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

Details of the Students

Sl.No	Hall Ticket Number	Name	Course
1	602919489001	BONAKOLLURI KAVYA	B.SC BT.Z.C
2	602919489002	BONAKOLLURI MOUNIKA	B.SC BT.Z.C
3	602919489003	KADULLA SHWETHA	B.SC BT.Z.C
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

Sl.No	Hall Ticket Number	Name	Course
1	602919489001	BONAKOLLURI KAVYA	B.SC BT.Z.C
2	602919489002	BONAKOLLURI MOUNIKA	B.SC BT.Z.C
3	602919489003	KADULLA SHWETHA	B.SC BT.Z.C
4	602919572002	CHATLAPALLY SRAVANTHI	B.SC BT.B.C
5	602919572005	GADDAM NIKHITHA	B.SC BT.B.C

Mr.Bhaskar Reddy

Lecturer in Biotechnology

P. V. Cleve Lain

Dr.P.V Uma Sasi

Principal

Index

S No	Content	Page No
1	Computational prediction of Chlamydia polymorphic membrane protein as a potential diagnostic markers for Bovine Trichomonosis Bonakolluri Kavya, And Bonakolluri Mounika	4-9
2	Computational prediction of Hypothetical protein TRFO 10391 as a potential diagnostic markers for Bovine Trichomonosis Chatlapally Sravanthi And Gaddam Nikhitha	10-17
3	Computational prediction of GP63-like protein as a potential diagnostic markers for Bovine Trichomonosis Kadulla Shwetha	18-24

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

SPTESPTESATXSATESPTESPTESATESPTESPTESATESPTESPTESATESPTESPTESAT

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

Predicted peptides:					
No. =	Start	End =	Poplide	Length	
1	22	22	¢	.1	
2	27	39	KWIVSKFVPDYNF	13	
3	64	68	TNSTG	5	
4	87	92	IKTYKD	6	
5	112	116	INTEG	5	
6	136	151	NSNNQMNLLKSSGEGS	18	
7	171	180	GSEAAVVEGK	10	
8	191	193	VGY	3	
9	195	195	ĸ	1	
10	203	228	SMSMDADIGTALFYSYHSSITTKSEG	26	
11	258	270	AADQWGDEGSNGG	13	
12	289	301	A005NLTLTVVA5	13	
13	312	338	LVIKKDSED0DGEDIVPTDKSQTLSRN	27	
14	365	373	KKRNQSPGF	9	

Average: 0.481 Minimum: 0.194 Maximum: 0.689

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.

