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IMMUNOINFORMATICS III : COMPUTATIONAL PREDICTION OF CHLAMYDIA POLYMORPHIC MEMBRANE PROTEIN - LIKE PROTEIN AS A DIAGNOSTIC MARKER FOR BOVINE TRICHOMONOSIS

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ABSTRACT

Bovine Trichomonosis is a venereal disease of cattle caused by *Trichomonas foetus*. Even though it is most common disease, a kit for identification of *Trichomonas foetus* infection is not yet available. During present investigation, transcriptomics and proteomics based data of the pathogen was evaluated. Simultaneously, Similar data on *Trichomonas vaginalis*, a closely related human parasite, were also analyzed. Clamydia polymorphic membrane protein -like protein was identified as potential virulent protein. TMMHM analysis revealed it to be as transmemebrane protein. It was further analyzed using Epitope Prediction and Analysis Tools, in Immune Epitope Database Analysis Resource. It was pointed out that above protein contained several large continuous epitopes, which can be exploited for developing suitable diagnostics

Key words: B-Cell epitope prediction, Trans membrane domains, Point of care diagnostics, Comparative genomics

Introduction

Trichomonosis is a wide spread venereal disease of cattle which remained neglected to some extent in India. A unicellular parasite, *Trichomonas foetus* is the casual agent of this disease. This disease results into early abortion in cows and increase in the number of cows which are unable to breed (Skirrow and Bondurrant,1988; Šlapeta, et. al. 2012; Tachezy, et. al, 2002; Yao, 2012) Identification of infected bull or cow, and its isolating from the herd, is the only available control strategy strategies (Yao, 2013). However, till now a kit has not been developed for identification of *Trichomonas foetus* infection. In the light of above information, it has been hypothesized that, screening of transcriptomics and proteomics based data of the pathogen, coupled with comparative genomic screening for *Trichomonas vaginalis*, a closely related human parasite, will be useful in developing markers. Therefore present investigation was undertaken,

Material and methods

Extensive review of literature was carried out, in order to study Transcriptomics and proteomics of *Trichomonas foetus* and *Trichomonas vaginalis.* Screening and listing of surface and virulent proteins involved in the pathogenesis of Bovine Trichomonosis was undertaken (Huang, et.al, 2013; Stroud, et. al. 2017;. 2013 Morin-Adeline, et.al. 2014; Ivone de, 2017 and *Kuo,et. 1l, 2014) For this purpose, virulent proteins of *T. vaginalis* were subjected to BLAST analysis, in order to compare and identify the corresponding proteins of *T. foetus* (Hirt, 2013; de Miguel, 2010). The accession numbers were obtained for highly expressed transcripts, ESTs and proteins from *T.foetus and T.vaginalis* from NCBI proteins data base. Clamydia polymorphic membrane protein -like protein was identified as virulent protein. By using accession number OHS93232.1 the FASTA

formats was downloaded from National Center for Biotechnology Information.

Confirmation of as surface proteins was done using TMMHM. FASTA sequence of the protein was submitted to "TMHMM Server v. 2.0" for prediction of transmembrane helices in proteins. Output was obtained in the form of graphics. The FASTA sequences were analyzed for putative antigenic domains of the listed virulent proteins using "*Epitope Prediction and Analysis Tools*" in "Immune Epitope Database Analysis Resource". "*B Cell Epitope Prediction Tools*" was used to identify the putative antigenic domains of proteins, which are more likely to be recognized as epitopes with regard to antibodies produced by B cell response. The "BepiPred-2.0" server was used to predict B-cell epitopes from the FASTA sequence of the protein.

Results and discussion

Chlamydia Polymorphic membrane protein-like protein was consistently expressed. Proteins in several transcriptomics and proteomics studies of *Trichomonas foetus* and BLAST analysis with *Trichomonas vaginalis* proteins revealed considerable similarity along with Retrieving FASTA Sequence. Genpept data sheet reveled that, it is a huge protein with 726 amino acids.

TMHMM analysis revealed that it has single trans membrane domain with a large extracellular domain measuring 726 amino acids (Fig.1.) Immunogenic nature of the protein was analyzed by IEDB resource, which is freely available resource for prediction of immunogenic domains maintained by National institute of Allergy and Infectious diseases, USA. B-Cell linear epitope prediction tools reveal that Chlamydia polymorphic membrane protein like protein has several large antigenic domains at 0.500 threshold value (Fig.2), with largest epitope domain measuring 333 amino acids as shown in Fig 3. Fig 2 provides graphic representation, which clearly indicated that It has several antigenic domains, above threshold value. There were several larger domains between 216-239 amino acids. The region between 304 and 636 are more suitable domains that can be used to produce recombinant proteins for use in diagnostics.

Chlamydia polymorphic membrane proteins of *T.vaginalis* are well known surface proteins which mediate host-pathogen interactions and cell aggregations leading to virulence. Pmps (Polymorphic Membrane Proteins) are a group of membrane bound surface exposed chlamydial proteins that have been characterized as autotransporter adhesins and are important in the initial phase of chlamydial infection. These Proteins were found to be mediating initial binding of the obligate intracellular pathogen and invasion into the host cell. Chlamydia polymorphic membrane protein like protein was the consistently highly expressed in several transcriptomics and proteomics studies of *Trichomonas foetus*. This protein contain several large continuous epitopes and highly immunogenic in nature, which suggest that they can be exploited for developing point of care diagnostics like, Lateral flow assay (LFA) Rapid kits to be useful for screening for *Trichomonas foetus* infection in resource limited stations and ELISA for bulk screening in organized farms.

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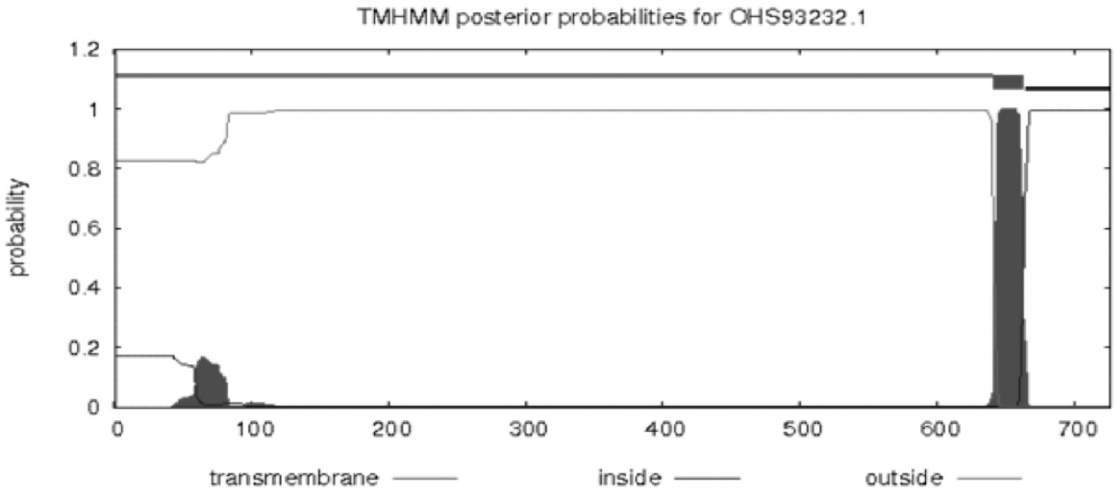

