# The contribution of *in silico* studies in Parasitology: A multifaceted approach

**Review Article**

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## ABSTRACT

The concept of a technical investigation using computational models (*in silico*) gained much attention of the scientific community over the past two decades. Though being relatively new, it succeeded in fulfilling several defects, and answering many problems that were left unresolved by the traditional experimental models such as *in vivo*, *ex vivo*, and *in vitro* studies. The drawbacks of *in vivo* models, for instance, include the use of animal models that may not exactly simulate humans; besides carrying risks to humans subjected to the experiment. Because both *ex vivo* and *in vitro* studies are conducted outside the body, they do not consider the interactive dynamics taking place inside the human body. Recently, the use of computational models evolved and was implemented in several areas of scientific research. The present review aims to highlight new model applications of *in silico* studies in parasitic diseases, in order to attain two main outcomes: understanding the parasite biology and the host-parasite relationship. This in return would lead to the identification of potential diagnostic biomarkers, drug targets, vaccine candidates, and new aspects of parasite epidemiology, molecular barcoding, phylogenetic tree construction, population epigenetics as well as suggested potential gene mutations linked to drug resistance.

**Keywords:** computational models; high throughput screening; *in silico*; parasitology; simulation; virtual screening.

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## INTRODUCTION

Over the past two decades, computational models were gradually introduced in various aspects of biological and medical fields such as diagnosis, treatment, and disease prevention. This was achieved by simulating real biological complex systems processes in a virtual environment, using a blend of mathematics with computer science. Computer modeling and simulations, referred to as "*in silico*", allow the study of multiple aspects and variables of the scientific experiment. Besides, their interactions with the stimuli in a system allow prediction of real-life scenario, reaching decisions, and probably recommends future actions[1]. As for biomedical research, multifaceted studies of the biological system matrices can be fulfilled by these simulation models, and the handling of huge raw data could be processed to add a piece to the puzzle of the building complex of the biomedical system[2].

*In silico* design has multidisciplinary usage in the biomedical field speeding up and simplifying target identification. Additionally, it determines the profiles of metabolism, excretion, and toxicity, thus eliminating any safety concerns[3]. Regulatory agencies and the pharmaceutical industry are working together to develop computational tools that will enhance the effectiveness and efficiency of drug discovery and developmental processes, thus reducing animal use, and improving predictability[4]. By using topological and 3-dimensional (3D) descriptors, it is now possible to correlate biological activities with the chemical structure, enabling the development of structure-activity relationship (SAR) models that can immediately assess the interaction of a ligand with its target molecule[5].

Drug development and vaccine discovery are two costly processes requiring immense time and resources. Pharmaceutical industries and researchers adopt computer-aided drug discovery techniques for simultaneous screening of compounds to locate new target molecules proposed as drug candidates (lead molecules)[6]. For this purpose, two approaches, direct or indirect, are used. Both of them use screening (virtual or high throughput), SAR, and molecular docking studies. The direct approach uses drug target structure (crystal structure or estimated 3-dimensional structure); whereas the pharmacophore data of the suspected drug target are used in the indirect approach[7].

Several confrontations face the establishment of *in silico* studies in parasitology. Among them is the lack of interest in investigating neglected tropical diseases (NTDs) by private organizations and large pharmaceutical companies[8]. Creation of these studies requires an efficient role of the academic communities to assist in the development of shorter approaches and more efficient strategies that drug manufacturers can optimize. From a chemical standpoint, molecules exhibiting bioactivity against NTDs have repeating structural scaffolds (e.g., privileged structures)[9]. These structural and molecular patterns received little attention even though, the technology to build

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more efficient structure-activity-phenotype techniques is now available, as well as the ability to forecast and propose shared targets across diseases. Such allocated structures present a starting point for creating novel therapeutic drugs because their synthetic feasibility can be assessed before biological testing. Another issue is creating models for predicting characteristics such as absorption, distribution, metabolism, excretion, and toxicity for these drugs[10,11].

The present review was conducted to enlighten the foundations of *in silico* applications in parasitic diseases, due to its importance in establishing an efficient understanding of several related issues. In return, additional familiarity with the available *in silico* applications would certainly accelerate development of novel therapeutic and protective strategies aiming to control and eradicate several parasitic diseases.

