatics provides powerful tools for rational vaccine design, its outputs must be interpreted cautiously and integrated with experimental immunology. Bridging the validation gap, improving parasite-specific pipelines, and expanding functional annotation are essential next steps to ensure that computational predictions can reliably translate into effective trematode vaccines.

Conclusion

Bioinformatics has revolutionized trematode vaccine design by enabling rapid and rational antigen discovery through genome mining, reverse vaccinology, epitope prediction, and structural modeling. Promising candidates have emerged for schistosomiasis, fascioliasis, clonorchiasis, opisthorchiasis, and paragonimiasis, with SchistoShield® demonstrating translational potential in clinical trials. However, the complexity of parasite biology, stage-specific antigen diversity, immune evasion, and the persistent gap between in silico predictions and in vivo validation remain major hurdles. Future progress will rely on integrating bioinformatics with experimental immunology, multi-omics resources, artificial intelligence, and innovative platforms such as mRNA and nanoparticles, guided by a One Health perspective. Together, these approaches hold transformative potential to deliver effective vaccines and sustainably reduce the global burden of trematodiases.

Acknowledgements
None

Conflict of Interest
We declare that we have no conflict of interest.

Financial Disclosure
The authors received no specific funding for this work.

Authors' contributions
Conceptualization: Fatima Noor Tanzeem; Literature search and interpretation: Fatima Noor Tanzeem; Manuscript Writing: Fatima Noor Tanzeem; Review and editing: Md Abdullah Yusuf; Supervision: Md Abdullah Yusuf. Both authors revised

the manuscript for important intellectual content, approved the final version, and agreed to be accountable for all aspects of the work.

Data Availability
Not Applicable

Ethics Approval and Consent to Participate
Not Applicable

How to cite this article: Tanzeem FN, Yusuf MA. Bioinformatics-Driven Vaccine Design for Trematodes: Advances, Challenges, and Future Directions. Bangladesh J Infect Dis 2025;12(1):174-180

ORCID
Fatima Noor Tanzeem: https://orcid.org/0009-0001-7840-8554
Md Abdullah Yusuf: https://orcid.org/0000-0002-8551-7185

Article Info
Received on: 1 March 2025
Accepted on: 20 April 2025
Published on: 1 June 2025

