Name of the 5th sem Topic: Cell Structure & function.

Name of the Student: Usha 1st year.

Date: 18/2/2021

18/2/21

Name of the Topic: Chromosomal structure.

Name of the student. Ch. Sowmya.

19/2/21

19/2/21

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S.NO	H.T NO	Name of the student	Signature
1	6029-17-572-001	B. Soumya	Soumya
2	6029 17572 -002	B. Mamatha	B. mamatha
3	6029 17572 - 003	B. Malavika	malavika
4	6029 17572 -004	C. Manisha	[illegible]
5	6029 17572 -005	D. Radhika	Radhika
6	6029 17572 -006	G. Sarika	Sarika
7	6029 17572 -007	G. Divya rani	Divya
8	6029 17572 -008	G. Jyothi	Jyothi
9	6029 17572 -009	M. Ramya	Ramya
10	" " -00	M. Ramya	Ramya
11	" " -022	S. Glory	Glory
12	" " -024	S. Pooja	Pooja
13	" " -025	T. Malleshwari	[illegible]
14	" " -026	T. Shirisha	Shirisha
15	" " -027	T. Swathi	Swathi
16	6029 17572 -029	V. Lavanya	Lavanya

Date: 19/2/2021

name of the topic. Deviation fragymendal's law.

name of the student: Osha. 1st year.

1. List of students

Sathvika
Anupama
Radhika
Karya.
Spandana Rekha.

19/2/2021

S.NO	H.T NO	Name of the student
1	6029-18-489-001	Shravya Sri
	002	Sony.
	003	Sanpoorna.
	004	Fathima.
	005	Swapna.
	006	Sowjanya
	007	Sowmya.
	008	Tejaswini
	009	Monika

S.NO	H.T. NO.	Name of Studen.
	6029-18-572-001	B. Preethi
	002	Bhargavi
	003	Uma Rani
	004	Bushra fathima.
	005	Shravanthi
	006	Vinoda.
	007	Vaishnavi
	008	Manasa.
	009	Monika.
	010	Archana.

Name of the topic: DNA Replication 21/2/2021

Name of the Student: Srivani 2nd year.

Date: 21/2/2021

Name of the topic: Mutation

Name of the student: Srivani 2nd year

21/2/2021

2020-2021 ✓ Date 4/2/21

1. steps involved in plant tissue Culture.
2. Transcripting mechanism.
3. production of synthetic seeds
4. application of r-DNA Technology

	HT No	Name of the student	Signature
1	6029185720021	B. Reethi	B. Reethi
2	-002	B. Bhargavi	B. Bhargavi
3	-003	B. Umarani	B. Umarani
4	-004	Bushra-fathima	Bushrafathima
5	-005	ch. Shravani	Shravani
6	-006	ch. Mounika	ch. Mounika
7	-007	D. Vinoja	D. Vinoja
8	-008	G. shirisha	G. Shirisha
9	-010	G. Srivani	Srivani
10	-012	G. Manasa	Manasa
11	-013	H. Mounika	H. Mounika
12	-014	K. Bhargavi	K. Bhargavi
13	-016	L. Archana	L. Archana
14	-017	M. Vyshnavi	M. Vyshnavi
15	-018	Md. Afreen	Md. Afreen
16	-019	N. Anusha	N. Anusha
17	-020	N. shirisha	shirisha
18	-021	N. Sumalatha	Sumalatha
19	-022	P. Jayasri	Jayasri
20	-023	R. Likitha	Likitha
21	-024	Sabahath kanam	Sabahath kanam
22	-025	U. Sunitha	Sunitha
23			
24	6029-18-489-002	A. Sravani	A. Sravani
2	-003	ch. Soumya	ch. Soumya
3	-006	ch. Prathima	ch. Prathima
4	-008	E. Soumya	E. Soumya
5	-009	G. Mounika	G. Mounika
6	-011	N. Soumya	N. Soumya
7	-012	Shahana	Shahana

2020-2021

1. Prokaryotic genome organization. Date 4/2/2021
2. Translation mechanism.
3. PCR Techniques.

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1	ch. Sravanthi	60291957	2002	ch. Sravanthi
2	D. Nikitha	"	003	D. Nikitha
3	G. Nikitha	"	005	G. Nikitha
4	G. Srivani	"	006	G. Srivani
5	K. Anjali	"	007	K. Anjali
6	k. prathyusha	"	008	k. Prathyusha
7	M. swathi	"	009	M. Swathi
8	M. shilpa	"	010	M. shilpa
9	P. keerthi sri	"	011	P. keerthi sri
10	P. shilpa shetty	"	012	P. shilpa shetty
11	R. sathvika	"	013	R. Sathvika
12	U. Anusha	"	015	U. Anusha
13	Y. Bhavana.	"	016	Y. Bhavana
14	B. kavya	6029 19 48	9001	B. Kavya
15	B. Mounika	"	002	B. Mounika
16	K. Swetha	"	003	K. Swetha
17	K. Srivani	"	004	K. Srivani
18	N. sony	"	005	N. Sony
19	T. spandhana	"	006	T. spandhana

4/2/21

4/2/2021

2020 - 2021

1. Bacterial cell structure.
2. mejosis.
3. Epistaxis.

H.NO	Name of the student	signature
1. 6029 - 20 - 572 - 003	D. Kalyani	D. Kalyani
2. - 004	G. Harshitha	G. Harshitha
3. - 005	K. Pooja	K. Pooja
4. - 006	K. Nagashreshta	K. Nagashreshta
5. - 007	M. shylaja	M. shylaja
6. - 008	N. Vinoda	N. Vinoda
7. - 009	P. Ramya	P. Ramya
8. - 010	S. Bhavani	S. Bhavani
9. - 011	S. Choosen Grace	S. Choosen Grace
10. - 012	S. Pavani	S. Pavani
1. 6029 - 20 - 489 - 001	Ch. Sowmya	Ch. Sowmya
2. - 002	D. Saideepthi	D. Saideepthi
3. - 003	G. usha	G. usha
4. - 004	J. Anupama	J. Anupama
5. - 005	K. Sandhya	K. Sandhya
6 - 006	K. prasanna	K. prasana
7 - 007	K. swetha	K. Swetha
8 - 009	M. shravani	M. shravani
9 - 010	M. madhulatha	M. Madhulatha