**Benefits of *in silico* studies in Parasitology research**

Application of *in silico* models in parasitic diseases, as a complementary method for the traditional experimental research, aimed at a better visualization of the parasite system biology, host-interactions, and dynamics[12]. It was implemented in several fields of research that included:

1. Cell models simulating spatial organization, cellular metabolic pathways, enzymatic processes, and gene expression in a spatial multidisciplinary way could replace *in vivo* models and overcome the ethical regulations concerning animal models or human subjects. It can also broaden the scope of *in vitro* studies by allowing numerous tests, and the isolation of the most effective variable in an experiment. Hence, traditional experiments are also needed for standardization, optimization, and validation, besides the ease of dealing with and storing of high throughput data provided by the omics studies[12]. All of this is provided using the correct scientific hypothesis[13]. It is worth mentioning that the creation of virtual tissue organs via simulation of complex physiological processes was achieved through highly advanced *in silico* simulation models[14].
2. *In silico* modeling can help understand the dynamics of parasitic diseases by enabling the study of specific biological interactions. Therefore, dynamics of parasitic diseases demand particular parameters in computational modelling that are related to the interactions between the parasite, host, and disease ecology. Parasite-related parameters are of great importance for the *in silico* modeling that includes infectivity, virulence, life-history, pathogenicity, and ability to evade the host immune system[15]. These parameters require input data for proper computational approaches. Several host-derived parameters are required such as host specificity, selection, genotype, general condition, sex, and age[15,16].
3. *In silico* models are vital for screening and optimization of novel anti parasitic drugs using large chemical libraries. Application of ligand-based approaches can be helpful to create models capable of clarifying the biological activity of anti-parasitic compounds[17]. Besides, molecular modeling for the prediction of receptors' interactions with the tested drug molecules allows a breakthrough in research involving product development as in drug discovery, delivery, biological activity, and end-organ interaction simulation, allowing safer and more effective end product[18].
4. Sequencing techniques and multi-omics approaches are applied in diagnosis, taxonomy, and phylogenetic studies especially with whole genome sequencing using bioinformatics for accurate species identification[19]. Construction of diagnostic immunoreagents is now achievable using a 3D structure-based *in silico* prediction of immunoreactive epitopes on the parasitic protein antigens[20,21]. Rapid advances in machine learning enabled various conformational epitopes to be accurately predicted and tested based on antigen sequences[22]. Likewise, these epitopes can help in further studies concerned with the stage of infection and disease progression[23]. It was observed that immunodiagnostic microarray platforms can be used for simultaneous detection of several target molecules in a single experiment[24]. The perception of using a single kit for a multiplexed immunodiagnostic platform ensures more rapid and accurate diagnosis in a simple setting, which is more suitable for large-scale patient examinations in developing countries[25].
5. Epidemiological studies of endemic and emerging parasitic diseases, update the status of the disease in populations. Discovery of new transmission patterns is mandatory to allow provision of efficient intervention strategies[26]. Spatiotemporal patiotemporal dynamics (geographical distribution and epidemiological data) of parasitic infections provided scientific data to enable *in silico* models contribution to assist in controlling disease spread by integrating prevention measures and vaccination[27].
6. Finally, interaction between parasite, host and disease geography add to understanding newly emerging and re-emerging parasitic diseases, pathogenesis, diagnosis, and treatment option[28].

In 2022, several studies included the application of *in silico* approaches in helminths and protozoan diseases. The following is a summary of the lastest updated *in silico* studies conducted during 2022.

***Plasmodium* spp.**

In search of novel drugs that can overcome chloroquine resistance, three pregnane glycosides isolated from *Gongronema latifolium* leafs were investigated against chloroquine-sensitive and resistant strains *in vitro*. It was demonstrated that iloneoside interacted with the catalytic sites of *P. falciparum* chloroquine resistance transporter, P-glycoprotein homology, plasmepsin IX, phosphatidylinositol

4-kinase, lactate dehydrogenase, and enoyl-acyl-carrier-protein reductase proteins in the same binding mechanism as the reference inhibitors[29]. Bokosi *et al*.[30] also investigated nine hybrid compounds of 7-substituted 4-aminoquinoline and cinnamic acid as anti-plasmodial drugs that demonstrated promising effectivity for further research.