Figure.1 TMHMM analysis Chlamydia polymorphic membrane protein of T.foetus

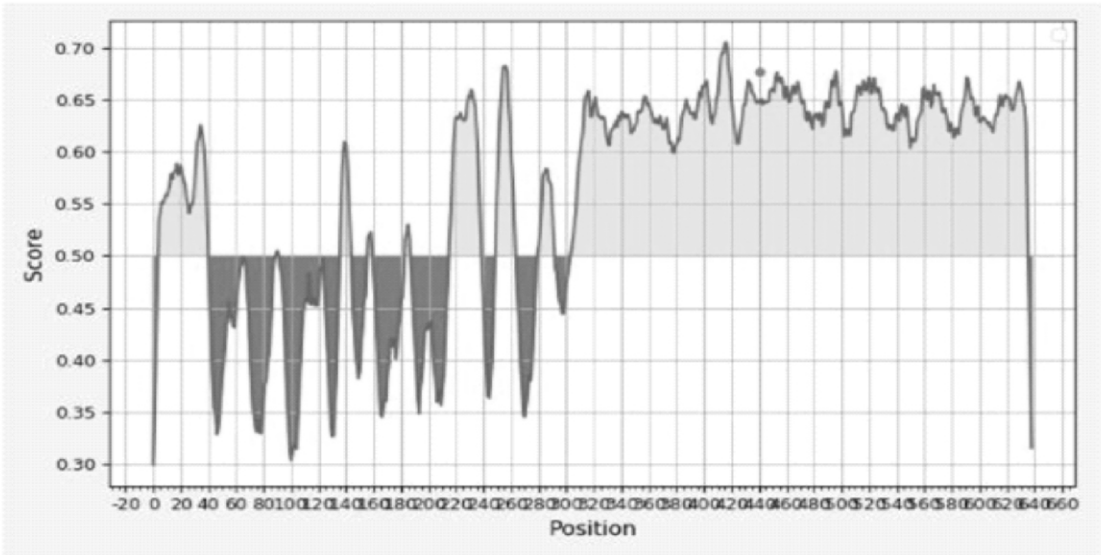

Figure.2 Graphic representations of epitope domains of Chlamydia polymorphic membrane protein of T.foetus

No.	Start	End	Peptide	Length
1	5	40	NRPNFKDLKDTTLDIDNKFRKLDRFSSQSFNKESRT	36
2	90	92	TKN	3
3	137	144	DSDDMKAQ	8
4	157	160	NEAK	4
5	184	187	NRAQ	4
6	216	239	RAFIQSNSFQPYQLKSFRKLNHLK	24
7	250	264	ENTRKDLTSDDYRAK	15
8	280	292	DSKLRTEPYNQGF	13
9	304	636	SYTDIFLNVKNVSLGGHMNTDFIFAGTTFYDQDTEWDTLTSNINQFFMVEERFP NLKSDIYDKLSVDKYEWPPTAALTDIPAGPVNNAAADSIDNNDLPANTAPATKL PERTTRGGQPAASTILPEWHVSSVSRTVHYVSPSMSPTESPTESATESATESP TESPTESATESATESPTESPTESATESPTESPTESATSATESPTESPTESATES ATESPTESPTESATESATESPTESPTESATESATESPTESP TESATESPTESPS LSPTESATQSATCSPSLSPSESATSSATESPSISATESATESPSLSSEIVDDNLE VEKDKSQFT	333

Figure.3 Antigenic domains of Chlamydia polymorphic membrane protein of *T.foetus*

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IMMUNOINFORMATICS IV : COMPUTATIONAL PREDICTION OF IMMUNO-DOMINANT VARIABLE SURFACE ANTIGEN-LIKE PROTEIN AS A DIAGNOSTIC MARKER FOR BOVINE TRICHOMONOSIS

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ABSTRACT

Bovine Trichomonosis is a venereal disease of cattle caused by *Trichomonas foetus*. Even though it is most common disease, a kit for identification of *Trichomonas foetus* infection is not yet available. During present investigation, transcriptomics and proteomics based data of the pathogen was evaluated. Simultaneously, similar data on *Trichomonas vaginalis*, a closely related human parasite, were also analyzed. Immuno-Dominant Variable Surface Antigen-Like Protein was identified as potential virulent protein. TMMHM analysis revealed it to be as transmemebrane protein. It was further analyzed using Epitope Prediction and Analysis Tools, in Immune Epitope Database Analysis Resource. It was pointed out that above protein contained several large continuous epitopes, which can be exploited for developing suitable diagnostics

Key words: B-Cell epitope prediction, Trans membrane domains, Point of care diagnostics, Comparative genomics

Introduction

Trichomonosis is a wide spread venereal disease of cattle which remained neglected to some extent in India. A unicellular parasite, *Trichomonas foetus* is the casual agent of this disease. This disease results into early abortion in cows and increase in the number of cows which are unable to breed (Skirrow and Bondurrant,1988; Šlapeta, et. al. 2012; Tachezy, et. al, 2002; Yao, 2012) Identification of infected bull or cow, and its isolating from the herd, is the only available control strategy strategies (Yao, 2013). However, till now a kit has not been developed for identification of *Trichomonas foetus* infection. In the light of above information, it has been hypothesized that, screening of transcriptomics and proteomics based data of the pathogen, coupled with comparative genomic screening for *Trichomonas vaginalis*, a closely related human parasite, will be useful in developing markers. Therefore present investigation was undertaken,

Material and methods

Extensive review of literature was carried out, in order to study Transcriptomics and proteomics of *Trichomonas foetus* and *Trichomonas vaginalis.* Screening and listing of surface and virulent proteins involved in the pathogenesis of Bovine Trichomonosis was undertaken (Huang, et.al, 2013; Stroud, et. al. 2017;. 2013 Morin-Adeline, et.al. 2014; Ivone de, 2017 and *Kuo,et. 1l, 2014) For this purpose, virulent proteins of *T. vaginalis* were subjected to BLAST analysis, in order to compare and identify the corresponding proteins of *T. foetus* (Hirt, 2013; de Miguel, 2010). The accession numbers were obtained for highly expressed transcripts, ESTs and proteins from *T.foetus and T.vaginalis* from NCBI proteins data base. Immuno-Dominant Variable Surface Antigen-Like Protein was identified as virulent protein. By using accession number OHT11175.1 the FASTA

formats was downloaded from National Center for Biotechnology Information.

Confirmation of as surface proteins was done using TMMHM. FASTA sequence of the protein was submitted to "TMHMM Server v. 2.0" for prediction of transmembrane helices in proteins. Output was obtained in the form of graphics. The FASTA sequences were analyzed for putative antigenic domains of the listed virulent proteins using "*Epitope Prediction and Analysis Tools*" in "Immune Epitope Database Analysis Resource". "*B Cell Epitope Prediction Tools*" was used to identify the putative antigenic domains of proteins, which are more likely to be recognized as epitopes with regard to antibodies produced by B cell response. The "BepiPred-2.0" server was used to predict B-cell epitopes from the FASTA sequence of the protein.

Results and discussion

Immuno-Dominant Variable Surface Antigen-Like Protein was consistently expressed. Proteins in several transcriptomics and proteomics studies of *Trichomonas foetus* and BLAST analysis with *Trichomonas vaginalis* proteins revealed considerable similarity along with Retrieving FASTA Sequence. Genpept data sheet reveled that, it is a huge protein with 270 amino acids.

TMHMM analysis revealed that it is a membrane associated protein, complete amino acid sequence is extracellular (Fig.1.) Immunogenic nature of the protein was analyzed by IEDB resource, which is freely available resource for prediction of immunogenic domains maintained by National institute of Allergy and Infectious diseases, USA. B-Cell linear epitope prediction tools reveal that Immuno-Dominant Variable Surface Antigen-Like Protein has several large antigenic domains at 0.500 threshold value (Fig.2), with largest epitope domain measuring 89 amino acids as shown in Fig 3. Fig 2 provides graphic representation, which clearly indicated that It has several antigenic domains, above threshold value. There were several larger domains between 41-129 amino acids. The region between 41-207 are more suitable domains that can be used to produce recombinant proteins for use in diagnostics.

Immuno-Dominant Variable Surface Antigen-Like protein of *T.vaginalis* are well known surface proteins which mediate host-pathogen interactions and cell aggregations leading to virulence. These Proteins were found to be mediating initial binding of the obligate intracellular pathogen and invasion into the host cell. Immuno-Dominant Variable Surface Antigen-Like Protein was the consistently highly expressed in several transcriptomics and proteomics studies of *Trichomonas foetus*. This protein contain several large continuous epitopes and highly immunogenic in nature, which suggest that they can be exploited for developing point of care diagnostics like, Lateral flow assay (LFA) Rapid kits to be useful for screening for *Trichomonas foetus* infection in resource limited stations and ELISA for bulk screening in organized farms.