4/2/2021

2020-21 Date: 5/2/2021

1. Animal tissue culture media
2. Somatic cell nuclear transfer
3. molecular marker
4. production of citric acid.

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	HT. No.	Name of the student	Signature
1	6029-18-572001	B. Preethi	B. Preethi
2	-002	B. Bhargavi	B. Bhargavi
3	-003	B. Umarani	B. Umarani
4	-004	Bushra-fathima	Bushrafathima
5	-005	Ch. Shravani	Ch. Shravani
6	-006	Ch. Mounika	
7	-007	D. Vinoja	D. Vinoja
8	-008	G. Shirisha	G. Shirisha
9	-010	G. Srivani	Srivani
10	-012	G. Manasa	G. Manasa
11	-013	H. Mounika	H. Mounika
12	-014	K. Bhargavi	K. Bhargavi
13	-016	L. Archana	L. Archana
14	-017	M. Vyshnavi	M. Vyshnavi
15	-018	Md. Afreen	Md. Afreen
16	-019	N. Anusha	N. Anusha
17	-020	N. Shirisha	N. Shirisha
18	-021	N. Sumalatha	N. Sumalatha
19	-022	P. Jayasri	P. Jayasri
20	-023	R. Likitha	R. Likitha
21	-024	Sabahath kanam	Sabahathkanam
22	-025	U. Sunitha.	U. Sunitha
1	6029-18-489-002	A. Sravani	A. Sravani
2	-003	Ch. Soumya	Ch. Soumya
3	-006	Ch. Prathima	Ch. Prathima
4	-008	E. Soumya	E. Soumya
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6	-011	N. Soumya	N. Soumya
7	-012	Shahana.	Shahana

5/2/21

2020-2021 17/2/2021

1. NCBI
2. BLAST
3. Probability Distribution.
4. ANOVA

1	Ch. Sravanthi	60291997 2002	Ch. Sravanthi
2	G. Nikitha	" 005	G. Nikitha
3	G. Srivani	" 006	G. Srivani
4	K. Anjali	" 007	K. Anjali
5	K. prathyusha	" 008	K. Prathyusha
6	M. Swathi	" 009	M. Swathi
7	M. Shilpa	" 010	M. Shilpa
8	P. Keerthi Sri	" 011	P. Keerthi Sri
9	P. Shipra shetty	" 012	R. Sathvika
10	R. sathvika	" 013	U. Anusha
11	U. Anusha	" 2015	Y. Bhavana
12	Y. Bhavana	" 016	B. Kavya
13	B. Kavya	6029 19 48 9001	B. Mounika
14	B. Mounika	" 002	K. Swetha
15	K. Swetha	" 003	K. Srivani.
16	K. Srivani	" 004	K. Srivani
17	N. Sony	" 005	N. Sony
18	T. Spandhana	" 006	T. Spandhana

Srivani

2020-2021 Date: 18-2-2021

1. Amino acids classification
2. michaelis-menton Equation.
3. Bright field microscope
4. Cultivation of anaerobic bacteria.

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-004	G. Harshitha	G. Harshitha
-005	K. Pooja	K. Pooja
-006	K. Nagashreshta	K. Nagashresta
-007	M. Shylaja	M. Shylaja
-008	N. Vinoda	N. Vinoda
-009	P. Ramya	P. Ramya
-010	S. Bhavani	S. Bhavani
-011	S. Choosen Grace	S. Choosen Grace
-012	S. Pavani	S. Pavani
1. 6029-20-489-001	Ch. Sowmya	Ch. Sowmya
-002	D. Saideepthi	D. Saideepthi
-003	G. Usha	G. usha.
-004	J. Anupama	J. Anupama
-005	K. Sandhya	K. Sandhya
-006	K. Prasanna	K. Prasanna
-007	K. Swetha	K. Swetha
-009	M. Shravani	M. shravani
-010	M. madhulatha	M. Madhulatha.

17/2/2021

Computational prediction of various membrane protein as a potential diagnostic markers for Bovine Trichomonosis

STUDENT STUDY PROJECT
2020-21

Submitted & Executed by

II B.Sc Biotechnology students
The Department of Biotechnology
Government Degree College (w), Gajwel, Siddipet Dist.

Project supervisor

Mr. Bhaskar Reddy
Lecturer in Biotechnology

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4	602919572002	CHATLAPALLY SRAVANTHI	B.SC BT.B.C
5	602919572005	GADDAM NIKHITHA	B.SC BT.B.C

GOVT. DEGREE COLLEGE (W) GAJWEL, SIDDIPET. DT.TELANGANA

CERTIFICATE

This is to certify that Students of II.B.Sc Biotechnology have successfully completed the Student Study project work titled " Computational prediction of various membrane protein as a potential diagnostic markers for Bovine Trichomonosis" offered by Dept of Biotechnology for the academic year 2020-21.