The interactions and stability of the alkaloids with *P. falciparum* dihydrofolate reductase (*Pf*DHFR) and dihydroorotate dehydrogenase (*Pf*DHODH) were studied using molecular docking and molecular dynamics simulations to examine the interactions and stability of the alkaloids shown to be potential inhibitors of *Pf*DHFR[31]. A series of heterocyclic chloroquine hybrids containing either a β-phenethylamine fragment or a 2-aminoindane moiety were synthesized and screened *in vitro*, *in vivo*, and *in silico*[31]. Results of the latter research revealed that these hybrids exhibited a promising inhibitory effect against falcipain-2, the major cysteine protease in *P. falciparum*. At the same time molecular dynamic modeling for the human globular C1q receptor (gC1qR) to the Duffy binding-like β12 (DBLβ12) domain of a *P. falciparum* erythrocyte membrane protein family 1 (*Pf*EMP1) was investigated[32]. The study identified potential inhibitors that may block interaction between cytoadherence receptor gC1qR and its known binder protein DBLβ12 of *Pf*EMP1. Accordingly, the investigators concluded that these compounds could lead to the development of new drugs for the treatment and management of severe *falciparum* malaria.

The crucial function of aspartyl protease (plasmepsin V, PMV) in protein export assigned it as a preferred therapeutic target. Ji *et al*.[33] carried out virtual screening against a library of 1,535,478 chemicals using homology modelling of PMV structure, molecular docking, and pharmacophore model analysis. The results validated non-peptidomimetic inhibitors of *Pf*PMV activity for further development into a more effective PMV inhibitor. Ommi *et al*.[34] looked at the potential impact of supplemental unsaturated fatty acids on the development of *P. falciparum*. According to their findings, the amount of unsaturation in polyunsaturated fatty acids (PUFAs) affects the inhibition degree of *Plasmodium* proliferation. The results showed that PUFAs suppressed *Plasmodium* growth in proportion to their degree of unsaturation.

Since antimalarial combinations therapy proved the possible reduction of resistance, Wicha *et al*.[35] created a novel *in vitro-in silico* combination strategy to identify the pharmacodynamics interactions between two antimalarial medications (artefenomel-piperaquine and artefenomel-ferroquine). The investigators concluded that this technology will greatly enhance the economic development of new malaria medication combinations[35].

***Toxoplasma gondii***

Drug discovery and development mainly used atoms with nitrogen in their fundamental structures as building blocks. Almutairi *et al*.[36] developed specific tris-1,3,4-thiadiazole derivatives, synthesized using a multi-step synthesis approach and molecular docking. Compound 7, exhibited decrease parasite counts in the brain, liver, and spleen tissues of infected mice by 82.6%, 65.3%, and 64.81%, respectively.

Ayariga *et al*.[37] applied an *in silico* analysis of the transcriptional, translational, and replication machinery of *T. gondii* to determine the binding potentials of dihydroquinine to a few top-choice enzymes. This was found to prevent *T. gondii* from growing, invading, or egressing; in addition to reactive oxygen species production in the tachyzoites. However, all of these hypotheses required further studies to be confirmed through *in vitro* tests.

Due to their essential involvement in tachyzoites invasion and reproduction, primers targeting genes encoding dense-granule proteins 1, rhoptry protein 1, and microneme 3 were also developed. Using these primers, it was easy to distinguish between typical parasites and other species and quantify parasite genetic diversity[38].

***Lieshmania* spp.**

To investigate anti-leishmanial efficacy, molecular docking of Riparin synthetic analogues was utilized to assess four basic pharmacokinetic profiles: absorption, distribution, metabolism, and excretion-toxicity. Accordingly, the 18 *L. major* molecular targets used for molecular docking revealed inhibitory activity for riparins C and E. Consequently, riparins were suggested for anti-leishmanial therapy[39].