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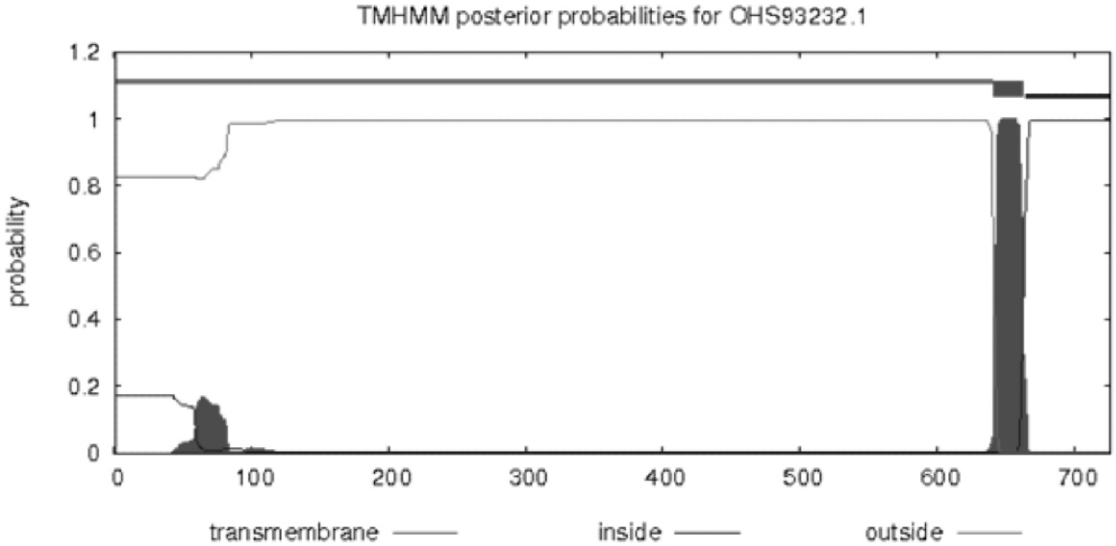

Figure.1 TMHMM analysis Chlamydia polymorphic membrane protein of T.foetus

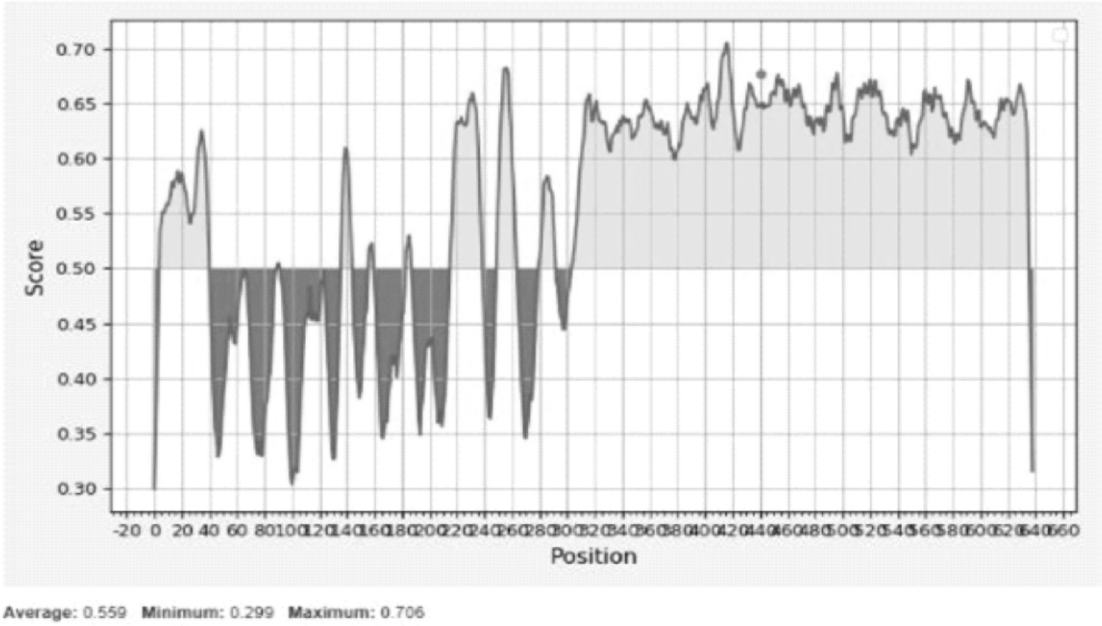

Figure.2 Graphic representations of epitope domains of Chlamydia polymorphic membrane protein of T.foetus

No.	Start	End	Peptide	Length
1	5	40	NRPNFKDLKDTTLDIDNKFRKLDRFSSQSFNKESRT	36
2	90	92	TKN	3
3	137	144	DSDDMKAQ	8
4	157	160	NEAK	4
5	184	187	NRAQ	4
6	216	239	RAFIQSNSFQPYQLKSFRKLNHLK	24
7	250	264	ENTRKDLTSDDYRAK	15
8	280	292	DSKLRTEPYNQGF	13
9	304	636	SYTDIFLNVKNVSLGGHMNTDFIFAGTTFYDQDTEWDTLTSNINQFFMVEERFP NLKSDIYDKLSVDKYEWPPTAALTDIPAGPVNNAAADSIDNNDLPANTAPATKL PERTTRGGQPAASTILPEWHVSSVSRTVHYVSPSMSPTESPTESATESATESP TESPTESATESATESPTESPTESATESPTESPTESATSATESPTESPTESATES ATESPTESPTESATESATESPTESPTESATESATESPTESP TESATESPTESPS LSPTESATQSATCSPSLSPSESATSSATESPSISATESATESPSLSSEIVDDNLE VEKDKSQFT	333

Figure.3 Antigenic domains of Chlamydia polymorphic membrane protein of *T.foetus*

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IMMUNOINFORMATICS I : COMPUTATIONAL ANALYSIS OF GP63-LIKE PROTEIN AS A POTENTIAL DIAGNOSTIC MARKERS FOR BOVINE TRICHOMONOSIS

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ABSTRACT

Bovine Trichomonosis is venereal disease of cattle in India caused by the infection of *Trichomonas foetus*. However, simple, inexpensive rapid kits have not yet developed for the identification of the infection by the pathogen. Transcriptomics and proteomics based data of the casual agent, as well as, genomics-based approach to screen *Trichomonas vaginalis*, a closely related human parasite were analyzed to identify GP-63 proteins. TMMHM tool revealed them to be as transmembrane protein and as membrane associated protein respectively. These proteins were analyzed using Epitope Prediction and Analysis Tools in Immune Epitope Database Analysis Resource. GP-63 proteins contain several large continuous epitopes which suggest that they can be exploited for developing diagnostics. Such computational predictions may help the researchers towards development of diagnostics and therapeutics.

Key words: B-Cell epitope prediction, Transmembrane domains, Point of care diagnostics, Comparative genomics, Rapid kits

Introduction

Bovine Trichomonosis is a venereal disease of cattle in India, characterized by early fetal death and infertility, The causal organism for this disease of *Trichomonas foetus,* a unicellular parasite. However, there is an urgent need of the development of simple procedure for identification of *Trichomonas foetus* infection into the cattle. During present investigation attempts were made towards screening of transcriptomics and proteomics based data of the causal agent. Similar data of closely related human parasite *Trichomonas vaginalis* was employed for comparison, which would enable to identify of potential markers.

Material and methods

An extensive literature survey in Pubmed was carried out to search for Transcriptomics and proteomics studies of *Trichomonas foetus* and *Trichomonas vaginalis.* Screening and listing of highly expressed surface proteins, and virulent proteins, involved in pathogenesis of Bovine Trichomonosis was done (Huang et. al., 2013; Stroud,et. al., 2017; Morin-Adeline,et. al., 2014; Ivone, et. al., 2017; Kuo, 2013) . Virulent proteins of *T.vaginalis* were subjected to BLAST analysis to compare and identify the corresponding proteins of *T.foetus.* (Hir, et.ai., 2013; de Miguel, et. al., 2010) Accession numbers for corresponding transcripts,, ESTs and proteins in *T.foetus and T.vaginalis* were obtained from NCBI proteins database, and the corresponding FASTA formats were downloaded.

These were confirmed as putative surface proteins using TMMHM. FASTA sequences of the proteins were submitted to “TMHMM Server v. 2.0” for the prediction of transmembrane helices in proteins. The output was obtained in the form of graphics. The FASTA sequences were analyzed for putative antigenic domains of the listed virulent proteins using “Epitope Prediction and Analysis Tools” in “Immune Epitope Database Analysis

Resource". "B Cell Epitope Prediction Tools" were used to identify the putative antigenic domains of proteins, which are more likely to be recognized as epitopes with regard to antibodies produced by B cell response. The "BepiPred-2.0" server was used to predict B-cell epitopes from the FASTA sequence of the protein.