Details of the Students

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5	602919572005	GADDAM NIKHITHA	B.SC BT.B.C

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Lecturer in Biotechnology

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Principal

Index

Computational prediction of Chlamydia polymorphic membrane protein as a potential diagnostic markers for Bovine Trichomonosis

Bonakolluri Kavya, And Bonakolluri Mounika

Abstract. Bovine Trichomonosis is the most neglected venereal disease of cattle in India. Identifying the infected bull or cow and isolating from the herd are the only available control strategies. Till today, there are no simple, inexpensive rapid kits developed for Identification of *Trichomonas foetus* infection. Transcriptomics and proteomics based data of the casual agent as well as comparative genomics based approach to screen for similar data of *Trichomonas vaginalis*, a closely related human parasite were analyzed to identify Adhesin proteins. TMMHM tool revealed them to be as transmemebrane protein and as membrane associated protein respectively. These proteins were also analyzed using Epitope Prediction and Analysis Tools in Immune Epitope Database Analysis Resource. Adhesin proteins contain several large continuous epitopes which suggest that they can be exploited for developing point of care diagnostics

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

Back ground

India is the leading country in the world with regard to cattle population. Live stock is threatened by several diseases, droughts and other environments stress. [1] There exists huge disparity with the as low as the existing fertility rate of cattle of 35% to the rapid increase in the demand for animal based products.[2],[3] Though the natural breeding methods are being replaced by Artificial insemination, the practice of semen screening for sexually transmitted diseases is posing great loss. [1]

Trichomonosis has wide spread epidemiology in the world.[4] Bovine Trichomonosis is the most neglected venereal disease of cattle in India. *Trichomonas foetus,* the casual agent of the disease is a single cellular parasite exists mostly in trophozoite form[5] and some times as pseudocyst.[6] Early abortions in cows and prescence of large number of unbred cows in the herd is the indication of Trichomonosis.[7], [8], [9], [10] Identifying the infected bull or cow and isolating from the herd are the only available control strategies.[11] Till today, there are no simple, inexpensive rapid kits developed for Identification of *Trichomonas foetus* infection. We hypothesized that, screening of transcriptomics and proteomics based data of the casual agent as well as comparative genomics based approach to screen for similar data of *Trichomonas vaginalis*, a closely related human parasite would enable to identify potential markers.

Materials and methods

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, virulent proteins proposed to be involved in pathogenesis of Bovine Trichomonosis was done.[12],[13],[14],[15],[16], Proposed potential virulent proteins of *T.vaginalis* are subjected to BLAST analysis to compare and identify the corresponding proteins of *T.foetus.*[17],[18] Obtained the accession numbers for corresponding highly expressed transcripts, ESTs and proteins in *T.foetus and T.vaginalis* from NCBI proteins data base. Downloaded the corresponding FASTA formats.[19]

Confirmation of listed proteins as potential putative surface proteins was done using TMMHM.[20]FASTA sequences of the proteins were submitted to "TMHMM Server v. 2.0" for prediction of transmembrane helices in proteins. Output was obtained with graphics. Analyzed the FASTA sequences for the putative antigenic domains of the listed virulent proteins using "Epitope Prediction and Analysis Tools" in "Immune Epitope Database Analysis Resource". [21] "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

Chlamydia polymorphic membrane protein are the consistently highly expressed. Proteins in several transcriptomics and proteomics studies of *Trichomonas foetus*. BLAST analysis with *Trichomonas vaginalis* proteins revealed that there exists considerable similarity.

Retrieving FASTA Sequence

FASTA sequence of Chlamydia polymorphic membrane protein with NCBI Protein accession number OHS93232.1was obtained.

>OHS93232.1 hypothetical protein TRFO_40483 [Tritrichomonas foetus]

MSKENRPNFKDLKDTTLDIDNKFRKLDRFSSQSFNKESRTERSYLESVVYITSTSF
TECFSYGSTYFLGC

GGAIFLCNSQLSIKGNTLFTKNSARVGGAIGLYASNVFIEGSSSSLVKFTSNKADF
LGGAIFSTQGDSDD

MKAQNFFQISFCDFTGNEAKEISGAVCIYSVFDSWIDHCIFDNNRAQHLSGALGF
YNCQGMFVFKSTFVN

NTSGHRAFIQSNSFQPYQLKSFRKLNHLKGGGGIFVQVKENTRKDLTSDDYRAK
LVFATQHCFFANNTCD

SKLRTEPYNQGFDILFGGIVTYQSYTDIFLNVKNVSLGGHMNTDFIFAGTTFYDQ
DTEWDTLTSNINQFF

MVEERFPNLKSDIYDKLSVDKYEWPPTAALTDIPAGPVNNAAADSIDNNDLPAN
TAPATKLPERTTRGGQ

PAASTILPEWHVSSVSRTVHYVSPSMSPTESPTESATESATESPTESPTESATESAT
ESPTESPTESATE

SPTESPTESATXSATESPTESPTESATESATESPTESPTESATESATESPTESPTESAT
ESATESPTESP

TESATESPTESPSLSPTESATQSATCSPSLSPSESATSSATESPSISATESATESPSLSS
EIVDDNLEVE

KDKSQFTTGKTAGLAIILFGFIVWVALVSFATNSIYKMKKARLEDPNDDNNGSSS
DRPETRMSNTETMSV

VSMNDTIDDPFAEDFDENIIQICAPL

Genpept data sheet reveled that, It is a huge protein with 726 amino acids.

Membrane protein prediction
TMHMM analysis reveals that it has single trans membrane domain with a large extracellular domain measuring 726 amino acids shown in the fig.1.