REFERENCES

- Compendium of Minimum standards of protocol & Standard operating procedures For Bovine breeding Government of India Ministry of Agriculture Department of Animal Husbandry, Dairying & Fisheries, INDIA (2014)
- 2. Document of The World Bank, National Dairy Support Project.(2012)
- 3. Annual report Department Of Animal Husbandry, Dairying & Fisheries Ministry Of Agriculture Government Of India New Delhi (2012-13)
- 4. Trichomoniasis. OIE Reference manual Chapter 3.04.15- Accessed on May 2019.
- 5. Warton, A., Honigberg, B,M.: Structure of trichomonads as revealed by scanning electron microscopy. J. Protozoot., 26, 56–6 (1979).
- 6. Mariante, R.M., Lopes, L.C., Benchimol, M.: Tritrichomonas foetus pseudocysts adhere to vaginal epithelial cells in a contact-dependent manner. *Parasitology Research*, 92, 303-312 (2003)
- 7. Skirrow, S,Z.,Bondurrant, R,H.: Bovine trichomoniasis. Vet. Bull., 58, 591–603. (1988)
- Slapeta, J., Müller, N., Stack, C.M., Walker, G., Lew, Tabor A., Tachezy, J.: Comparative analysis of Tritrichomonas foetus (Riedmüller, 1928) cattle genotype and Tritrichomonas suis (Davaine, 1875) at 10 DNA loci. Int. J. Parasitol., 42, 1143– 1149. (2012)
- Tachezy, J., Tachezy, R., Hampl, V., Se, Dinova, M., Vanacova, S., Vrlik, M., Van, Ranst, M., Flegr J. Kuldaa J.:Cattle pathogen Tritrichomonas foetus (Riedmuller, 1928) and pig commensal Tritrichomonas suis (Gruby & Delafond, 1843) belong to the same species. J. Eukaryot. Microbiol., 49, 154–163. (2002)
- 10. Yao C.: Opportunistic human infections caused by Tritrichomonas species: a minireview. Clin. Microbiol. News, 34, 127–131. (2012).
- 11. Yao C.: Diagnosis of Tritrichomonas foetus-infected bulls, an ultimate approach to eradicate bovine trichomoniasis in US cattle? J. Med. Microbiol., 62, 1–9. (2013)
- 12. Huang, K,Y., Shin, J,W., Huang, P,J., Ku,F,M., Lin, W,C., Lin, R., Hsu, W,M., Tang,
 P.: Functional profiling of the Tritrichomonas foetus transcriptome and proteome. Mol Biochem Parasitol.187(1):60-71. (2013)
- 13. Stroud, L, J., Slapeta, J., PadulaM, P., Druery, D., Tsiotsioras, G., Coorssen, J, R., Stack, C , M.: Comparative proteomic analysis of two pathogenic Tritrichomonas foetus genotypes: there is more to the proteome than meets the eye. Int J Parasitol. 47(4):203-213. (2017)
- 14. Morin-Adeline, V., Lomas, R., O'Meally, D., Stack, C., Conesa, A., Šlapeta, J.: Comparative transcriptomics reveals striking similarities between the bovine and feline isolates of Tritrichomonas foetus: consequences for in silico drug-target identification. BMC genomics, 15(1), 955. (2014)
- 15. Ivone de, Andrade, Rosa, I., Caruso MB, de Oliveira Santos E, Gonzaga L, Zingali RB, de Vasconcelos ATR, de Souza W, Benchimol M The costa of trichomonads: A

complex macromolecular cytoskeleton structure made of uncommon proteins Biol Cell. 109(6):238-253. (2017)

- 16. Kuo, Yang, Huanga, b., Jyh, Wei, Shinc., Po, Jung, Huangd., Fu, Man, Kua., Wei, Chen, Lina., Rose, Lina., Wei, Min, Hsua, Petrus, Tanga.: Functional profiling of the Tritrichomonas foetus transcriptome and proteome Molecular & Biochemical Parasitology 187, 60–71. (2013)
- 17. Hir,t R. P.: Trichomonas vaginalis virulence factors: an integrative overview. Sexually transmitted infections, 89(6), 439–443. (2013)
- 18. de Miguel, N., Lustig, G., Twu, O., Chattopadhyay, A., Wohlschlegel, J. A., & Johnson, P. J.: Proteome analysis of the surface of Trichomonas vaginalis reveals novel proteins and strain-dependent differential expression. Molecular & cellular proteomics: MCP, 9(7), 1554–1566. (2010)
- 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/
- 22. Everett KD. *Chlamydia* and *Chlamydiales*: more than meets the eye. *Vet Microbiol* 2000; 75:109–26; PMID:10889402;
- 23. Brunham, R. C., and J. Rey-Ladino. 2005. Immunology of *Chlamydia* infection: implications for a *Chlamydia trachomatis* vaccine. *Nat. Rev. Immunol*.5:149-161
- 24. Caldwell, H. D., J. Kromhout, and J. Schachter. 1981. Purification and partial characterization of the major outer membrane protein of *Chlamydia trachomatis*. *Infect. Immun*.31:1161-1176
- 25. Carlson, J. H., S. F. Porcella, G. McClarty, and H. D. Caldwell. 2005. Comparative genomic analysis of *Chlamydia trachomatis* oculotropic and genitotropic strains. *Infect. Immun*. 73:6407-6418