Results and discussion

Several transcriptomics and proteomics studies of *Trichomonas foetus* revealed that GP63-like proteins are consistently expressed. During present study, BLAST analysis of *Trichomonas vaginalis* proteins, revealed considerable similarity with those of *Trichomonas foetus*.

T. vaginalis is an extracellular parasite, which adheres to the epithelial lining or extracellular matrix of urogenital tract (Warton and Honigberg, 1979).

TMHMM analysis revealed that it had single transmembrane domain containing extracellular protein containing 561 amino acids (Fig.1).Such extracellular domain offers antigenicity as well as cell to cell communication, and thus can be exploited for epitope prediction.

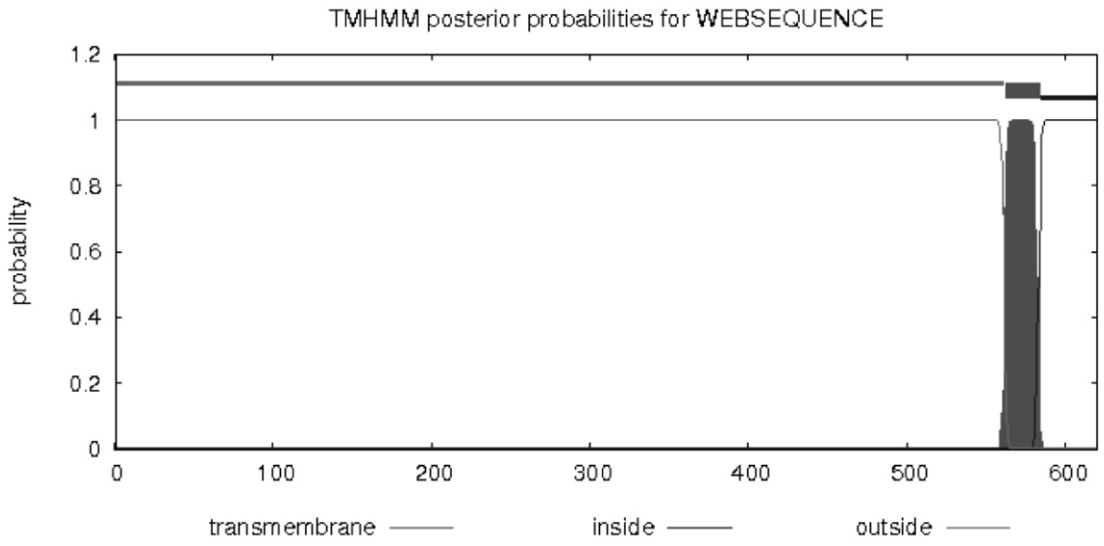

Figure.1 TMHMM analysis GP-63 protein of T.foetus

Immunogenic nature of the protein was analyzed by IEDB resource, maintained by the National Institute of Allergy and Infectious diseases, USA. B-Cell linear epitope prediction tools revealed that GP-63 like protein had several large antigenic domains at 0.5 threshold value (Fig.2), with largest epitope domain containing 52 amino acids (Fig 3).

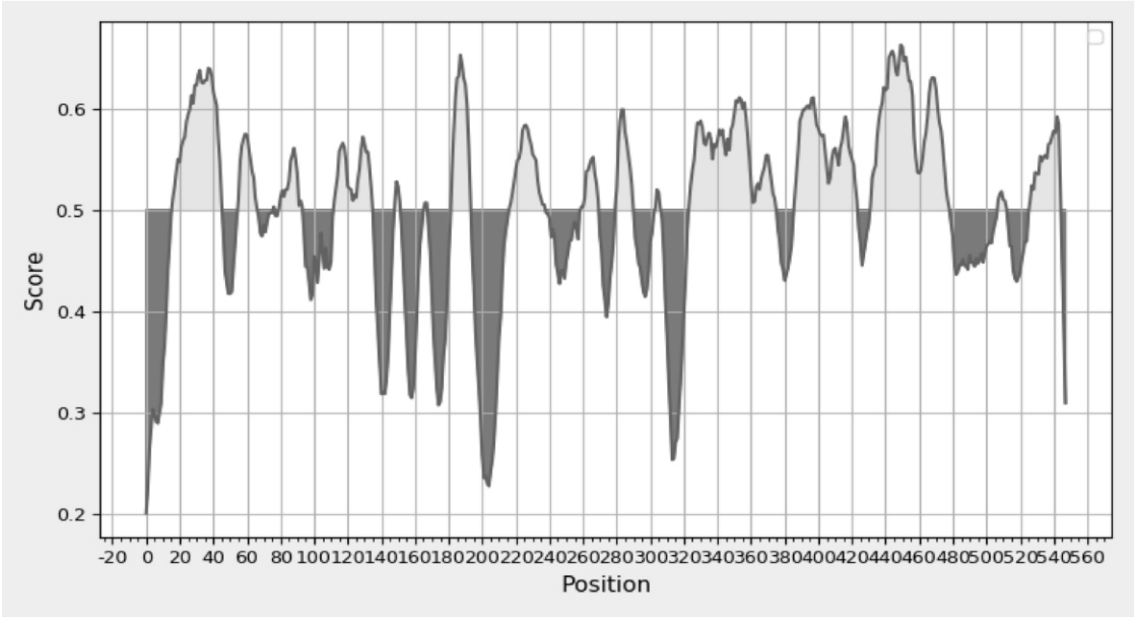

Figure.2 Graphic representations of epitope domains of GP-63 protein of T.foetus

Average: 0.500 **Minimum:** 0.200 **Maximum:** 0.663

Predicted peptides:

No.	Start	End	Peptide	Length
1	17	46	DSLQSQINIRKLQFPPDPGLMDEDEIIERE	30
2	56	67	SLTSDYDPSVCR	12
3	77	77	G	1
4	80	94	EICTKDDIITPEKIS	15
5	113	135	VTRLKGGFDISNITDITVLERHV	23
6	149	152	GTHR	4
7	167	168	VN	2
8	183	194	IPAEPQNESSFD	12
9	217	238	PSWIDPNTNQPYEHLPIIEYSA	22
10	260	270	ERFGVEYFAPD	11
11	280	290	GGGVGTFGSHA	11
12	304	307	TIGQ	4
13	324	375	YDVSYEKAEKSAWGLGESLNLSPLTTFPNTAPQHAFPKHYMCDPSDIDTDVC	52
14	387	424	GVKVDCDLPSDEDDQKFCEMRNFTDPLRIGLRGRSEVH	38
15	432	478	PYSNSRCSDISRNTDSAYKNGELYGGESLCFMSTLLRSSFSFYTYYH	47
16	508	513	QKLSFS	6
17	527	545	ACGIRKFYGIVGPTPVPSP	19

Figure.3 Antigenic domains of GP-63 of *T.foetus*

The graphic representation illustrated in Fig 2 clearly indicates that GP-63 had several antigenic domains, above threshold value. There are several larger domains between 324-375 and 432-478 amino acids. The regions between 320 and 480 were more suitable for producing recombinant proteins which can be used as diagnostics.

GP63 is second-largest group of surface proteins, with 48 members in case of *Trichomonas vaginalis* (Lina et. al.,2011). During present study, BLAST Analysis indicated similarity between the two pathogens. The adherence was species as well as cell specific.

T. vaginalis can adhere to human vaginal epithelial cells (hVECs) and produce cytotoxic effects, but neither adherence nor cytotoxicity has been observed when *T. vaginalis* is exposed to human vaginal fibroblasts or bovine vaginal epithelial cells. Similarly, the bovine parasite *Tritrichomonas foetus* had no cytotoxic effects on hVECs (Ryan et. al.,2011). Both of the proteins contain several large continuous epitopes and both of them are highly immunogenic in nature, which suggest that they can be exploited for developing diagnostics in the form of Lateral flow assay (LFA). Rapid kits are useful for screening of *Trichomonas foetus* infection.

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IMMUNOINFORMATICS II : COMPUTATIONAL ANALYSIS OF HYPOTHETICAL PROTEIN AS A POTENTIAL DIAGNOSTIC MARKER FOR BOVINE TRICHOMONOSIS

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ABSTRACT.