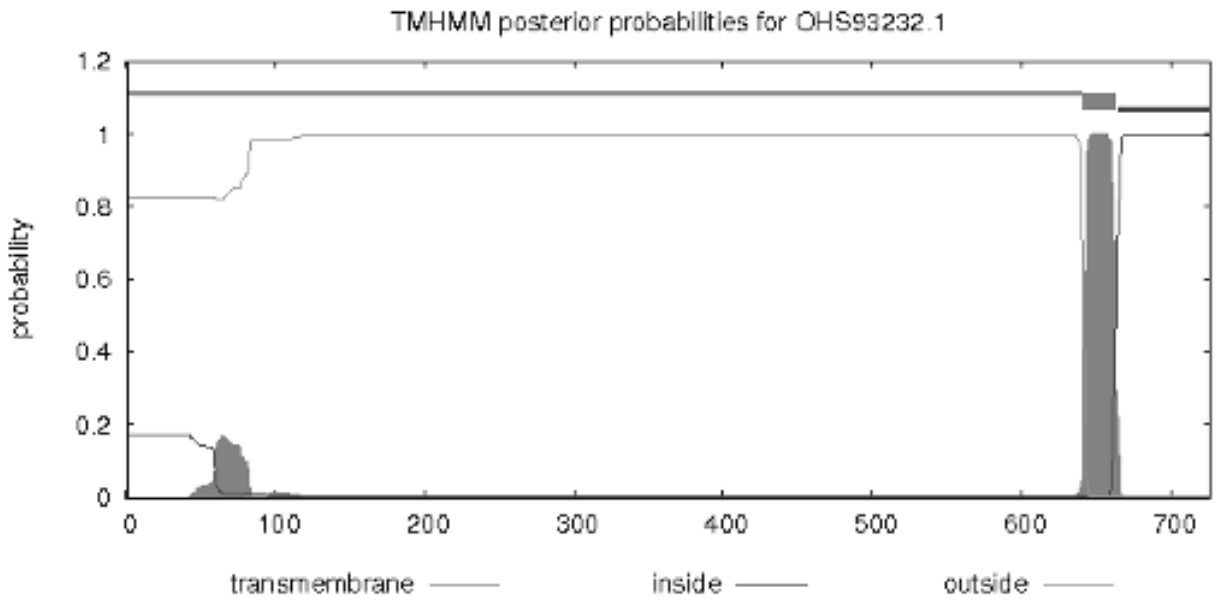

Figure.1 TMHMM analysis Chlamydia polymorphic membrane protein of T.foetus

Immunogenic domain analysis

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 has several large antigenic domains at 0.500 threshold value shown in Fig.2, with the largest epitope domain measuring 333 amino acids shown in the Fig 3

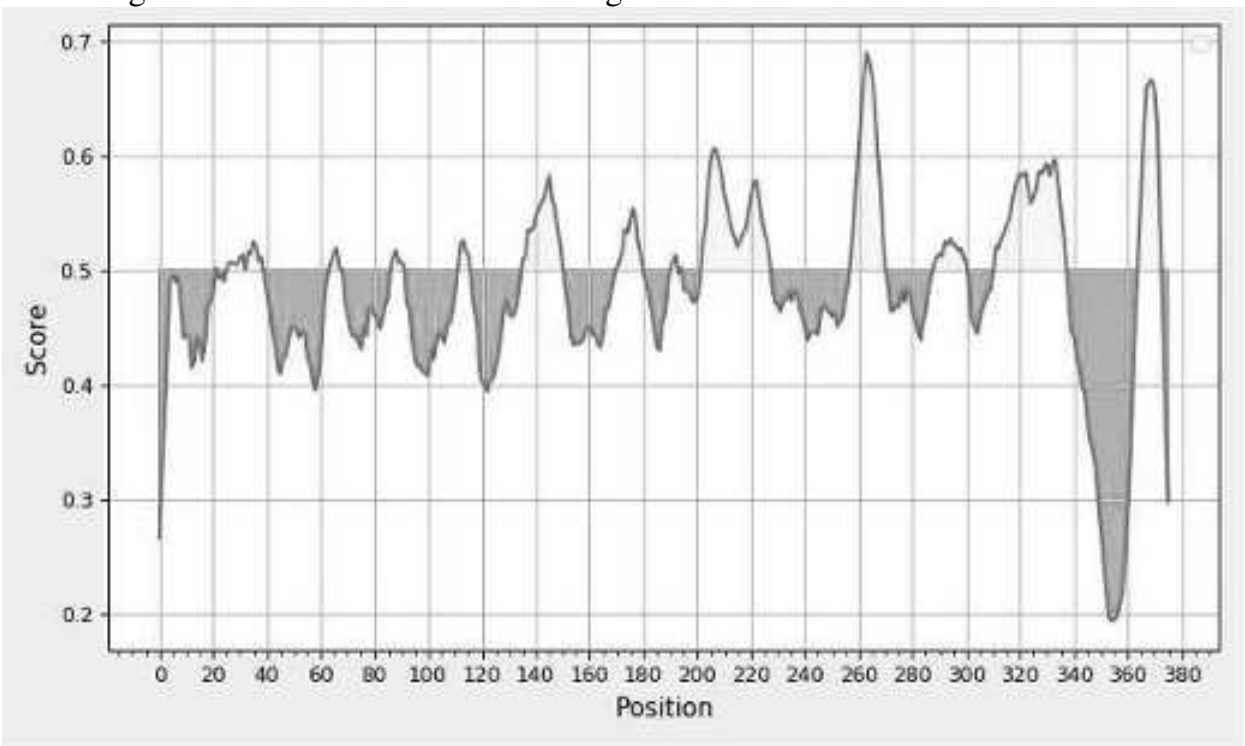

Figure.2 Graphic representations of epitope domains of Chlamydia polymorphic membrane protein of T.foetus

Average: 0.481 **Minimum:** 0.194 **Maximum:** 0.689

Predicted peptides:

No.	Start	End	Peptide	Length
1	22	22	C	1
2	27	39	KWIVSKFVPDYNF	13
3	64	68	TNSTG	5
4	87	92	IKTYKD	6
5	112	116	INTEG	5
6	136	151	NSMNQMNLLKSSGEGS	16
7	171	180	QSEAAVVEGK	10
8	191	193	VGY	3
9	195	195	K	1
10	203	228	SMSMDADIGTALFYSYHSSITTKSEG	26
11	258	270	AADQWGDEGSNGG	13
12	289	301	ADDSNLTLTVVAS	13
13	312	338	LVIKKDSEDDDGEDIVPTDKSQTLSRN	27
14	365	373	KKRNQSPGF	9

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

The above graphic representation in Fig2. clearly indicates that adhesions have several antigenic domains shown above the threshold value. There are several larger domains between 203-228 amino acids and 312-338 amino acids. The region between 200 and 340 are more suitable domains that can be used to produce recombinant proteins for use in diagnostics..