<u>Computational prediction of Hypothetical protein TRFO 10391 as a potential</u> <u>diagnostic markers for Bovine Trichomonosis</u>

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] "<u>B Cell Epitope Prediction Tools</u>" 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

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

Membrane protein prediction

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





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

No	Start =	End +	Peptide	Length
1	6	28	SELTREQHQSGGEYDNVREANAR	23
z	47	52	DTQRCF	6
3	57	75	TLRNRDGGAGLVPGSKSSV	19
4	93	94	ID	2
5	104	123	SCYDEPSGLFRDSPESEPSI	20
6	154	169	NOHLNDDHFEFDGV5L	16
7	185	195	LTVPYHRISEF	-11
8	204	212	IKDNKLONE	9
9	223	241	LFGDEAIPEQLSASFKSSG	19
10	276	285	LIDFEKOGLT	10:
31	315	323	IGDEAPTTE	9
12	325	347	LKIDYQTGLFNSQR15SINKLGQ	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	EVPANLALSOV	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	Ð
28	781	610	GNAVAELPLAQMKKGSRLSHESGDAKGEYK	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 hostpathogen 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.

REFERENCES

- 1. Compendium of Minimum standards of protocol & Standard operating procedures For Bovine breeding Government of India Ministry of Agriculture Department of Animal Husbandry, Dairying & Fisheries, INDIA (2014)
- 2. Document of The World Bank, National Dairy Support Project.(2012)
- 3. Annual report Department Of Animal Husbandry, Dairying & Fisheries Ministry Of Agriculture Government Of India New Delhi (2012-13)
- 4. Trichomoniasis. OIE Reference manual Chapter 3.04.15- Accessed on May 2019.
- 5. Warton, A., Honigberg, B,M.: Structure of trichomonads as revealed by scanning electron microscopy. J. Protozoot., 26, 56–6 (1979).
- 6. Mariante, R.M., Lopes, L.C., Benchimol, M.: Tritrichomonas foetus pseudocysts adhere to vaginal epithelial cells in a contact-dependent manner. *Parasitology Research*, 92, 303-312 (2003)
- 7. Skirrow, S,Z.,Bondurrant, R,H.: Bovine trichomoniasis. Vet. Bull., 58, 591–603. (1988)
- 8. Šlapeta, J., Müller, N., Stack, C.M., Walker, G., Lew, Tabor A., Tachezy, J.: Comparative analysis of Tritrichomonas foetus (Riedmüller, 1928) cattle genotype

and Tritrichomonas suis (Davaine, 1875) at 10 DNA loci. Int. J. Parasitol., 42, 1143–1149. (2012)