Bovine trichomonosis is venereal sickness of cattle caused by *Tritrichomonas foetus*. In farm animals, the parasite infects urogenital tract and cause symptoms which are similar to those due to the infection of *Trichomonas vaginalis* in humans. In cats, the parasite colonizes gastrointestinal tract and results into prolonged watery diarrhea which can result in abandonment or euthanasia. However, lack of rapid kits for identification of *Trichomonas foetus* infection is not yet available. For this purpose, transcriptomics and proteomics based information of *Trichomonas vaginalis* was analyzed during present investigation, to obtain hypothetical proteins. TMMHM method revealed them as transmembrane and membrane related proteins respectively. These proteins were analyzed using epitope prediction and evaluation equipment. The hypothetical proteins indicated that they can be exploited as diagnostics.

Key words: B-Cell epitope prediction, Trans membrane domains, Point of care diagnostics, Comparative genomics

Introduction

Trichomonosis is wide spread venereal disease of cattle in India.. The casual organism of the disease is *Trichomonas foetus,* a unicellular parasite (Warton and Honigberg, 1979). Identifying the infected animals and isolating them is the only method of its control. There is an urgent need of developing a kit for identification of *Trichomonas foetus* infection. It has been assumed that, screening of transcriptomics and proteomics based data of the casual organism and its genomic comparison with *Trichomonas vaginalis* (a closely related human parasite) may help in the developing of markers**.**

Material and methods

Extensive literature survey was carried out in order to search Transcriptomics and proteomics studies of *Trichomonas foetus* and *Trichomonas vaginalis.* Screening of virulent surface proteins, responsible for pathogenesis of Bovine Trichomonosis was also undertaken (Huang, et. al., 2013; Stroud,et. al, 2017; Morin-Adeline, et. al., 2014; Ivone et al., 2017 and Kuo,et. al., 2013). Virulent proteins of *T.vaginalis* were subjected to BLAST analysis for comparison and identification corresponding proteins of *T. foetus (Hirt, 2013*; *de Miguel, 2010).* The accession numbers for corresponding transcripts, ESTs and proteins in *T. foetus and T. vaginalis* were obtained from NCBI proteins data base. FASTA sequence of the Hypothetical protein with the accession number OHS95735.1 was downloaded from National Center for Biotechnology Information (NCBI).

This protein was confirmed as potential putative surface protein using TMMHM. FASTA sequences of the protein were submitted to “TMHMM Server v. 2.0” for prediction of transmembrane helices in proteins, in the form of graphics. FASTA sequences for the putative antigenic domains of the above protein was analyzed using

"*Epitope Prediction and Analysis Tools*" in "*Immune Epitope Database Analysis Resource*".

"*B Cell Epitope Prediction Tools"* were used to identify putative antigenic domains of proteins, which were more likely to be recognized as epitopes with regard to antibodies produced by B cell response. The "BepiPred-2.0" server was used to predict B-cell epitopes from the FASTA sequence of the protein.

Results and discussion

Hypothetical protein was highly expressed consistent proteins in several transcriptomics and proteomics studies of *Trichomonas foetus*. BLAST analysis with *Trichomonas vaginalis* proteins revealed considerable similarity with those from *Trichomonas foetus*. The genotypic data sheet reveled that, it is made up of 870 amino acids.

TMHMM analysis revealed that it has single transmembrane domain with a large extracellular domain measuring 870 amino acids (Fig. 1) The immunogenic nature of the protein was analyzed by IEDB resource maintained by National institute of Allergy and Infectious diseases, USA. B-Cell linear epitope prediction tools revealed that the hypothetical protein had several large antigenic domains at 0.5 threshold value (Fig. 2), with largest epitope domain measuring 33 amino acids (Fig. 3)

The graphic representation, illustrated in Fig 2 clearly indicates that Hypothetical protein have several immunogenic domains above threshold value. There are several larger domains between 520 - 552 and 781 - 810 amino acids. The regions in between 520 and 810 amino acids are more suitable domains, which can be used to produce recombinant proteins, suitable for use in diagnostics.

Several Hypothetical proteins of *T. vaginalis* are well known surface proteins, which are responsible for host-pathogen interactions and cellular aggregations, leading to virulence. Speculative proteins, on the other hand, are detected in various transcriptomics and proteomics exploration of *Trichomonas foetus*. These proteins may be evaluated develop vaccine. Such computational predictions, thus assist researchers for creating diagnostics and therapeutics.

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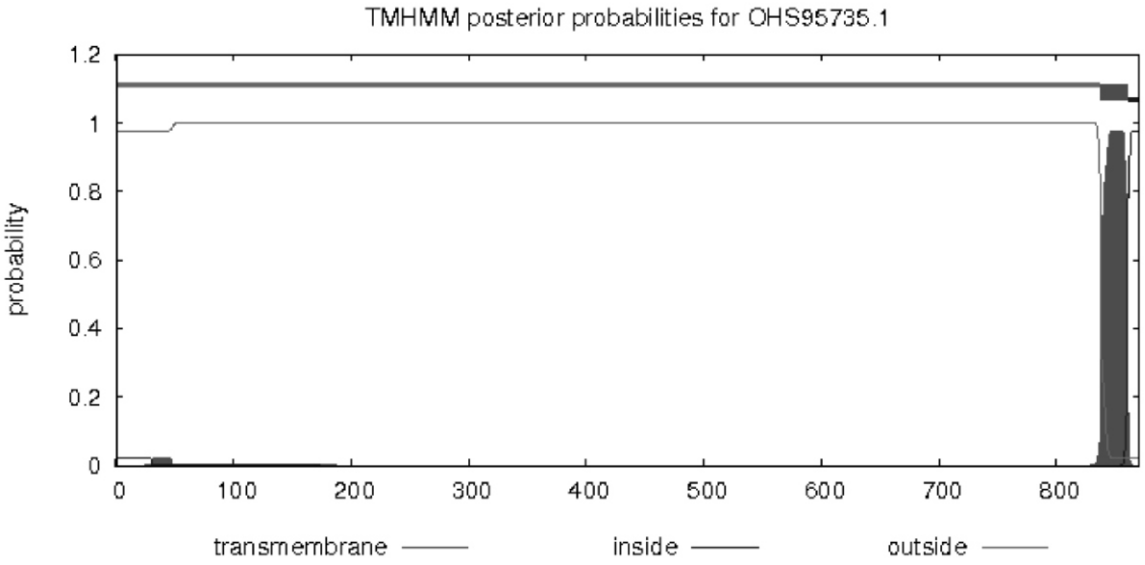

Figure.1 TMHMM analysis Hypothetical protein of T.foetus

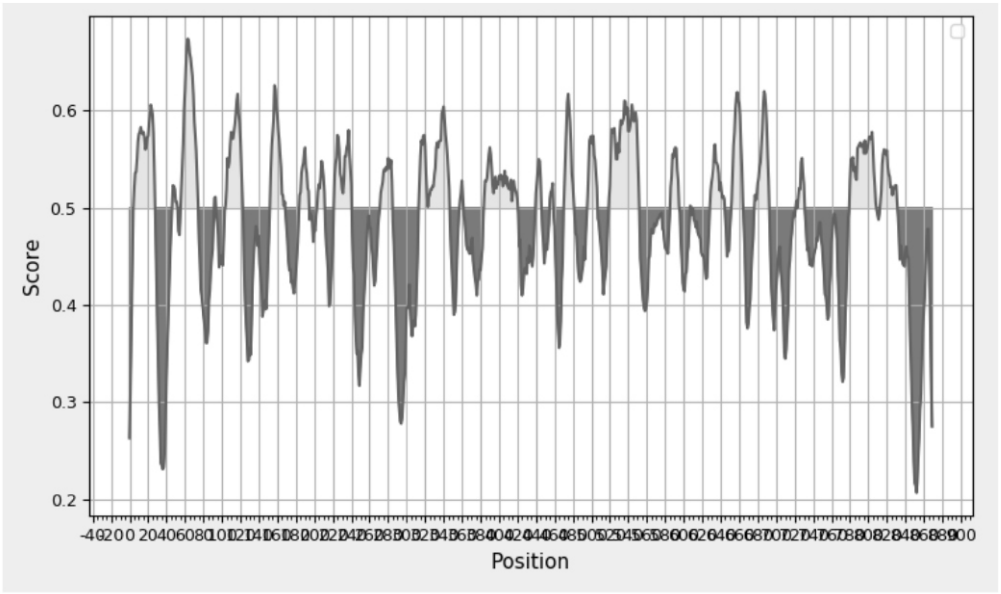

Figure.2 Graphic representations of epitope domains of Hypothetical Protein of T.foetus