Conclusion and future prospects

Chlamydia polymorphic membrane proteins of *T.vaginalis* are well known surface proteins mediate host-pathogen interactions and cell aggregations leading to virulence. These Proteins were found to be mediating the initial binding of the obligate intracellular pathogen and eventual invasion into the host cell. Chlamydia polymorphic membrane protein are the consistently highly expressed in several transcriptomics and proteomics studies of Trichomonas foetus. Both the proteins contain several large continuous epitopes. Both the proteins are 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. These proteins can be further evaluated to develop vaccine.

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Computational prediction of Hypothetical protein TRFO 10391 as a potential diagnostic markers for Bovine Trichomonosis

Chatlapally Sravanthi And Gaddam Nikhitha

Abstract.

Bovine Trichomonosis is the most neglected venereal disease of cattle in India.Tritrichomonas foetus is a bovine and feline parasite and a porcine commensal. This organism is the causative agent of bovine and feline trichomonosis. In cattle, the parasite colonizes the urogenital tract and causes similar symptoms to those caused by Trichomonas vaginalis in humans. In cats, the parasite colonizes the gastrointestinal tract and produces a protracted watery diarrhea. In cattle, this parasite can lead to abortions

and substantial herd loss due to culling of infected animals, whereas in cats prolonged courses of diarrhea can lead to abandonment or euthanasia. Identifying the infected bull or cow and isolating from the herd are the only available control strategies. Till today, there are no simple, inexpensive rapid kits developed for Identification of *Trichomonas foetus* infection. Transcriptomics and proteomics based data of the casual agent as well as comparative genomics based approach to screen for similar data of *Trichomonas vaginalis*, a closely related human parasite were analyzed to identify Hypothetical proteins. TMMHM tool revealed them to be as transmembrane protein and as membrane associated protein respectively. These proteins were also analyzed using Epitope Prediction and Analysis Tools in Immune Epitope Database Analysis Resource. Hypothetical proteins contain several large continuous epitope which suggest that they can be exploited for developing point of care diagnostics

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

Back ground

India is the leading country in the world with regard to cattle population. Live stock is threatened by several diseases, droughts and other environments stress. [1] There exists huge disparity with the as low as the existing fertility rate of cattle of 35% to the rapid increase in the demand for animal based products.[2],[3] Though the natural breeding methods are being replaced by Artificial insemination, the practice of semen screening for sexually transmitted diseases is posing great loss. [1]

Trichomonosis has wide spread epidemiology in the world.[4] Bovine Trichomonosis is the most neglected venereal disease of cattle in India. *Trichomonas foetus,* the casual agent of the disease is a single cellular parasite exists mostly in trophozoite form[5] and sometimes as pseudo cyst.[6] Early abortions in cows and presence of large number of unbred cows in the herd is the indication of Trichomonosis.[7], [8], [9], [10] Identifying the infected bull or cow and isolating from the herd are the only available control strategies.[11] Till today, there are no simple, inexpensive rapid kits developed for Identification of *Trichomonas foetus* infection. We hypothesized that, screening of transcriptomics and proteomics based data of the casual agent as well as comparative genomics based approach to screen for similar data of *Trichomonas vaginalis*, a closely related human parasite would enable to identify potential markers.

Materials and methods

Extensive literature survey in Pub med was carried out to search for Transcriptomics and proteomics studies of *Trichomonas foetus* and *Trichomonas vaginalis.* Screening and listing of highly expressed surface proteins, virulent proteins proposed to be involved in pathogenesis of Bovine Trichomonosis was done.[12],[13],[14],[15],[16], Proposed potential virulent proteins of *T.vaginalis* are subjected to BLAST analysis to compare and identify the corresponding proteins of *T.foetus.*[17],[18] Obtained the accession numbers for corresponding highly expressed transcripts, ESTs and proteins in *T.foetus and T.vaginalis* from NCBI proteins data base. Downloaded the corresponding FASTA formats.[19]

Confirmation of listed proteins as potential putative surface proteins was done using TMMHM.[20]FASTA sequences of the proteins were submitted to "TMHMM Server v. 2.0" for prediction of transmembrane helices in proteins. Output was obtained with graphics. Analyzed the FASTA sequences for the putative antigenic domains of the listed virulent proteins using "Epitope Prediction and Analysis Tools" in "Immune Epitope Database Analysis Resource". [21] "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

Hypothetical proteins are the consistently highly expressed. Proteins in several transcriptomics and proteomics studies of *Trichomonas foetus*. BLAST analysis with *Trichomonas vaginalis* proteins revealed that there exists considerable similarity.

Retrieving FASTA Sequence

FASTA sequence of Hypothetical with NCBI Protein accession number OHS95735.1 was obtained.

>OHS95735.1 hypothetical protein TRFO_10391 [Tritrichomonas foetus]

MGKEVSELTRFQHQSGGFYDNVREANARDTFHAIWISSIYGAFQYIDTQRCFRWFTTLRNRDGGAGLVPG

SKSSVFATYCHFNLAAIISPDAIDVARIIEFLKSCYDEPSGLFRDSPESEPSIEATYYAYELLSRFRNAE

ITWLTSYNLQMYINDHLNDDHFEFDGVSLMKAQLWAGSIAKFVSLTVPYHRISEFIVNHLNQAIKDNKLD

NEDAAAAARILKLFGDEAIPEQLSASFKSSGSLADLFYINQILVATGEVTKFFEVHVHSLSGDNHLIDFE

KDGLTYGQIARPALAITALGRFINTMLQVNVTTHIGDEAPTTETLKIDYQTGLFNSQRISSINKLGQMQI

DVVAWLATEFGTPVVITKSVVSRVSLPIDVTSEAWLSADEPIPVGGEIVPGVNFRVQLNGKLDDIIDKLE

DTTAATFQVTDPAGAVLYHKFEDFKGQLEFTWQLPSLALPAGDLHVTVEIGDKVNGIHTHKEFTYKVSST

MAASGVEVPANLRLSDVLRVKMVPALIVNEAPVPFTNEKFFEGDLRDATGEAFYPQTASEAQRYTMRVKV

GDVVVKTVEGEVSVDDQNKLSVEFESNVNENLDFATGFSIDFLFNAEGSEPVLLDLEKEIFVQVSSKVVV

EAQPLVSGAVDYGSKITSEFRLKDEDSGNYLEAGRAYPVIAILRASDRTVLLEKKAKILSDKYKAKLTVT

AAVESGNVIVAILIRKGDDLVPVQTAQGSPFESAVTVSGQIQFDAQVVEARKYVVVDFTTTYKGKALRGT

AFMCRVVDAQGNAVAELPLAQMKKGSRLSWESGDAKGEYKLELRRLSATEGAPIFVKSISVESPILSIIH

HLPVEGITLAVAFAIFVWSIRVRKQIQSTR

- Genpept data sheet reveled that, it is a huge protein with 870 amino acids.

