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Immunoinformatics prediction of linear epitopes from *Taenia solium* TSOL18

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Abstract:
Cysticercosis is a public health problem in several developing countries. The oncosphere protein TSOL18 is the most immunogenic and protective antigen ever reported against porcine cysticercosis, although no specific epitope has been identified to account for these properties. Recent evidence suggests that protection might be associated with conformational epitopes. Linear epitopes from TSOL18 were computationally predicted and evaluated for immunogenicity and protection against porcine cysticercosis. A synthetic peptide was designed based on predicted linear B cell and T cell epitopes that are exposed on the surface of the theoretically modeled structure of TSOL18. Three surface epitopes from TSOL18 were predicted as immunogenic. A peptide comprising a linear arrangement of these epitopes was chemically synthesized. The capacity of the synthetic peptide to protect pigs against an oral challenge with *Taenia solium* proglottids was tested in a vaccine trial. The synthetic peptide was able to produce IgG antibodies in pigs and was associated to a reduction of the number of cysts, although was not able to provide complete protection, defined as the complete absence of cysts in necropsy. This study demonstrated that B cell and T cell predicted epitopes from TSOL18 were not able to completely protect pigs against an oral challenge with *Taenia solium* proglottids. Therefore, other linear epitopes or eventually conformational epitopes may be responsible for the protection conferred by TSOL18.

Background:
Taeniasis and cysticercosis are infectious parasitic diseases caused by different stages of the tapeworm *Taenia solium*. An infection with the larval stage in humans frequently causes neurocysticercosis, which is an important contributor to neurological morbidity in developing countries **[1]**, and a presumable principal cause of acquired epilepsy in the world **[1, 2]**. Given the essential role of the pig as the obligate intermediate host, porcine vaccines are promising tools in a sustained control of taeniasis/cysticercosis and are crucial to achieve possible eradication of this disease. TSOL18 is a recombinant protein which has been shown repeatedly to be highly protective when used as a vaccine against *T. solium* infection in pigs **[3, 4]**. TSOL18, along with other oncosphere antigens, has previously been identified as comprising a fibronectin type III domain but no specific role has been attributed yet **[5]**. It would be of great value if the protective epitopes associated with this protein were known and, if they could be produced synthetically, this would be likely to offer cost and quality consistency advantages over the current vaccine that is produced in *Escherichia coli* **[3]**.

Synthetic peptides are being tested as potential vaccines in a wide range of infectious diseases **[6]**. It has been shown that the use of synthetic peptides/epitopes could offer an important contribution to protection against *T. solium* cysticercosis in pigs **[7-9]**. Current immunoinformatics tools are able to predict human B cell and T cell linear epitopes with high accuracy. These tools are playing an important role in understanding the molecular basis of immunity and notably in the development of peptide vaccines, immunotherapy against cancer and autoimmune diseases **[10-14]**. Similar immunoinformatics approaches are being developed for other organisms, and will constitute the basis for the design of new generation epitope vaccines. This manuscript concerns the computational prediction of immunogenic epitopes of the TSOL18 protein from *T. solium* and the design of a synthetic peptide vaccine candidate. We hypothesize that TSOL18 comprises a linear epitope able to protect pigs against an oral challenge with proglottids with an efficacy close to 100%. We used molecular modeling and immunoinformatics tools to predict linear B cell and T cell immunogenic epitopes of TSOL18. The theoretically predicted structure of TSOL18 was used to identify the epitopes exposed on the protein surface. A synthetic peptide encompassing three predicted epitopes was predicted and tested in a porcine vaccine trial.

Methodology:
Cellular localization of TSOL18:
The amino acid sequence of TSOL18 was obtained from the Genbank (accession code AF017788). The prediction of the cellular localization was performed with the web servers TMHMM **[15]** and TMPred **[16]**. The presence of a signal peptide was tested with SignalP V1.1 **[17]**. Transmembrane helices

were predicted by HMMTOP **[18]**, SOSUI **[19],** TMpred **[16]**, TopPred **[20]** and PredictProtein **[21].** The hydrophobicity profile of TSOL18 was predicted with ProtScale **[22]** using the Kyte-Dolittle scale **[23]**.