No.	Start	End	Peptide	Length
1	6	28	SELTRFQHQSGGFYDNVREANAR	23
2	47	52	DTQRCF	6
3	57	75	TLRNRDGGAGLVPGSKSSV	19
4	93	94	ID	2
5	104	123	SCYDEPSGLFRDSPESEPSI	20
6	154	169	NDHLNDDHFEFDGVSL	16
7	185	195	LTVPYHRISEF	11
8	204	212	IKDNKLDNE	9
9	223	241	LFGDEAIPEQLSASFKSSG	19
10	276	285	LIDFEKDGLT	10
11	315	323	IGDEAPTTE	9
12	325	347	LKIDYQTGLFNSQRISSINKLGQ	23
13	360	362	FGT	3
14	385	404	WLSADEPIPVGGEIVPGVNF	20
15	406	421	VQLNGKLDDIIDKLED	16
16	441	447	FEDFKGQ	7
17	456	459	SLAL	4
18	472	482	DKVNGIHTHKE	11
19	497	507	EVPANLRLSDV	11
20	520	552	EAPVPFTNEKFFEGDLRDATGEAFYPQTASEAQ	33
21	587	597	NVNENLDFATG	11
22	608	609	GS	2
23	611	611	P	1
24	630	645	VEAQPLVSGAVDYGSK	16
25	653	665	KDEDSGNYLEAGR	13
26	678	693	RTVLLEKKAKILSDKY	16
27	723	731	VQTAQGSPF	9
28	781	810	GNAVAELPLAQMKKGSRLSWESGDAKGEYK	30

Figure.3 Antigenic domains of Hypothetical Protein of *T.foetus*

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Insilico Functional Annotation for Antigenic Proteins of *Trichomonas Foetus*

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ABSTRACT

Introduction: Bovine Trichomonosis is considered as one of the most neglected diseases of cattle in that are known to have sexually transmission. Though cattle remain asymptomatic initially, but eventually leads to frequent abortions, and finally to complete reproductive failure. Lack of point of care diagnostics remains the major hurdle for screening for Trichomoniasis. In our earlier work, we have identified several potential immunogenic proteins that can be targeted as diagnostic markers. In this current study, we have chosen Cysteine protease 8, Surface antigen BspA-like Protein, Chlamydia polymorphic membrane protein-like, Circumsporozoite protein precursor, hypothetical protein, Immuno-dominant variable surface antigen-like, Ser/Thr protein phosphatase as frequently expressed proteins of *Trichomonas foetus* for functional annotation.

Results and discussion: Gene ontology resource for functional annotations reveal that all the proteins are involved in host pathogen interaction and establishing virulence. Except for surface antigen BspA-like Protein, all the above proteins are relatively small to medium size proteins with 300 to 800 amino acids length and are suitable for in- vitro studies. Based on sub-cellular localization predictions,. Clan SB, family S8, subtilisin_like serine peptidase, Hypothetical protein, Immuno-dominant variable surface antigen-like Ser/Thr protein phosphatase, Adhesin are mostly confined to plasma membrane and out side the membrane.

Conclusions: *Trichomonas foetus* was found to have several immuogenic proteins with large outer membrane protein domains. Such proteins are most likely to have major role in host pathogen interaction. The proteins may have multiple large epitopes and exhibit efficient immune reactions. Heterologous expression of above proteins would promise to develop point of care diagnostics like Lateral flow assays and ELISA.

Keywords: Sub cellular location, Heterologous expression, functional annotation, Protein stability, Host pathogen interaction

Introduction

The animal health is the key contributor of any country`s economy as well as sustainability. But in this fast global pandemic situations, animal health and well being is paid very less attention. Animal population is under severe stress with regard to quality feed, hygiene, drastic changes in the climate conditions and various disease out breaks. If unattended at least from now, future world will have to struggle for good quality animal based food products (Standard operating procedures For Bovine breeding 2014). In view of zoonotic potential of several animal pathogens, there is a great need to develop easily available, simple and easy to perform diagnostics (OIE Reference manual 2018). Sexually transmitted diseases are major

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threat to Bovine community. As per the Minimum standard Operating procures of Bovine breeding, in recent times, there is little attention being made in developing, point of care diagnosis and treatment of some diseases like, Bovine tuberculosis (Pucken 2017). Para tuberculosis (Rudrama 2019), Brucellosis (Mallikarjuna 2017, Manasa 2019).

Bovine Trichomonosis is among the highly neglected sexually transmitted disease in cattle is responsible for huge losses in the cattle industry. (OIE Reference manual 2018). *Trichomonas foetus (T.foetus)* is a flagellate parasitic organism is the causative organism. It exists as trophozoite in most of its life cycle, can also take a pseudocyst form (Warton2018). Though cattle remain asymptomatic initially, but eventually leads to frequent abortions, and finally to complete reproductive failure. Presence of large number of infertile cows, requirement of multiple semen injections are the major symptoms of infestation with *T.foetus* (Schwebke 2004*). Trichomonas vaginalis* (*T.Vaginalis)* is a closely related with *T.foetus*, known to have sexual transmission in human, causing Trichomonosis. Based on several studies on surface proteome of *T.Vaginalis (*de Miguel 2010), and analysis for virulence factors (Hirt R 2013) in a comparative omics based approach it was possible to search for targets in *T.foetus*. Recently available transcriptomics and proteomics data of *T.foetus* (Huang 2013, Morin-Adeline 2014, Stroud2017, de, Andrade 2017) have further enhanced our possibility of suitable diagnostic markers and drug targets.

In our earlier studies, using comparative Omics based approach, we have identified several consistently highly proteins as possible biomarkers of Bovine Trichomonosis (Karli G 2020). As the genome sequencing of the *T.foetus* was done very recently, there is no availability of functional annotations for the proteins of this organism. In this current study, In this current study, we have chosen Cysteine protease 8, Surface antigen BspA-like Protein, Chlamydia polymorphic membrane protein-like, Circumsporozoite protein precursor, hypothetical protein, Immuno-dominant variable surface antigen-like, Ser/Thr protein phosphatase as frequently expressed proteins of *Trichomonas foetus* for further analysis. We have used several bioinformatics tools for functional annotations of proteins, prediction of physico-chemical properties of proteins, identification of most probable sub cellular localization of the protein as well as prediction of extracellular membrane proteins.. Using computational tools now it is very much possible to successfully predict various properties of proteins that aid in maximum expression of the proteins in heterologous host systems like, E.coli, Yeast or mammalian cells.

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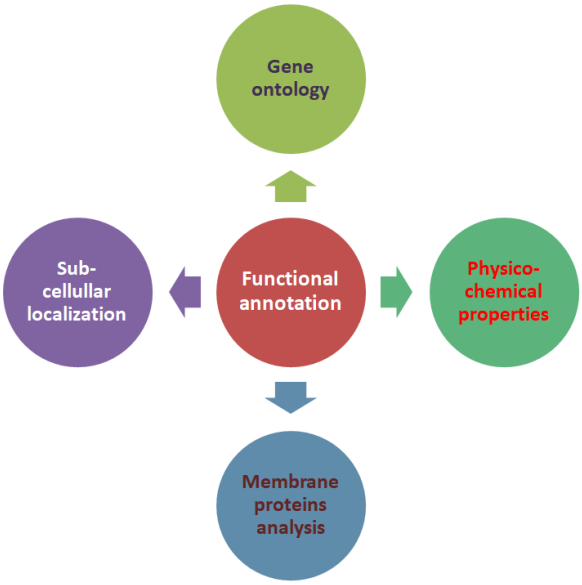

Fig.1. Over view of Functional annotation of newly identified protens. Gene ontology reveals the biological and molecular functions, sub-cellular localization for the cellular location of the protein, Physico- chemical properties for functional analysis and membrane protein prediction for possible role in pathogenicity.