<u>Membrane protein prediction</u>

TMHMM analysis reveals that it has single transmembrane domain with a large extracellular domain measuring 870 amino acids shown in the fig.1.

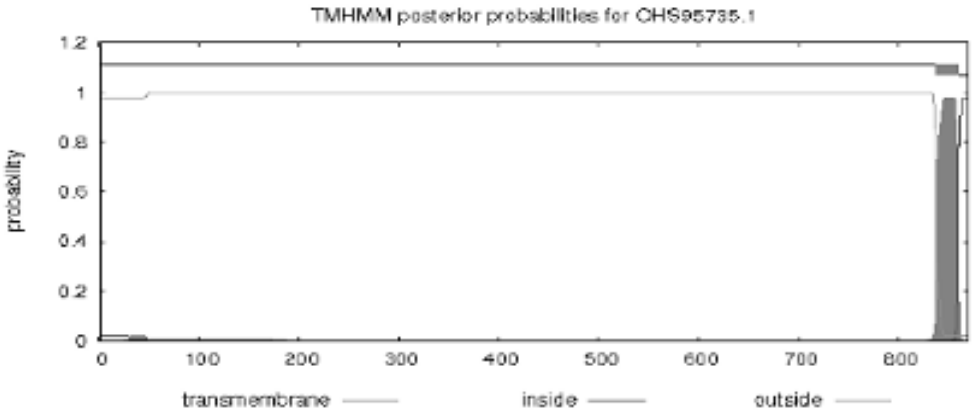
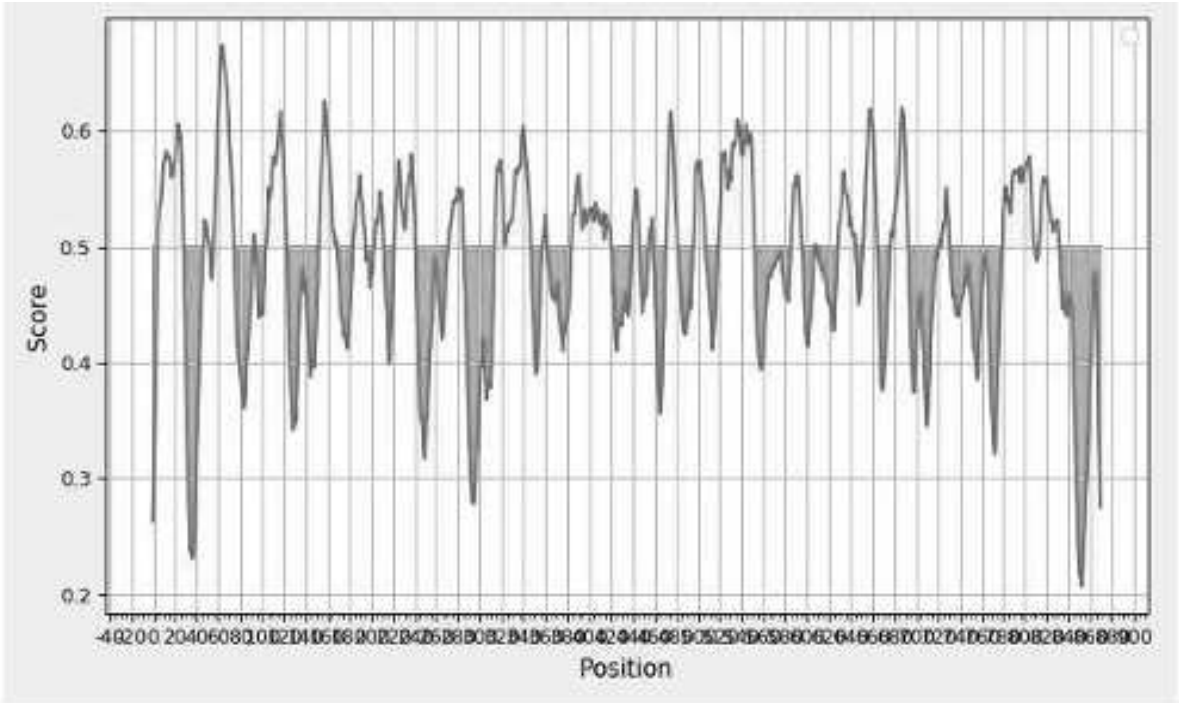


Figure.1 TMHMM analysis Hypothetical protein of T.foetus

Immunogenic domain analysis

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 Hypothetical protein has several large antigenic domains at 0.5 threshold value shown in Fig.2, with the largest epitope domain measuring 33 amino acids shown in the Fig 3



Average: 0.484 Minimum: 0.207 Maximum: 0.674

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

Predicted peptides:

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	GNAVAELPLAQPKKGSRLSWESGDAKGEYK	30

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

The above graphic representation in Fig2. Clearly indicates that adhesions have several antigenic domains shown above the threshold value. There are several larger domains between 520-552 amino acids and 781-810 amino acids. The region between 200 and 340 are more suitable domains that can be used to produce recombinant proteins for use in diagnostics..