- Tachezy, J., Tachezy, R., Hampl, V., Se, Dinova, M., Vanacova, S., Vrlik, M., Van, Ranst, M., Flegr J. Kuldaa J.:Cattle pathogen Tritrichomonas foetus (Riedmuller, 1928) and pig commensal Tritrichomonas suis (Gruby & Delafond, 1843) belong to the same species. J. Eukaryot. Microbiol., 49, 154–163. (2002)
- 10. Yao C.: Opportunistic human infections caused by Tritrichomonas species: a minireview. Clin. Microbiol. News, 34, 127–131. (2012).
- 11. Yao C.: Diagnosis of Tritrichomonas foetus-infected bulls, an ultimate approach to eradicate bovine trichomoniasis in US cattle? J. Med. Microbiol., 62, 1–9. (2013)
- Huang, K,Y., Shin, J,W., Huang, P,J., Ku,F,M., Lin, W,C., Lin, R., Hsu, W,M., Tang,
 P.: Functional profiling of the Tritrichomonas foetus transcriptome and proteome. Mol Biochem Parasitol.187(1):60-71. (2013)
- 13. <u>Stroud,L,J., Slapeta,J., PadulaM,P., Druery,D., Tsiotsioras,G., Coorssen,J,R., Stack,C., M.</u>: Comparative proteomic analysis of two pathogenic Tritrichomonas foetus genotypes: there is more to the proteome than meets the eye. Int J Parasitol. 47(4):203-213. (2017)
- 14. Morin-Adeline, V., Lomas, R., O'Meally, D., Stack, C., Conesa, A., Šlapeta, J.: Comparative transcriptomics reveals striking similarities between the bovine and feline isolates of Tritrichomonas foetus: consequences for in silico drug-target identification. BMC genomics, 15(1), 955. (2014)
- 15. Ivone <u>de</u>, <u>Andrade</u>, <u>Rosa</u>, <u>I.</u>, <u>Caruso MB</u>, <u>de Oliveira Santos E</u>, <u>Gonzaga L</u>, <u>Zingali</u> <u>RB</u>, <u>de Vasconcelos ATR</u>, <u>de Souza W</u>, <u>Benchimol M</u> The costa of trichomonads: A complex macromolecular cytoskeleton structure made of uncommon proteins Biol Cell. 109(6):238-253. (2017)
- 16. Kuo, Yang, Huanga, b., Jyh, Wei, Shinc., Po, Jung, Huangd., Fu, Man, Kua., Wei, Chen, Lina., Rose, Lina., Wei, Min, Hsua, Petrus, Tanga.: Functional profiling of the Tritrichomonas foetus transcriptome and proteome Molecular & Biochemical Parasitology 187, 60–71. (2013)
- 17. Hir,t R. P.: Trichomonas vaginalis virulence factors: an integrative overview. Sexually transmitted infections, 89(6), 439–443. (2013)
- 18. de Miguel, N., Lustig, G., Twu, O., Chattopadhyay, A., Wohlschlegel, J. A., & Johnson, P. J.: Proteome analysis of the surface of Trichomonas vaginalis reveals novel proteins and strain-dependent differential expression. Molecular & cellular proteomics: MCP, 9(7), 1554–1566. (2010)
- 19. National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov
- 20. Immune Epitope Database <u>https://www.iedb.org/</u>
- 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

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]<u>FASTA</u> 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] "<u>B Cell Epitope Prediction Tools</u>" 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, microorganisms 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.





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
----------------	----------------	----------------

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

Predicted peptides:

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.

REFERENCES

- 1. Compendium of Minimum standards of protocol & Standard operating procedures For Bovine breeding Government of India Ministry of Agriculture Department of Animal Husbandry, Dairying & Fisheries, INDIA (2014)
- 2. Document of The World Bank, National Dairy Support Project.(2012)
- 3. Annual report Department Of Animal Husbandry, Dairying & Fisheries Ministry Of Agriculture Government Of India New Delhi (2012-13)
- 4. Trichomoniasis. OIE Reference manual Chapter 3.04.15- Accessed on May 2019.
- 5. Warton, A., Honigberg, B,M.: Structure of trichomonads as revealed by scanning electron microscopy. J. Protozoot., 26, 56–6 (1979).
- 6. Mariante, R.M., Lopes, L.C., Benchimol, M.: Tritrichomonas foetus pseudocysts adhere to vaginal epithelial cells in a contact-dependent manner. *Parasitology Research*, 92, 303-312 (2003)
- 7. Skirrow, S,Z.,Bondurrant, R,H.: Bovine trichomoniasis. Vet. Bull., 58, 591–603. (1988)
- 8. Šlapeta, J., Müller, N., Stack, C.M., Walker, G., Lew, Tabor A., Tachezy, J.: Comparative analysis of Tritrichomonas foetus (Riedmüller, 1928) cattle genotype and Tritrichomonas suis (Davaine, 1875) at 10 DNA loci. Int. J. Parasitol., 42, 1143– 1149. (2012)
- Tachezy, J., Tachezy, R., Hampl, V., Se, Dinova, M., Vanacova, S., Vrlik, M., Van, Ranst, M., Flegr J. Kuldaa J.:Cattle pathogen Tritrichomonas foetus (Riedmuller, 1928) and pig commensal Tritrichomonas suis (Gruby & Delafond, 1843) belong to the same species. J. Eukaryot. Microbiol., 49, 154–163. (2002)
- 10. Yao C.: Opportunistic human infections caused by Tritrichomonas species: a minireview. Clin. Microbiol. News, 34, 127–131. (2012).
- 11. Yao C.: Diagnosis of Tritrichomonas foetus-infected bulls, an ultimate approach to eradicate bovine trichomoniasis in US cattle? J. Med. Microbiol., 62, 1–9. (2013)
- Huang, K,Y., Shin, J,W., Huang, P,J., Ku,F,M., Lin, W,C., Lin, R., Hsu, W,M., Tang,
 P.: Functional profiling of the Tritrichomonas foetus transcriptome and proteome. Mol Biochem Parasitol.187(1):60-71. (2013)
- 13. <u>Stroud, L, J.</u>, <u>Slapeta, J.</u>, <u>PadulaM, P.</u>, <u>Druery, D.</u>, <u>Tsiotsioras, G.</u>, <u>Coorssen, J, R.</u>, <u>Stack, C.</u>, <u>M.</u>: Comparative proteomic analysis of two pathogenic Tritrichomonas foetus genotypes: there is more to the proteome than meets the eye. Int J Parasitol. 47(4):203-213. (2017)