Methods

Gene Ontology annotation

Protein sequences were obtained in FASTA format from NCBI protein (NCBI Resource Coordinators 2018) are used for analysis. Gene ontology annotation was used to retrieve the molecular functions and Biological processes associated with the targetable antigenic proteins of *Trichomonas foetus*. The data was obtained from Uniprot resource (https://www.uniprot.org/help/gene_ontology) (Ashburner, M 2000, UniProt Consortium 2015). FASTA sequences of the proteins are available in Supplementary file 1.0

Prediction of Physico-chemical properties of proteins

Expasy ProtParam tool was used to obtain various physico-chemical properties of the putative antigenic proteins of Trichomonas foetus.(https://web.expasy.org/protparam) (Gasteiger E 2005). These results were obtained based on the computational prediction and comparison of various chemical parameters like protein molecular weight, Isoelectric point, Composition, proportion of various amino acids as well as Physical parameters like the extinction coefficient, Half life of proteins in different cell systems, estimate of stability of proteins, Extent of aliphatic, hydropathicity etc. that are available from Swissprot.

Sub cellular localization prediction of proteins

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WoLF PSORT tool was used to predicts the intra cellular localization of proteins.(https://wolfpsort.hgc.jp/) (Horton P 2007). This tool predicts based upon the sub cellular sorting signals as well as some amino acid sequence specific features mostly obtained through Uniprot. Depending on the predicted signals, cellular locations like, cytoplasmic, nuclear, mitochondrial, Endoplasmic reticular spaces, lysosomes as well plasma membrane proteins will be predicted.

Transmembrane protein analysis

Using Wolf PSORT majority of the proteins were predicted to be most likely present on the plasma membrane. These proteins were further analyzed by submitting the FASTA sequence to the TMMHM V.2.0 (http://www.cbs.dtu.dk/services/TMHMM/) (Krogh A 2001) to identify the number of transmembrane domains and extent of extra cellular domain.

Results

GO Annotations

All the proteins are predicted to be highly antigenic proteins involved in both pathogen interactions. Cysteine Protease 8 is the extrcellularly secreted protein involved in damaging the bovine genial tract. There are several surface antigenic proteins listed in the most expressed group may be involved in virulence. Adhesins, Surface Antigen Bspa-Like Protein, Clan SB, FamilyS8,Subtilisin_Like Serine Peptidase, Chlamydia Polymorphic Membrane Protein-Like, Immuno-Dominant Variable Surface Antigen-Like, Ser/Thr Protein Phosphatase may have a role in host pathogen interaction of the parasite to the genital epithelium. Functional annotations of the various highly expressed antigenic proteins using Gene Ontology resource (http://geneontology.org/) are given in the Table.1

Table 1: Functional Annotation of Putative Antigenic Proteins of *T.foetus*

Name of the protein	Functional Annotation
Cysteine Protease 8	Highly expressed extracellular protease, plays a vital role in host–parasite interactions such as virulence, adherence, nutrition acquisition and inflammation
Surface Antigen Bspa-Like Protein	Highly expressed Transmembrane protein Highly antigenic
Clan SB, FamilyS8,Subtilisin_Like Serine Peptidase	Serine –Type endo-peptidase activity
Chlamydia Polymorphic Membrane Protein-Like	As auto transporter adhesins and are important in the initial phase of infection
Hypothetical Protein	Identified as hypothetical protein with transmembrane domain and antigenic domains in the *T. foetus*, potential diagnostic candidate gene.
Circumsporozoite Protein	A similar Circumsporozoite protein is the immunodominant surface

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Precursor	antigen on the sporozoite (the infective stage of the malaria parasite that is transmitted from the mosquito to the vertebrate host
Immuno-Dominant Variable Surface Antigen-Like	Protein involved in effective strategy involved in Immune evasion
Ser/Thr Protein Phosphatase	A protein with hydrolase activity
Tetraspaninfamily protein	Novel candidate cell surface virulence factors , These are membrane proteins involved in signaling modulating adhesion, motility and tissue invasion in host systems
Adhesin AP65	Membrane associated protein involved in adhesion and pathogenesis.

Physico-chemical Properties:

Expasy protpram analysis of FASTA sequences of these proteins are listed in Table 2.1 and 2.2 reveal that except for surface antigen BspA-like Protein, all the above proteins are relatively small to medium size proteins with 300 to 800 aa length.

Table.2.1 Chemical Properties of Potential Antigenic Proteins of *Trichomonas foetus*

NCBI ID	Name of the Protein	No of Amino acids	Molecular weight	Theoretical pI	-vely charged residues (Asp + Glu)	+velycharged residues (Arg + Lys)
OHT13704.1	Cysteine protease 8	320	35412.54	4.99	32	23
OHS99292.1	Surface antigen BspA-like Protein	3567	403762.44	4.75	448	276
OHT04136.1	Clan SB, family S8, subtilisin_like serine peptidase	866	95993.72	4.82	104	63
OHS93232.1	Chlamydia polymorphic membrane protein-like	264	29223.69	7.07	21	21
OHS95735.1	Hypothetical protein	870	96017.99	5.14	112	83
OHT08051.1	Circumsporozoite protein precursor	241	27316.93	6.24	16	11
OHT11175.1	Immuno-dominant variable surface antigen-like	270	30673.01	5.92	31	26
OHT00560.1	Ser/Thr protein phosphatase	506	58573.05	5.4	62	48
OHS99351.1	Tetraspanin family protein	208	23156.85	7.34	20	21

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OHT02241.1	Adhesin AP65	376	40253.29	5.04	37	28

SB-Seven stranded beta helix; Asp-Aspartic acid; Glu-Glutamic acid; Arg-Arginine;Lys-Lysine; Pi-Isolectric pH

Table 2.2 Physical Properties Potential Antigenic Proteins of *Trichomonas foetus*

NCBI ID	Ext. coefficient	Abs Cys residues form cystines	Ext.coefficient	All Cys reduced	Estimated half-life	Instability index	Aliphatic index	(GRAVY)
OHT13704.1	81415	2.299	80790	2.281	30hours	25.67	68.34	-0.218
OHS99292.1	232190	0.575	223940	0.555	30,20,10	45.36	98.96	-0.022
OHT04136.1	75720	0.789	714720	0.778	30,20,10	40.66	85.1	-0.255
OHS93232.1	23880	0.817	23380	0.8	2.8,10,2	23.8	67.23	-0.118
OHS95735.1	87000	0.903	86750	0.903	30,20,10	32.69	95.14	-0.04
OHT08051.1	35410	1.296	35410	1.296	30,20,10	48.5	72.32	-0.509
OHT11175.1	62005	2.021	61880	2.017	30,20,10	47.76	83.04	-0.382
OHT00560.1	92640	1.582	92140	1.573	30,20,10	34.42	75.32	-0.473
OHS99351.1	27805	1.201	26930	1.163	30,20,10	31.58	126.11	0.754
OHT02241.1	44725	1.111	44350	1.102	30,28,10	27.34	86.57	-0.026

Cys-Cysteine; GRAVY- Grand average of hydropathicity

Theoretical Iso-electric point of all the a proteins is in the acidic range except for like Chlamydia polymorphic membrane protein-like and Tetraspanin family protein which fall in alkaline range. These proteins exhibit varied extinction coefficients. Except for Chlamydia polymorphic membrane protein-like, all the proteins are stable for 30 hrs in mammalian reticulocyte cells, 20 hrs in yeast cells and 10 hrs in E.coli. All the proteins are stable for in-vitro studies

Sub cellular localization:

Cellular distributions of various target proteins of *T.foetus* are consolidated with their respective probability distribution scores in WOLF PSORT tool are listed in Table.3.