Structural model building:
TSOL18 does not yet have any crystallized homologue protein. The TSOL18 structure was modeled by threading using servers Fugue **[24]** and 3D-PSSM **[25, 26]**. The amino acid sequence used to model the structure did not include the signal peptide. To correct the integrity of the threaded structure, internal discontinuities of the amino acid sequence were manually linked and missing amino acids in the carboxy- and amino- terminals were introduced as random coils. This was done using Swisspdbviewer **[27]** and Chimera **[28]**. The structure was refined by energy minimization using the steepest descent approach. Further refinement was performed with a 1 ns run of molecular dynamics (MD) by means of an explicit periodic box, with water molecules and using the GROMACS software package with the force field GROMOS96 G43a1 **[29]**

Immunogenic and protective linear epitopes:
Immunoinformatics approaches to predict linear epitopes are well developed for the human HLA-DR restriction element. The prediction of linear epitopes in porcine proteins should be done using the SLA-DR molecule; however, the immunoinformatics tools for pigs have not been developed yet. HLA and SLA are homologues molecules but the range of diversity within the peptide binding grooves is not identical. However, given the similarity between the human and porcine immune systems and the similarity of their major histocompatibility complex, it is likely that immunogenic epitopes might be shared **[30]**. As a first approach, potentially immunogenic porcine epitopes will be identified with human immunoinformatics tools. The prediction of TSOL18 B cell epitopes was performed with the web server ABCpred **[12]**. Recurrent neural network score was calculated for each 10-amino acid length peptide. The B cell predicted epitopes above threshold value (0.45) were chosen. Linear T cell epitopes were identified as a first approximation, by prediction of human MHC class II linear epitopes with the web server Propred **[31]**. An immunogenicity score was estimated as the frequency in which an epitope was classified as immunogenic, among the total number of MHC alleles. We considered this probability as the MHC-II immunogenicity index. The accessibility to the solvent of each amino acid residue was calculated from the TSOL18 structure using the Lee and Richards algorithm implemented in the program ASAView **[32]**. We selected the accessible residues as those with a solvent accessibility beyond 30%. Accessible epitopes with the highest scores were considered as potentially protective.

Multiepitopic peptide design:
Three epitopes were selected and were associated to a random-coil in the TSOL18 theoretical structure. A peptide comprising the three selected epitopes was designed. The secondary structure of the peptide was monitored during a 1 ns MD run in a water periodic box at 310 K. We confirmed that the peptide showed a random coil structure during the 1 ns MD run. The selected peptide was chemically synthesized with 95% purity and lyophilized (Alpha Diagnostic Intl. Inc, San Antonio, TX). The peptide was synthesized and conjugated to Keyhole Limpet Hemocyanin (KLH).

Vaccine trial:
The synthetic peptide conjugated to KLH was used to immunize pigs. The vaccinated group consisted of four two-month old female pigs that received three doses. Each of the three doses included 2 ml of antigen (0.15 mg/ml) with 1 mg saponin as adjuvant in PBS. The placebo group consisted of five pigs of similar ages, two male and three female. For this group each of the three doses consisted of 2 ml PBS, KLH (0.3 mg), and 1 mg saponin. In all cases the second dose was given 2 weeks after the first dose and the third dose was given 1 week after the second dose. Animals were challenged by oral infection with one gravid proglottid 15 days after the third dose. Gravid proglottids were obtained from two different tapeworms and were evenly distributed between the vaccinated and placebo groups. We collected pre-immune and post-immune sera. The latter was collected the day before challenge. Ninety days after the challenge, the pigs were humanely euthanized and the entire carcass and brain were examined for the presence of cysticerci **[33, 34]**. Cysts were classified as either viable, if a defined cyst structure with liquid content was still present, or degenerated, if this had been replaced by semisolid contents or inflammatory scar tissue **[34]**. The collected sera were evaluated using an ELISA assay to test the reaction against the peptide, as described previously **[35, 36]**. The ELISA assay used 1 μg/ml of peptide as antigen. Sera were diluted 1/100. A 1/2000 dilution of anti-pig-IgG-conjugated with peroxidase (Bio-Rad Laboratories, Rockville Centre, NY) was used.