Conclusion and future prospects

Hypothetical proteins of *T.vaginalis* are well known surface proteins mediate host-pathogen interactions and cell aggregations leading to virulence. These Proteins were found to be mediating the initial binding of the obligate intracellular pathogen and eventual invasion into the host cell. Hypothetical proteins are the consistently highly expressed in several transcriptomics and proteomics studies of Trichomonas foetus. The application of proteomics to uncover proteins that are differentially expressed on the surface of parasite strains with varying pathogenic properties has similar limitations and requires biochemical analyses of identified proteins to reveal their functions. Both the proteins contain several large continuous epitopes. Both the proteins are 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. Although recent analyses of the *T. vaginalis* genome have provided clues to possible surface proteins with potential roles in pathogenesis, such data are limited as localization or proposed activity on the basis of sequence similarity to proteins of known function does not necessarily confer these properties. These proteins can be further evaluated to develop vaccine.

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19. National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov
20. Immune Epitope Database https://www.iedb.org/
21. TMHMM Server v. 2.0 http://www.cbs.dtu.dk/services/TMHMM/

Computational prediction of GP63-like protein as a potential diagnostic markers for Bovine Trichomonosis

Kadulla Shwetha

Abstract

Bovine Trichomonosis is the most neglected venereal disease of cattle in India. Identifying the infected bull or cow and isolating from the herd are the only available control strategies. Till today, there are no simple, inexpensive rapid kits developed for Identification of *Trichomonas foetus* infection. Transcriptomics and proteomics based data of the casual agent as well as comparative genomics based approach to screen for similar data of *Trichomonas vaginalis*, a closely related human parasite were analyzed to identify GP-63 proteins. TMMHM tool revealed them to be as transmemebrane protein and as membrane associated protein respectively. These proteins were also 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 point of care diagnostics

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

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Identification of *Trichomonas foetus* infection. We hypothesized that, screening of transcriptomics and proteomics based data of the casual agent as well as comparative genomics based approach to screen for similar data of *Trichomonas vaginalis*, a closely related human parasite would enable to identify potential markers.

Materials and methods

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Confirmation of listed proteins as potential putative surface proteins was done using TMMHM.[20]FASTA sequences of the proteins were submitted to "TMHMM Server v. 2.0" for prediction of transmembrane helices in proteins. Output was obtained with graphics. Analyzed the FASTA sequences for the putative antigenic domains of the listed virulent proteins using "Epitope Prediction and Analysis Tools" in "Immune Epitope Database Analysis Resource". [21] "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

GP63-like proteins are the consistently highly expressed. Proteins in several transcriptomics and proteomics studies of *Trichomonas foetus*. BLAST analysis with *Trichomonas vaginalis* proteins revealed that there exists considerable similarity." To survive as an extracellular parasite, *T. vaginalis* adheres to the epithelial lining or extracellular matrix components of the urogenital tract [5]. Attachment to cells, micro-organisms or other surfaces drives a transition of the ovoid free-swimming parasite into an amoeboid form [5,14] that may be highly adherent [5]"[22]

Retrieving FASTA Sequence
FASTA sequence of GP63-like protein with NCBI Protein accession number OHS97275.1 was obtained >OHS97275.1 Clan MA, family M8 [Tritrichomonas foetus]

MIFAIIQIVSATNCFHDSLQSQINIRKLQFPPDPGLMDEDEIIEREPIRIVFDISSLTSD
YDPSVCRTIEQAVSWGGREEICTKDDIITPEKISSLNTTLNNVNNYLSSVLNVTRL
KGGFDISNITDITVLERHVDCDTFITVTTRPFGTHRSTSLASAFYEITDPVNGRPVQ
GAIVVNAANIPAEPQNESSFDRIYFTTLLHELVHALGVSYRAIPSWIDPNTNQPYE
HLPIIEYSATKYPHKVFRILQTKNVHEFAAERFGVEYFAPDVPAGLELEDGGGVG
TFGSHAEARVYIDDMFVGLTIGQNRISKLVFALLADTGWYDVSYEKAEKSAWGL
GESLNLSPLTTFPNTAPQHAFPKHYMCDPSDIDTDVCTYDFLGIALCKGVKVDCD
LPSDEDDQKFCEMRNFTDPLRIGLRGRSEVHDYLLYKAPYSNSRCSDISRNTDSA
YKNGELYGGESLCFMSTLLRSSFSFYTYYHGACHRSICDENGSLIVYVDGIGKICE
KANQKLSFSGFKGEIICPEPSYACGIRKFYGIVGPTPVPSPPNPTPIWEGFSLDSNQT
IIIAVFASVTGLIIFMAVVMQVRAKKAADAAKEAEEGVGPSMEGKDDPYEAVKP
PLVL GenPept data sheet reveled that, It is a huge protein with 620 amino acids.

Membrane protein prediction

TMHMM analysis reveals that it has single trans membrane domain with a Single transmembrane domain containing protein a large extracellular domain measuring 561 amino acids shown in the fig.1.

The extracellular domain offers antigenicity, cell to cell communication. This can be exploited for epitope prediction.