Table 3. Prediction of Sub Cellular Localization of Antigenic Proteins of *Trichomonas foetus*

Name of the Protein	Output of Localization	Predominant localization	Membrane localization
Cysteine protease 8	Nucl: 13, Cyto_Nucl: 11, Cyto: 6.5, Cyto_Pero: 6.16667, Mito: 6, Extr: 4,	Nucleus	No
Surface antigen BspA-like	Nucl: 25, Plas: 3, Cyto: 2, E.R.: 1, Golg: 1	Nucleus	No
Clan SB, family S8,	Plas: 32	Plasma	Yes

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subtilisin_like serine peptidase		membrane	
Chlamydia polymorphic membrane protein-like	Cyto: 10, Plas: 9, Cyto_Nucl: 8, Nucl: 4, Extr: 3, Mito: 3, Lyso: 2, Golg: 1	Cytoplasm &Plasma membrane	Yes
Hypothetical protein	Cyto: 15, Plas: 12, Nucl: 3, Cysk:1, Golg: 1	Cytoplasm &Plasma membrane	Yes
Circumsporozoite protein precursor	Cyto_Nucl: 15, Cyto: 14.5, Nucl: 10.5, Extr: 4, Mito: 1, Pero: 1, Cysk: 1	Cytoplasm	No
Immuno-dominant variable surface antigen-like	Cyto_Nucl: 15.6667, Cyto: 15, Cyto_Plas: 9.66667, Nucl: 8, Mito: 3, Pero: 3, Extr: 2	Cytoplasm& Plasma membrane	Yes
Ser/Thr protein phosphatase	Plas: 23, Extr: 5, E.R.: 2, Pero: 1, Lyso: 1	Plasma membrane	Yes
Tetraspaninfamilyprotein	Extr: 31, Plas: 1	Extracellular	Yes
Adhesin AP65	Plas: 32	Plasma membrane	Yes

Nucl- Nucleus, Cyto- Cytoplasmic, Pero-Peroxisome, Mito-Mitochondria, Extr-Extracellular, Plas-Plasma membrane

Several proteins like Cysteine protease 8, Surface antigen BspA-like Protein, Chlamydia polymorphic membrane protein-like, Circumsporozoite protein precursor, hypothetical protein, Immuno-dominant variable surface antigen-like, Ser/Thr protein phosphatase have multiple sub cellular localizations, where as Clan SB, family S8,subtilisin_like serine peptidase, Tetraspannins and Adhesins are mostly confined to plasma membrane and out side the membrane.

Transmembrane protein analysis of T.foetus proteins

The Table 4 below describes the length of various domains of protein in the Exterior membrane region, within the membrane and in the cytoplasmic side.

Table 4. Membrane protein analysis using TMHMM analysis tool

Name of the protein	Total number of Amino acids	Outer membrane region (No of AA)	Trans membrane region (No of AA)	Cytoplasmic region (No of AA)
Clan SB, family S8, subtilisin-like serine peptidase	860	1-819	820-842	843-866
Chlamydia polymorphic membrane protein-like	726	1-640	641-663	664-726

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Hypothetical protein	870	1-837	838-860	861-870
Immuno-dominant variable surface antigen	270	1-270	--	--
Ser/Thr protein phosphatase	506	1-64	65-87	88-506
Adhesin AP65	376	1-340	341-363	364-376

The graphical representations of the TMHMM V.2.0 analysis of one of the largest plasma membrane proteins, Clan SB, family S8, subtilisin-like serine peptidase are shown in Fig2. A single vertical bar represents that, it has 1 trans membrane domain predicted at the probability cut off value at 1.0 . For the above protein in the total 860 amino acids, this tools predicts it to have 819 amino acids in the extracellular domain only. Proteins with large extracellular domains tend to involve in host pathogen interaction. The figures of other trans membrane proteins are available in Supplementary file 2.

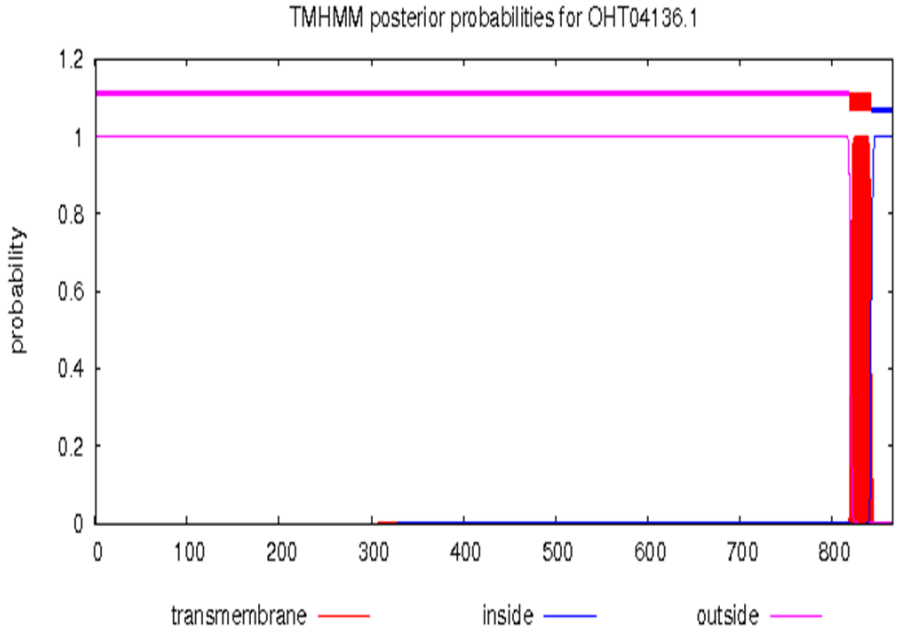

Fig.2. TMHMM analysis for Clan SB, family S8, subtilisin-like serine peptidase showing a largest extracellular domain and single trans membrane domain

Discussion

Omics based studies are helping the scientists towards a comprehensive understanding of the causative agent and possible mechanism/ mediators pathogenesis of any disease in recent times. Available data from a well studied closely related pathogen to *T.foetus* as well as few transcriptomics and proteomics have inspired us to screen the data to identify highly expressed proteins as well as consistently expressed proteins. Availability of freely accessible

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Bioinformatics tools like Gene ontology resource have made us to understand the function of the target protein. The above analysis clearly indicates that, majority of amino acid sequences are falling in the exterior membrane region. It is very advantageous in the immunity/antigenicity point of view to have large outer membrane protein domains. The proteins may have multiple large epitopes and exhibit good host pathogen interaction.

In immune diagnostics point of view, it is always mandate to choose cell surface proteins as target proteins for antibodies as well as vaccine candidate. Insilico tool like, WOLF PSORT as well as TMHMM V.2.0 helped us to predict and shortlist few potential membrane proteins with moderately large amount of protein towards exterior of the cell. Extracellular portion of the proteins are more suitable to develop subunit vaccines, for developing point of care sero-diagnostics such as rapid kit LFA, ELISA as well as drug targets. Though above analysis helps in developing fast track tools, the results needs further validation with at least two more similar analytical tools for each parameter, before starting the lab experiments,

Conclusions

Trichomonas foetus is among the neglected tropical parasitic diseases. There are no diagnostics and therapeutics developed for point of care use. The above predictions would aid the scientists to perform various experiments like, cloning, expression and purification of proteins in order to develop diagnostics as well as therapeutics. Cysteine protease 8 is most abundantly expressed as well as extra cellular secretary protein of the parasite pose to induce immune response in the host. Hence using the above parameters, it can be expressed in suitable heterologous host like, E.coli, Yeast for developing serological assays. Circumsporozoite protein precursor was well exploited for sero-diagnosis in malarial infection (Zhao J 2016) which was also found to a vaccine target.

Limitations and future prospects

Insilico analysis warrants further invivo protein production and antigenicity testing in lab animals. Based on the above understanding, *T.foetus* Circumsporozoite protein need to be further exploited and characterized as therapeutic and vaccine candidate. Several proteins such as Immuno-dominant variable surface antigen-like, Tetraspanin family protein, Chlamydia polymorphic membrane protein-like, Clan SB, family S8,subtilisin_like serine peptidase, Surface antigen BspA-like Protein etc have plasma membrane localization. They can be well characterized as diagnostic targets, for vaccine development as well as drug targets.

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List of Abbreviations

T.foetus (Trichomonas foetus); *T.vaginalis* (*Trichomonasvaginalis);* AA – Amino acids; BspA-Bacteroids Spirochetes surface antigen ; LFA –Lateral flow assay; ELISA-Enzyme linked Immunosorbent assay

Additional Information

Supplementary file 1.

Amino acid sequences of Target antigenic proteins of Trichomonas foetus in FASTA.

Supplementary file 2.

Graphic representations of TMHMM analysis for Chlamydia polymorphic membrane protein-like,Hypothetical protein, Immuno-dominant variable surface antigen-like, Ser/Thr protein phosphatase, Adhesin AP65.

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***Authors' contributions*:** GK has selected the list of immunogenic proteins and performed the functional annotation, and prediction of Physico-chemical properties and prepared the draft manuscript. RP has done the sub cellular localization analysis and critical analysis of results and KV has done transmembrane protein analysis and conclusions. authors have read and approved the manuscript

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