Data analysis:
The number of healthy and degenerated cysts and the number of scars were compared between the vaccinated and placebo groups. Means and 95% confidence intervals were calculated based on a Poisson distribution. To account for over dispersion and to adjust for the age and sex of the pigs, as well as the identity of the taenia used for the infection, the number of cysts was modeled in a multiple Negative Binomial regression. A paired *t*-test and non-parametrical tests were used to compare the ELISA OD means between the pre-immune and post-immune sera.

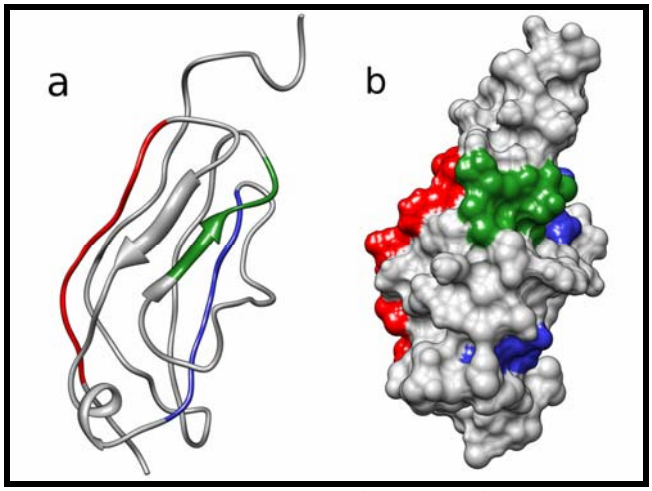

Figure 1: Theoretical structure predicted for TSOL18 of *Taenia solium* **(a)** Ribbon representation, **(b)** surface representation. In color, the selected epitopes: Blue (Ep1) (EEIKVKVEK), green (Ep2) (VIIRDL), and red (Ep3) (IFVPYLRCFAL).

Results and Discussion:
A signal peptide including the first 18 amino acids (MVCRFALIF LVAVVLASG) was predicted. No trans-membrane helices were detected, and an average hydrophobicity of 0.07 was estimated in the Kyte-Dolittle scale. This confirms previous studies that showed TSOL18 being a secreted protein **[3]**. The amino acid sequence of TSOL18 did not have any homologue with a known structure (E-value =2.9 for the best hit), and no other putative functional conserved domain except fibronectin type III, was assigned. The 3D structure was obtained using as query a fibronectin type III structural protein (PDB Id: 1QG3). The modeled structure of TSOL18 comprises a fibronectin type III domain **(Figure 1)** as was previously proposed **[3]**. HP6 from *Taenia saginata* was associated with a fibronectin type III domain and was described as an adherence protein **[37]**. TSOL18 and HP6 are homologs according to the high similarity shared (61%) **[3]**. Our independent analysis after a rigorous structure refinement approach confirms the association of TSOL18 with a fibronectin type III domain. These predictions suggest that TSOL18 may participate in the intestinal adherence of oncospheres, an event preceding the intestinal wall degradation and penetration. It can be speculated that the protection conferred by TSOL18 could be associated to the prevention of the adherence of the oncosphere to the intestinal wall mediated by the action of IgA antibodies at the intestinal epithelium. The three best B cell linear epitopes showing the highest score and that are exposed on the surface of TSOL18 structure, coincidently resulted to be predicted as potential linear T cell epitopes too. The epitope NH3-EEIKVKVEK-COOH in position 56-64 (Ep1) had the highest B cell epitope score (0.83). The epitope NH3-VIIRDL-COOH in position 83-88 (Ep2) had the second highest score (0.72) and was exposed on the protein surface. The third surface epitope selected was NH3-IFVPYLRCFAL-COOH in position 26-36 (Ep3), with a score of 0.46, and also was exposed on the protein surface. The fusion peptide (NH3_EEIKVKVEKIFVPYLRC FALVIIRDL_COOH) preserved the average random coil secondary structure during the 1 ns MD run and was selected as the antigen for the vaccine trial. In the vaccine trial all challenged animals harbored live parasites; therefore, a complete protection was not observed in any given pig. Complete protection is understood as an absolute absence of cysts in the vaccinated group. Although, a trend towards reduced number of total cysticerci in the vaccinated group

