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Research article

Letter to the editor of Heliyon re: Bioinformatics-based prediction and screening of immunogenic epitopes of *Toxoplasma gondii* rhoptry proteins 7, 21 and 22 as candidate vaccine target [Heliyon, 9 [7] July 2023, e18176]

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1. Dear editor

a recently-published article evaluated some of the immunoinformatics properties and immunogenic epitopes of three *Toxoplasma gondii* (*T. gondii*) rhoptry proteins (ROP7, ROP21 and ROP22) [1]. Next-generation vaccine design studies demand an orchestrated utilization of specific online web servers and/or standalone software for enhanced prediction of immunoinformatics features of vaccine candidate antigens and their immunogenic epitopes, based on the immune responses elicited naturally against respective pathogen [2]. As a result, potent multi-epitope vaccine candidates can be precisely engineered and designed [3]. Due to the complex life cycle of *T. gondii* infection and the diversity of the involved antigens as potential vaccine candidates, multi-epitope vaccine strategies are important for vaccine design strategies against toxoplasmosis [4,5].

In introduction section of this paper, the authors claimed that "developing vaccine pipelines are of utmost importance for both cattle and humans, due to global burden of latent disease". As we know, identification of viable *T. gondii* parasites and even clinical toxoplasmosis have rarely been documented in cattle, which is in sharp contrast with infection/disease in small ruminants [6]. Accordingly, toxoplasmosis vaccine pipeline seems to be cost-beneficial and clinically-effective in those population of warm-blooded animals that experience clinical sequelae of the disease and are of public health importance, including humans, small ruminants (intermediate hosts) as well as cats (definitive hosts) [7]. In Material and Methods section, the protein sequence retrieval was done using GenBank database; however, in case of the existence of specific databases such as ToxoDB (as *Toxoplasma* Informatics Resource) [8] the authors must extract protein sequences from such databases, not general ones. Although ElliPro tool can predict linear B-cell epitopes in a given protein sequence, but it is principally used to predict conformational B-cell epitopes [9,10]. Linear B-cell epitope prediction should be performed using multiple servers, applying multiple machine-learning prediction methods, to more cover potential epitopes, including SVMTriP, ABCpred, BepiPred 2.0, LBtope, etc. [11]. Hence, relying on a sole web server is not appropriate during *in silico* epitope mapping and should be avoided. Accordingly, the linear B-cell epitopes predicted in the present study are not reliable to be used in a multi-epitope vaccine design strategy. The major issue in the present study was immunogenicity evaluation for continuous B-cell epitopes! The authors utilized IEDB server class I immunogenicity, available at http://tools.iedb.org/immunogenicity/for this purpose. As the help section of this tool indicates, "This tool uses amino acid properties as well as their

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position within the peptide to predict the immunogenicity of a peptide MHC (pMHC) complex, particularly for 9-mer peptides". Also, the name of the online tool evidently suggests to immunogenicity evaluation of peptides with binding potential to class I major histocompatibility complex (MHC-I) [12]. Hence, this server has been erroneously used for immunogenicity prediction of B-cell epitopes and the results are not valid. In general, predicted linear B-cell epitopes in vaccine design studies must be screened in terms of antigenicity, allergenicity and peptide water solubility [13], while the latter was not done in the present study. A research paper that deals with determination of immunogenic epitopes should consider both humoral- and cellular-associated epitopic regions. The greatest disappointment in the present *in silico* study was lacking of epitope prediction for cell-mediated immune responses. Based on evidences, cell-mediated immunity is the most limiting response against intracellular microorganisms, including *T. gondii* [14], so that helper T-lymphocyte (HTL) and cytotoxic T-lymphocyte (CTL) epitopes should be predicted and screened during *in silico* epitope mapping of potential *T. gondii* vaccine candidates, along with B-cell epitopes. In conclusion, we believe that current manuscript done by Ayub et al. (2023), dealing with preliminary *in silico* properties of *Toxoplasma* ROP7, ROP21 and ROP22 proteins and determination of their immunogenic epitopes does not qualify the required standards, particularly for server-based epitope mapping sections.

Declarations

Both authors equally contributed to this work.

CRediT authorship contribution statement

Hamidreza Majidiani: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Conceptualization. **Amir Fotovati:** Writing – review & editing, Writing – original draft, Methodology, Investigation.

Declaration of competing interest

The author declares no conflict of interest.

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