The tryptanthrin alkaloid's *in vitro* and *in silico* pharmacokinetic and toxicological potentials against intracellular amastigotes and promastigotes were evaluated[40]. Two series of thiazolidine compounds (7a-7e, and 8a-8e) were proposed by Gouveia *et al*.[41] against *L. infantum*, *in vitro* and *in silico*. Besides, investigation of the biological effects of gamma-terpinene (GT) on *Leishmania* emphasized its possible anti-leishmanial action, cytotoxicity, induction of apoptosis, modification of gene expression, and antioxidant activity. Regarding promastigotes and amastigotes of *L. major*, GT showed considerable dose-dependent anti-leishmanial effects[42]. Moreover, the anti-leishmanial activity of five xanthone analogues was investigated, along with their cytotoxicity testing by *in silico* research. The molecular mechanism of action of the produced compounds was examined using molecular docking. It was discovered that these compounds have strong binding affinities with various leishmanial enzymes that are crucial for the disease's critical process[43].

Since chalcones, whether natural or synthetic, are known to exhibit various biological activities, five analogs were investigated for their *in vitro* and *in silico* efficacy as possible anti-leishmanial agents by suppression of the trypanothione reductase enzyme. The highest level of inhibition against promastigotes was demonstrated by Chalcone 4[44]. Febrifugine dihydrochloride was also studied as a novel anti-leishmanial drug and was found to target *L. donovani*'s antioxidant system. Structure distortion and an increase in ROS levels were seen in the treated parasites compared to the control group[45]. Pyne *et al*.[46] also tested six phytochemicals found in *Garcinia cowa* against three characteristic *L. donovani* enzymes, i.e., O-acetylserine sulfhydrylase, trypanothione reductase, and N-myristoyltransferase as target proteins, compared to pentamidine. The study revealed that the *Garcinia cowa* plant has leishmanicidal properties. Additionally, Sinha *et al*.[47] recognized that *L. donovani*'s myo-inositol-1-phosphate synthase (MIP synthase) is a site of valproate action and can be considered as a target for anti-leishmanial medications and preventive measures.

It is worth mentioning that self-assembled peptide nanoparticles (SAPNs) are composed of monomers of short or repetitive amino acid sequences that unite to form nanostructures. Due to their multi-valency, SPPNs received mush interest in the field of vaccine design. A SAPN nanovaccine using a variety of immunoinformatics techniques was designed. Immunological and structural evaluations of the designed SAPN vaccine revealed that it protected against visceral leishmaniasis through online servers that apply different prediction methods. However, the investigators recommended further experimental validations to assess its efficacy[48].

***Trypanosoma* spp.**

The potential pathogenic and prognostic differentially expressed genes (DEGs) associated with human trypanosomiasis were identified through *in silico* bioinformatics analysis of genetic profiles from infected patients[11]. The investigators claimed that these genes have a variety of roles in the development, progression, and severity of trypanosomiasis and concluded that the identified DEGs results might serve as the basis for a better knowledge of trypanosomiasis in humans.

For drug discovery, Aminu *et al*.[49] examined the effects of oral administration of β-ionone to *T. congolense*-infected rats. Molecular docking study was also conducted to determine the mode of interaction of the ellagic acid to the catalytic domain of *T. rangeli* sialidase. Improvement of anemia and organ failure, as well as inhibitory effects on trypanosomal sialidase were noted. Muscat *et al*.[50] offered a thorough computational analysis of the *T. cruzi* unstructured region of cytochrome b catalytic site in order to illuminate the molecular mechanisms underlying the actions of known inhibitors and substrates. The researchers concluded that the cytochrome bc1's Qi catalytic site is a possible therapeutic target for *T. cruzi*.

**Free-living amoeba**

The effectiveness of modified alpha-mangostin against the tubulin protein of *Acanthamoeba* was investigated by Ongtanasup *et al*.[51]. The amino acid sequence from the National Center for Biotechnology Information was used to create a homology model of Acanthamoeba beta-tubulin (BT). Utilizing *in silico* study, the investigators demonstrated that modified alpha-mangostin compounds were bound to BT more effectively than alpha mangostin.

Ahmed *et al*.[52] published the synthesis of a library of ten functionally diverse quinazolinone derivatives (Q1-Q10) to evaluate their activity against *A. castellanii*. The excystation assays revealed that these compounds significantly inhibited *A. castellanii*'s morphological transformation. The *in silico* analysis confirmed that the drug target was sterol 14-alpha-demethylase that is essential in ergosterol synthesis pathway.