compared to the placebo group was observed. In all vaccinated animals, the ELISA readings confirmed an increased reactivity of the post-immune sera compared to the pre-immune sera against the vaccine antigen. The identity of the taenia was significantly associated with the total number of cysts ($P < 0.0001$), suggesting that the infectivity of the two tapeworms was different. After adjusting for the taenia identity, the age and sex of the pig, the number of total cysts, viable cysts and scars, were significantly lower in the vaccinated group ($P = 0.018$, $P=0.001$, and $P=0.001$ respectively) **(Table 1 see supplementary material)**. No significant difference was observed in the number of degenerated cysts between the placebo and vaccinated groups ($P=0.156$). For some time, available evidence has suggested that the protective epitopes of TSOL18 were likely to be conformational, based on similarities with the EG95 vaccine protein **[38, 39]**. These studies examined the nature of the antigenic epitopes of the protective oncosphere protein EG95 of *Echinococcus granulosus*, which suggested that its host-protective epitopes are conformational rather than linear **[38, 39]**. EG95 and TSOL18 are both host-protective oncosphere antigens of taeniid cestode parasites and both contain a fibronectin type III domain. Supporting these results, recently, Assana et al. (2010) suggested that the principal antibody specificities raised by TSOL18 in pigs is against conformational epitopes **[40]**. Although no porcine-specific MHC-epitopes prediction tools are available, the prediction of TSOL18 immunogenic epitopes using human MHC class II profiles is justified by the high level of similarity between human and porcine class II molecules antigens **[30]**. It would be important to do further studies to develop a porcine-specific MHC matrix to have a more accurate prediction of SLA epitopes. The epitopes predicted in the present study, were identified using a hypothetical structure model of the TSOL18. It is possible that the model did not perfectly reflect the real structure of the protein. It would be important to determine a crystallographic 3-dimensional structure of TSOL18 in order to obtain more accurate predictions.

Conclusion:

A synthetic peptide comprising the best theoretically predicted protective linear epitopes using immunoinformatics tools based on human MHC matrixes failed to completely protect pigs against porcine cysticercosis. Although a trend to a decreased number of total cysts was observed in the vaccinated group. It is likely that the total protection conferred by TSOL18 could be associated with conformational epitopes rather than with linear epitopes.

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Supplementary material:

Table 1: Results of the immunization trial measured during necropsy of pigs.

Group pig (id)	Age (days)	Sex	Taenia[a]	Viable cysts		Degenerated cysts		Total cysts		Scars	
Placebo											
1P	200	F	2	2 [b]	793.6 [c]	1419 [b]	857.2 [c]	1421 [b]	1650.8 [c]	3 [b]	20 [c]
2P	199	M	2	3922	[769.1,	43	[831.7,	3965	[1615.4,	7	[16.3,
3P	198	F	2	2	818.7]	1582	883.2]	1584	1686.8]	70	24.0]
4P	200	F	1	0		1104		1104		20	
5P	199	M	1	42		138		180		0	
Vaccine											
6V	200	F	2	554	166.3	850	359.3	1404	525.5	0	5.25
7V	198	F	2	0	[153.9,	346	[340.9,	346	[503.3,	9	[3.30,
8V	199	F	1	108	179.4] [c]	187	378.3] [c]	295	548.4] [c]	8	8.0] [c]
9V	198	F	1	3		54		57		4	
P-value				0.001		0.156		0.018		0.001	

[a]Taenia used in challenge. [b]Number of cysts and scars and its measure of variation. [c]Mean and 95% CI for a Poisson distribution. *P*-values correspond to the adjusted comparisons between the placebo and the vaccinated group with multiple negative binomial regressions.