- 14. Morin-Adeline, V., Lomas, R., O'Meally, D., Stack, C., Conesa, A., Šlapeta, J.: Comparative transcriptomics reveals striking similarities between the bovine and feline isolates of Tritrichomonas foetus: consequences for in silico drug-target identification. BMC genomics, 15(1), 955. (2014)
- 15. Ivone <u>de</u>, Andrade, Rosa, I., Caruso MB, <u>de Oliveira Santos E</u>, <u>Gonzaga L</u>, <u>Zingali</u> <u>RB</u>, <u>de Vasconcelos ATR</u>, <u>de Souza W</u>, <u>Benchimol M</u> The costa of trichomonads: A complex macromolecular cytoskeleton structure made of uncommon proteins Biol Cell. 109(6):238-253. (2017)
- 16. Kuo, Yang, Huanga, b., Jyh, Wei, Shinc., Po, Jung, Huangd., Fu, Man, Kua., Wei, Chen, Lina., Rose, Lina., Wei, Min, Hsua, Petrus, Tanga.: Functional profiling of the Tritrichomonas foetus transcriptome and proteome Molecular & Biochemical Parasitology 187, 60–71. (2013)
- 17. Hir,t R. P.: Trichomonas vaginalis virulence factors: an integrative overview. Sexually transmitted infections, 89(6), 439–443. (2013)
- 18. de Miguel, N., Lustig, G., Twu, O., Chattopadhyay, A., Wohlschlegel, J. A., & Johnson, P. J.: Proteome analysis of the surface of Trichomonas vaginalis reveals novel proteins and strain-dependent differential expression. Molecular & cellular proteomics: MCP, 9(7), 1554–1566. (2010)
- 19. National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov
- 20. Immune Epitope Database <u>https://www.iedb.org/</u>
- 21. TMHMM Server v. 2.0 http://www.cbs.dtu.dk/services/TMHMM/
- 22. Ryan, C. M., de Miguel, N., & Johnson, P. J. (2011). Trichomonas vaginalis: current understanding of host-parasite interactions. *Essays in biochemistry*, *51*, 161–175. https://doi.org/10.1042/bse0510161
- 23. Lina Ma, Wei-Hung Cheng, Qingshu Meng, Petrus Tang, JunYu, Songnian Hu, Yunju Sung.,

Involvement of the GP63 protease in infection of Trichomonas vaginalis Article in Parasitology Research \cdot July(2011)