***Entamoeba histolytica***

The mitosome, a mitochondrion-related organelle, is an essential organelle for cellular differentiation and disease transmission, and thus plays an important role in *E. histolytica* lifestyle. A new membrane contact site between mitosomes and endosomes was identified as *Entamoeba* transmembrane mitosomal proteins 1 (ETMP1) and the *E. histolytica* domain 1 (*Eh*D1). The unique ETMP1-*Eh*D1 interaction suggested that this mitosome-endosome membrane contact point may be involved in a variety of physiological activities such as lipid and ion transport, and mitosome fission. It regulates protein exit from the endocytic recycling compartment to the plasma membrane. Similar to ETMP1-*Eh*D1, the investigators reported two examples in *P. falciparum* and *Dictyostelium discoideum*. The *Pf*EHD involved in endocytosis creates endocytic vesicles on the plasma membrane. These vesicles are subsequently directed to the neutral lipid generation/storage site, situated near the feeding vacuole. In the free-living amoebozoan *D. discoideum*, a single gene expressing EHD was identified as crucial in phagosome maturation. Its deletion caused abnormalities in intraphagosomal proteolysis and acidification, as well as early delivery of lysosomal enzymes[53]. Ghosh *et al*.[54] presented an *in silico* investigation of the presence of peptides or proteins with homology to S-phase kinase-associated protein 1 (Skp1) in *E. histolytica*. Since Skp1 is involved in cell cycle regulation, the study was conducted to understand its role in centrosome maturation, spindle assembly, mitotic exit, and cytokinesis. Based on the obtained results, the investigators proposed *Eh*Skp1 a novel drug target against amoebiasis.

Heat shock factor 7, a transcription factor, was identified specifically bound to the multidrug resistance (MDR) *E. histolytica* P-glycoprotein 5 gene promoter that could regulate MDR gene at the transcriptional

level in response to emetine administration[55]. López-Luis *et al*.[56] studied circular RNAs (circRNAs) from *E. histolytica* through *in silico* models and reported 143 and 605 reverse overlapping circRNAs from *E. invadens* and *E. histolytica* libraries, respectively. The results allowed the designing of a working hypothesis to evaluate the links between circRNAs and microRNA-like molecules in determining virulent/nonvirulent phenotypes and the discovery of other regulatory mechanisms during amoebic encystment.

Apte *et al*.[57] conducted an *in silico* study for the potential role of *E. histolytica* macropinocytosis in developing novel drugs against amoebiasis. Notably, macropinocytosis is an evolutionarily conserved process of bulk endocytosis in which cells absorb extracellular fluid and form huge, irregularly shaped vesicles, termed macropinosomes. Results revealed that lysophosphatidic acid that triggered cytoskeleton reorganisation to enhance macropinocytosis, was regulated by phosphoinositide kinase- *E. histolytica* guanine nucleotide exchange factor 2 and *E. histolytica* Rho5.

***Giardia Lamblia***

In an attempt to synthesize novel bioactive compounds against giardiasis, Xavier *et al*.[58] introduced thymol derivatives with superior anti-giardial action compared to thymol. The investigators utilized *in silico* prediction to demonstrate their theoretical oral bioavailability. In the *G. duodenalis* genome, a study discovered a symporter $H^+$:inorganic phosphate (Pi)-type of open reading frame (ORF) sequence, known as *Gd*PHO84. Since *Gd*PHO84 absorption was essentially required for *Giardia* physiology and metabolism, the investigators succeeded in using an H+-dependent inhibitor of Pi transport (PAA), and by *in silico* demonstrated inhibition of *G. duodenalis* cell proliferation[59].

***Cryptosporidium* spp.**

Building a 2D and 3D quantitative structure-activity relationship (QSAR) model against inosine 5' monophosphate dehydrogenase was studied[60]. Purine nucleotide biosynthesis in *Cryptosporidium* spp. is dependent on its inosine 5'monophosphate dehydrogenase (*Cp*IMPDH) enzyme. Hence, distortion of parasite IMPDH was pursued as a compelling strategy for curbing cryptosporidiosis due to its different kinetics from the host enzyme. To discover novel ligand molecules with noticeable activity against *Cp*IMPDH, Katiyar *et al*.[61] proposed using the molecular docking and molecular dynamics simulation analysis.