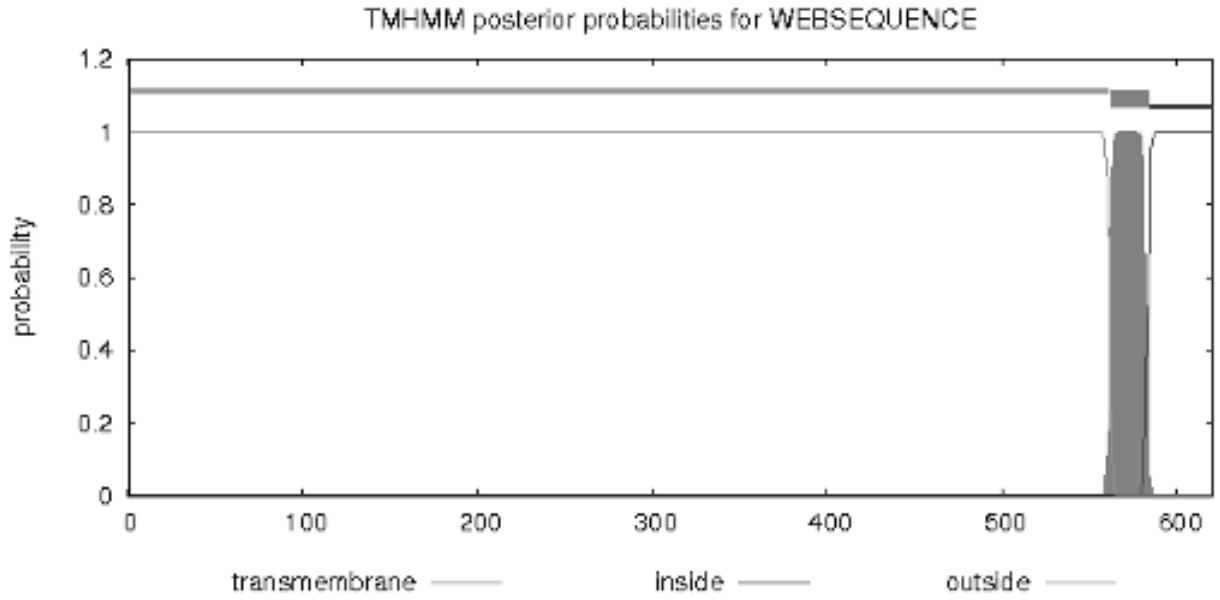

Figure.1 TMHMM analysis GP-63 protein of T.foetus

Immunogenic domain analysis

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 GP-63like protein has several large antigenic domains at 0.5 threshold value shown in Fig.2, with the largest epitope domain measuring 52 amino acids shown in the Fig 3

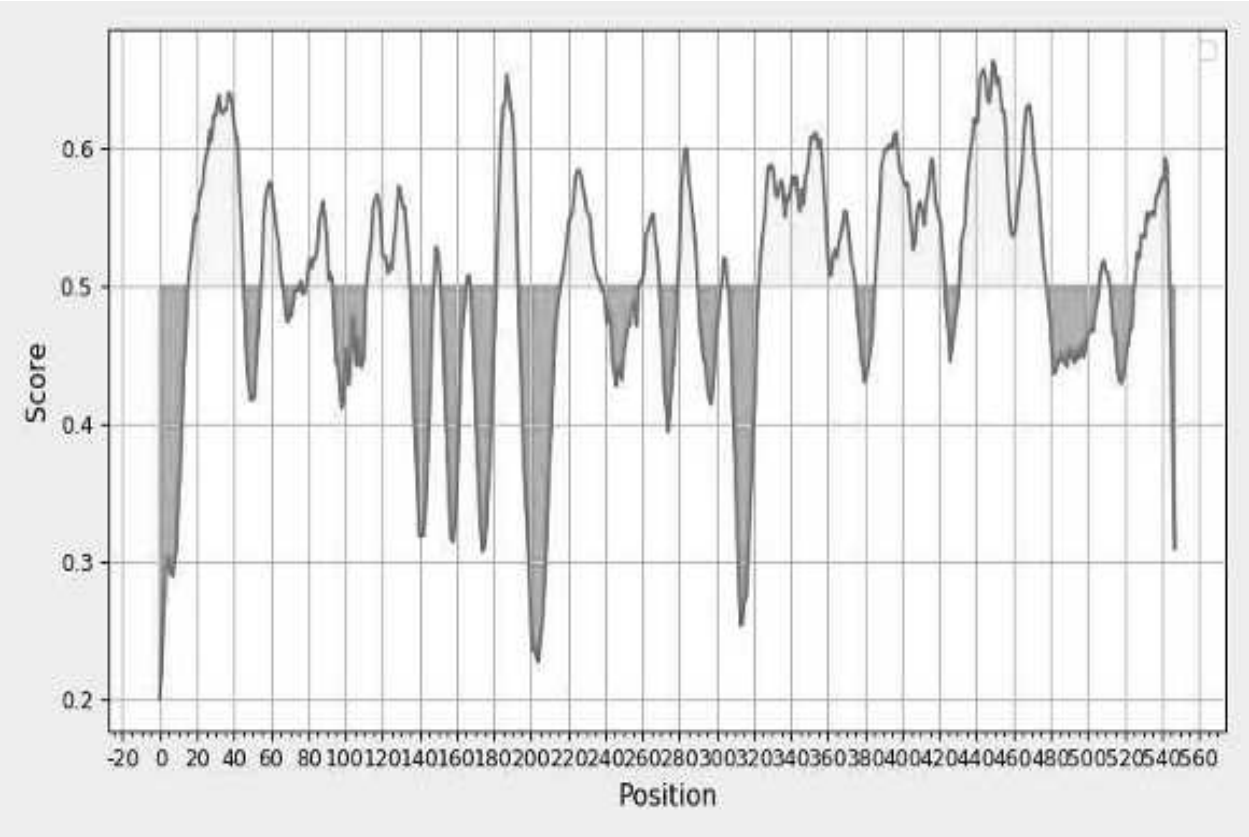

Figure.2 Graphic representations of epitope domains of GP-63 protein of T.foetus

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 above Graphic representation in Fig2. clearly indicates that GP-63 have several antigenic domains shown above the threshold value. There are several larger domains between 324-375 amino acids and 432-478 amino acids. The region between 320 and 480 are more suitable domains that can be used to produce recombinant proteins for use in diagnostics..

Conclusion and future prospects

GP63 protease family is the largest surface protease family and the second largest surface protein family, There are 48 members of the GP63 protease family in Trichomonas vaginalis [23]according to our BLAST Analysis it is predicted that there is similarity between the two. Further experiments have indicated that adherence is also cell-specific and species 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. [23] Both the proteins contain several large continuous epitopes. Both the proteins are 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. These proteins can be further evaluated to develop vaccine.

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