References

1. Keiser J, Utzinger J. Food-borne trematodiases. Clinical Microbiology Reviews. 2009;22(3):466-83
2. Hu Y, Zhan RJ, Lu SL, Zhang YY, Zhou MY, Huang H, et al. Global distribution of zoonotic digenetic trematodes: a scoping review. Infectious Diseases of Poverty. 2024;13(1):46
3. Gebrehana DA, Molla GE, Endalew W, Teshome DF, Mekonnen FA, Angaw DA. Prevalence of schistosomiasis and its association with anemia in Ethiopia, 2024: A Systematic Review and Meta-Analysis. BMC Infectious Diseases. 2024;24(1):1040
4. Pirzaman AT, Sepidarkish M, Alizadeh F, Al-Obidy S, Ebrahimi P, Kianifard N, et al. Prevalence of human Schistosoma mansoni infection in endemic regions (2010–2024): A Systematic Review And Meta-Analysis. EClinicalMedicine. 2024;77:102855
5. Bennett AP, Robinson MW. Trematode proteomics: recent advances and future directions. Pathogens. 2021;10(3):348
6. Rosas-Hostos Infantes LR, Paredes Yataco GA, Ortiz-Martínez Y, Mayer T, Terashima A, Franco-Paredes C, et al. The global prevalence of human fascioliasis: a systematic review and meta-analysis. Therapeutic advances in infectious disease. 2023;10:20499361231185413
7. Caravedo MA, Cabada MM. Human fascioliasis: current epidemiological status and strategies for diagnosis, treatment, and control. Research and Reports in Tropical Medicine. 2020:149-58
8. Xu M, Jiang Y, Yin J, Cao S, Shen Y, Cao J. Risk factors for Clonorchis sinensis infection in residents of Binyang, Guangxi: a cross-sectional and logistic analysis study. Frontiers in Public Health. 2021;9:588325
9. Yoo WG, Sohn WM, Na BK. Current status of Clonorchis sinensis and clonorchiasis in Korea: epidemiological perspectives integrating the data from human and intermediate hosts. Parasitology. 2022;149(10):1296-305
10. Domatskiy VN, Sivkova EI. Opisthorchiasis-is an urgent medical and social problem in Russia. Journal of Biochemical Technology. 2022;13(4-2022):20-9
11. Kopolrat KY, Worasith C, Wongphutorn P, Techasen A, Eamudomkarn C, Sithithaworn J, Loilome W, Namwat N, Titapun A, Tawarungruang C, Thinkhamrop B. Large-scale epidemiology of opisthorchiasis in 21 provinces in Thailand based on diagnosis by fecal egg examination and urine antigen assay and analysis of risk factors for infection. PLOS Neglected Tropical Diseases. 2025;19(7):e0013095
12. Tidman R, Kanankege KS, Bangert M, Abela-Ridder B. Global prevalence of 4 neglected foodborne trematodes targeted for control by WHO: A scoping review to highlight the gaps. PLoS Neglected Tropical Diseases. 2023;17(3):e0011073
13. Blair D. Lung flukes of the genus Paragonimus: ancient and re-emerging pathogens. Parasitology. 2022;149(10):1286-95
14. Amaro DE, Cowell A, Tuohy MJ, Procop GW, Morhaime J, Reed SL. Cerebral paragonimiasis presenting with sudden death. The American journal of tropical medicine and hygiene. 2016;95(6):1424
15. Blair D. Paragonimiasis. InDigenetic Trematodes 2019 Jul 12 (pp. 105-138). Cham: Springer International Publishing.
16. Marcos L, Maco V, Terashima A. Triclabendazole for the treatment of human fascioliasis and the threat of treatment failures. Expert Review of Anti-infective Therapy. 2021;19(7):817-23
17. Bless PJ, Schär F, Khieu V, Kramme S, Muth S, Marti H, Odermatt P. High prevalence of large trematode eggs in schoolchildren in Cambodia. Acta tropica. 2015;141:295-302
18. Condeng YH, Katu S, Bakri S, Aman AM, Iskandar H. The impact of diagnosing and treating Schistosomiasis. Arch Balkan Med Union. 2024;59(1):112-7
19. Driciru E, Koopman JP, Cose S, Siddiqui AA, Yazdanbakhsh M, Elliott AM, Roestenberg M. Immunological considerations for Schistosoma vaccine development: transitioning to endemic settings. Frontiers in immunology. 2021;12:635985
20. Konhar R, Das KC, Nongrum A, Samal RR, Sarangi SK, Biswal DK. In silico design of an epitope-based vaccine ensemble for fasliolopsiasis. Frontiers in Genetics. 2025;15:1451853
21. Sanches RC, Tiwari S, Ferreira LC, Oliveira FM, Lopes MD, Passos MJ, et al. Immunoinformatics design of multi-epitope peptide-based vaccine against Schistosoma mansoni using transmembrane proteins as a target. Frontiers in immunology. 2021;12:621706
22. Sanches RC, Tiwari S, Ferreira LC, Oliveira FM, Lopes MD, Passos MJ, Maia EH, Taranto AG, Kato R, Azevedo VA, Lopes DO. Immunoinformatics design of multi-epitope peptide-based vaccine against Schistosoma mansoni using transmembrane proteins as a target. Frontiers in immunology. 2021;12:621706
23. Soleymani N, Sadr S, Santucciu C, Dianaty S, Lotfalizadeh N, Hajjafari A, Heshmati F, Borji H. Unveiling Novel Insights in Helminth Proteomics: Advancements, Applications, and Implications for Parasitology and Beyond. Biologics. 2024;4(3):314-44
24. Kim J, Davis J, Lee J, Cho SN, Yang K, Yang J, Bae S, Son J, Kim B, Whittington D, Siddiqui AA. An assessment of a GMP schistosomiasis vaccine (SchistoShield®). Frontiers in tropical diseases. 2024;5:1404943.
25. Alhassan HH, Ullah MI, Niazy AA, Alzarea SI, Alsaidan OA, Alzarea AI, Alsaidan AA, Alhassan AA, Alruwaili M, Alruwaili YS. Exploring glutathione transferase and Cathepsin L-like proteinase for designing of epitopes-based vaccine against Fasciola hepatica by immunoinformatics and biophysics studies. Frontiers in Immunology. 2024;15:1478107

26. Shi Y, Li X, Liang K, Lu T, Chen Y, Lai Y, Li Y, Wei S, He S, Tang L, Liu D. Characteristics and immunoprotective functions of three cysteine proteases from Clonorchis sinensis. Frontiers in Immunology. 2025;16:1550775
27. Kafle A, Ojha SC. Advancing vaccine development against Opisthorchis viverrini: A synergistic integration of omics technologies and advanced computational tools. Frontiers in Pharmacology. 2024;15:1410453.
28. Rosa BA, Choi YJ, McNulty SN, Jung H, Martin J, Agatsuma T, Sugiyama H, Le TH, Doanh PN, Maleewong W, Blair D. Comparative genomics and transcriptomics of 4 *Paragonimus* species provide insights into lung fluke parasitism and pathogenesis. GigaScience. 2020;9(7):giaa073.
29. Alzarea AI, Alhassan HH, Alzarea SI, Alanazi AS, Alotaibi FO, Alsaidan OA, Bakkari MA. Designing a Precision Epitope-Based Vaccine Against *Paragonimus westermani* Using Immunoinformatics, Biophysical Analysis, and Molecular Modeling Approaches. Journal of Computational Biophysics and Chemistry. 2026;25(8):1159-76.
30. Trujillo-Rodríguez I, López-Abán J, Alonso-Sardón M, Vicente-Santiago B, Muro-Álvarez A, Manzano-Román R. Current Efficacy of Multiepitope Vaccines Against Helminths: A Systematic Review. Biomolecules. 2025;15(6):867.
31. Sobrino I, Villar M, de la Fuente J. The path to anti-vector vaccines: current advances and limitations in proteomics and bioinformatics. Expert Review of Proteomics. 2024;21(12):497-500
32. Rehman A, Ahmad S, Shahid F, Albutti A, Alwashmi AS, Aljasir MA, Alhumeed N, Qasim M, Ashfaq UA, Tahir ul Qamar M. Integrated core proteomics, subtractive proteomics, and immunoinformatics investigation to unveil a potential multi-epitope vaccine against schistosomiasis. Vaccines. 2021;9(6): 658