***Trichomonas vaginalis***

Concerned with the aspect of treatment, Alves *et al*.[62] discussed the effectiveness of two 2,8-bis(trifluoromethyl) quinolines (QDA-1 and QDA-2) against *T. vaginalis in vitro* and *in silico*. In order to assess the selectivity profile, molecular docking, biochemical, and cytotoxicity investigations were carried out. The outcome showed that QDA-1 significantly inhibited *T. vaginalis* growth.

Additionally, a novel nitric oxide synthase (NOS) identification and functional verification of *T. vaginalis* was attained using a hybrid-alignment annotation method and *in silico* models. The impact of a particular peptide generated from *T. vaginalis* transporter (*Tv*ZIP8) on the activation of macrophages was examined. The results revealed that the transporter caused generation of NO and H2O2, an upregulation of inducible NOS (iNOS) and nicotinamide adenine dinucleotide phosphate oxidase 2 (NOX-2) genes in murine macrophages, as well as marked rise in pro-inflammatory cytokines. The investigators proposed *Tv*ZIP8 a potential antigen for inducing a particular macrophage response against trichomoniasis[63]. Urbaski *et al*.[64] investigated the biochemical and structural characteristics of *T. vaginalis* beta-carbonic anhydrase (*Tv*CA-β). Its identification offered crucial information for developing selective inhibitors targeting *Tv*CA-β.

***Schistosoma* spp.**

Proposal of a suitable target for *Schistosoma* miRNA that modulates the host immune response was suggested to enhance the anti-schistosomal protective immunity[65]. In search of novel drugs, i.e., alternative for Praziquantel in the treatment of schistosomiasis, several *in silico* studies were conducted. Molecular docking studies identified glucose transport protein (SGTP4)[66], cathepsin B1[67], and thioredoxin glutathione reductase[68] as promising drug targets. In addition, 17 active compounds gave satisfactory drug-like properties in molecular docking models of five shistosomal protein kinases[69]. Menezes *et al*.[70] utilized structure-based virtual screening approach, as molecular docking simulation to detect potentially active alkaloids against *Schistosoma* spp. suggesting two of them as potential drug candidates. A study of the phenotypic screening of histone deacetylase inhibitors potency against *Schistosoma* was conducted by Hassan *et al*.[71]. Results revealed minimal potency against both adult *S. mansoni* and their newly transformed schistosomula activities. Besides, *Cucurbita maxima* compounds[72], *Ganoderma lucidum*[73], and eugenol derivatives[74] were also *in silico* studied as potential novel drugs. It was concluded that all exhibited the potentiality of serving as anti-schistosomal drugs, but required further *in vitro* and *in vivo* validation assays[72-74].

Considering the control aspect, the myco-synthesized nano-selenium antioxidant potentiality as a molluscicide for *Biomphlaria alexandrina* snails was suggested[75]. From the diagnostic aspect, 21 screening diagnostic antigens of schistosome membrane were identified[76]. The investigators proposed their usefulness in developing more reliable serological diagnosis and the recommending of serological evaluation. A new synthetic protein with several immunodominant B cell epitopes was proposed as a diagnostic kit[77]. On the vaccination level, development

of a potential 3D model vaccine targeting *Schistosoma* heat shock protein was proposed for safe use[78].

***Fasciola* spp.**

A vaccination candidate was designed *in silico* using four excretory/secretory antigens with promising results[79]. Twenty novel Ly6 family members were characterized in search for protective immunity development in vaccination trials[80]. Notably, Ly6 family members, also termed lymphocyte antigen 6, are complement cascade inhibitors that disrupt host complement dysregulation by preventing membrane attack complex formation as a sort of protective immunomodulation. To understand genetic diversity and phylogeny of *Fasciola*, Celik *et al*.[81] conducted an *in silico* approach utilizing mitochondrial cytochrome c oxidase subunit 1 (mt-CO1) gene fragments. The investigators identified 35 haplotypes most of which distinguished between different hosts and among various countries of origin. Accordingly, the *in silico* approach highlighted the potential for the emergence of novel *F. hepatica* strains in the future.

***Taenia* spp.**

A drug docking study was conducted to target seven anti-cysticidal proteases[82]. The investigators recommended 1,10-phenanthroline for further assessment in treatment of neurocysticercosis. An analysis of cytochrome oxidase for haplotype diversities conducted for epidemiological analyses, identified fifty-one haplotypes in relation to the geographical distribution. However, no special haplotype pattern was related to the host, which requires further studies[83].

***Echinococcus* spp.**

The major histocompatibility complex (MHC)-binding epitopes of *Eg*P29 protein as a potential multi-epitope vaccine candidate were suggested for *E. granulosus*[84] and for *E. cyclophilin*[85]. Both studies suggested that the properties of the candidate proteins allowed their usage in developing multi-epitope vaccines that require further validation. Since *E. multilocularis* possesses a proviral integration site for murine leukemia virus (*Em*PIM), Koike *et al*.[86] utilized an *in silico* modelling to discriminate between mammalian and parasite PIM sequences for discovery of kinase-specific inhibitors with better selectivity and less toxicity. Maglioco *et al*.[87] modeled and validated four histones representing different viability stages of *E. granulosus* using a three-dimensional structure of the parasite and characterized the presence of B-cell epitopes of histones H4 and H2A in the parasite genome.

In another study of the hydatid fluid proteomic profile of *E. granulosus* and *E. ortleppi* pulmonary bovine cysts, Dos Santos *et al*.[88] recognized the molecular tools used in survival strategies affecting host-parasite relationship, and that may be considered as targets for novel therapies development. Another study[89] conducted *in silico* comparative analysis of gene expression in *E. multilocularis* primary cell culture and primary cells under electroporated conditions after 48 h of culture. The investigators noted that ~15% of genes showed a significant alteration in gene expression level between them including highly upregulated genes in electroporated cells. The significant changed genes encoded calcium ion binding, proteolysis, glucose metabolism, and microtubule processing. The investigators advised that understanding gene function responsible for cellular distribution and decreased formation of cellular aggregates can help the scientists to improve the genetic studies of host-parasite interaction.

**Tissue nematodes causing lymphatic filariasis**

As revealed by Kumar *et al.*[90] in *in silico* and *ex vivo* studies, the use of the nutraceutical emodin as an immune-modulatory protein food in the treatment of filariasis showed better response than albendazole and diethyl carbamazine. The investigators recommended further studies to explore the molecular action of other nutraceutical foods. On the vaccine production level, a study targeting *Wuchereria* thioredoxin as a multi-epitope peptide-based vaccine was attempted by simulation and molecular docking. The investigators concluded that this result offers future hope for filarial elimination and recommended experimental and clinical validation[91]. Another *in silico* approach based on molecular docking, searched the effect of the phytocompound N-octanoate in the medicinal plant, *Calotropis procera* against filarial glutathione-s-transferase enzyme. The investigators recommended further *in vitro* and *in vivo* validation to consider n-octanoate as a potential drug candidate for lymphatic filariasis treatment[92].

***Onchocerca volvulus***

Assessment of the diagnostic value of the multi-epitope *O. volvulus* antigen (*Ov*MCBL02) revealed significant distinction between sera of patients from different areas in Africa and did not react with sera from other helminthic infections. The researchers suggested further *Ov*MCBL02 characterization to consider it as a new tool for diagnosis and elimination of onchocerciasis[93]. Results of another *in silico* docking analysis of *Onchocerca* superoxide dismutases to accentuate the potentiality of species-specific drug development showed structural diversity of the enzyme that can serve as an attractive drug target for combating onchoserciasis[94].

***Trichinella spiralis***

Molecular docking and *in silico* stimulation of *T. spiralis* membrane-associated progesterone receptor component 2 (*Ts*-MAPRC2) and human progesterone receptor membrane component 1 (PGRMC1) protein proved the formation of stable complexes, thus potentiating the use of *Ts*-MAPRC2 for the development of drugs and vaccines to treat trichinosis[95]. A co-evolution and phylogenetic study using molecular

docking for *T. spiralis* progesterone receptor membrane component-2 (PGRMC2-Ts) protein, proposed that its presence in the worm's ovarian oocytes is crucial for the life cycle and may promote the design of new specific drugs[96]. The joining of *in silico* and proteomics in the analysis of the muscle-stage *Trichinella* excretory-secretory products (ESPs) defined a regulatory motif associated with stichosome-derived protein that may be used to predict the potential metabolic functions in the regulation of the host immune system[97]. *In silico* analysis of antitumor peptides of *Trichinella* infective larvae extracts tested against human hepatocellular carcinoma suggested their antineoplastic activity[98].

**Intestinal nematodes**

For the diagnosis of strongyloidiasis, selected B cell epitopes of L3Nie.01 and IgG immunoreactive proteins were employed to design a multi-epitope protein and to study the physiochemical properties that qualifies it for use in diagnostic ELISA[99]. Another study predicted models for coproantigens testing to be used as potential specific diagnostic assay of *Strongyloides*[100]. In *Ascaris* spp., benzimidazole resistance associated with mutations in the β-tubulin protein family was identified by molecular docking simulations[101]. On the other hand, vaccine candidates against ascariasis were elaborated using a reverse vaccinology approach for both humans and pigs. The study predicted four proteins in *Ascaris* muscle or ovaries with strong affinity epitopes for both T-cells and B-cells. The investigators recommended further *in vitro* and *in vivo* experimental studies to prove the efficacy of these targets before the pharmaceutical medicinal research takes over[102].

**Other nematodes**

The extract of *Calophyllum macrophyllum* flowering plant was evaluated as a drug against *Angiostrongylus* eosinophilic meningitis by pharmacophore and molecular docking. The study observed that the pharmacokinetic profile of flavan-3-ols compound isolated from the extact could be presented as a potential anti-*Angiostrongylus* that needs further studies for better understanding of the mechanism of action *in vitro* and *in vivo* before presenting the proposal for pharmaceutical industry[103]. Using bioinformatics analysis and *in silico* characterization, the first global analysis of *Anisakis simplex* L3 larvae excretory-secretory proteins proposed the better understanding of L3 larvae survival and invasion strategies[104].

**The future vision for implementation of *in silico* studies in parasitic diseases**

In view of the global changes of ecological factors, the spread and transmission of parasitic diseases necessitates the development of novel research approaches. Therefore, this review recommends the following:

1. The implementation of the *in silico* approach in studying parasitic diseases is required when the dynamic nature of the parasite-host relationship cannot be simulated by conventional experimental models.
2. The alliance between *in silico* studies with different experimental models and clinical trials is needed for rapid and effective intervention to reduce morbidity and mortality of parasitic diseases.
3. The importance of applying these models in diagnosis, control, and treatment of parasitic diseases in relation to the cost-effectiveness is emphasized considering the global economic impacts of such diseases and the emergence of resistance to the current chemotherapeutics, and the absence of confirmed vaccinations.
4. The development of powerful predictive computer-aided tools to integrate high throughput data in complementary research, and the enlisting of *in silico* along with the "omics" techniques, is urgent to overcome the challenge of proper data extraction and integration.
5. A multidisciplinary collaborative effort between parasitology, computer science, bioinformatics, and data science are advised for standardization and implementation of these predictive models to be applied in personalized medicine.

## CONCLUDING REMARKS

1. *In silico* studies gained interest in the past decade since they allowed for quick predictions for a large number of chemicals in a high-throughput approach. Besides, they can make predictions based on a compound's structure even before it has been manufactured.
2. The interaction between the host and parasite creates an unstable dynamic in which successive waves of parasitemia can result in long-term parasite persistence. For example, *P. falciparum* can evolve a strategy known as clonal antigenic variation. This necessitates the systematic utilisation of a broad variety of host immune response targets.
3. Regarding diagnostic targets, *Ov*MANE1 reacts selectively with sera from individuals infected with *O. volvulus*.
4. *In silico* studies opened up new possibilities for the discovery of various novel drug classes with antiparasitic activity. Several drug targets in *Plasmodium*, *Leishmania* and *Schistosoma* spp. were identified, and *in silico* screening studies succeeded to develop selective inhibitors.
5. *In silico* studies enabled advanced, comprehensive databases that allow quick access to protein structure and function, peptide folding prediction, and precise molecular dynamics simulations. Advanced technology improved progress in vaccine development against *falciparum* malaria, leishmaniasis, schistosomiasis and hydatid cyst.
6. Yet this type of studies requires new partnerships with companies and governments for more funding and support for wet lab implementation.

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