Comparative Omics based approach to identify putative antigenic Proteins of *Trichomonas foetus*

STUDENT STUDY PROJECT
2021-22

Submitted & Executed by

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

Project supervisor

Mrs. Karli Geethanjali
Asst Professor of Biotechnology

Details of the Students

Sl.No	Hall Ticket Number	Name	Course
1	602920489001	CH.SOWMYA	B.SC BT.Z.C
2	602920489002	D.SAI DEEPTHI	B.SC BT.Z.C
3	602920489003	G.USHA	B.SC BT.Z.C
4	602020489004	ANUPAMA	B.SC BT.Z.C
5	602920489005	K.SANDHYA	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 Comparative Omics based approach to identify putative antigenic Proteins of *Trichomonas foetus" in the* Dept of Biotechnology for the academic year 2021-22.

Details of the Students

Sl.No	Hall Ticket Number	Name	Course
1	602920489001	CH.SOWMYA	B.SC BT.Z.C
2	602920489002	D.SAI DEEPTHI	B.SC BT.Z.C
3	602920489003	G.USHA	B.SC BT.Z.C
4	602020489004	ANUPAMA	B.SC BT.Z.C
5	602920489005	K.SANDHYA	B.SC BT.B.C
6	602920489001	CH.SOWMYA	B.SC BT.B.C

Mrs.K.Geethanjali

Asst. Professor in Biotechnology

Dr.P.V Uma Sasi

Principal

Index

Comparative Omics based approach to identify putative antigenic Proteins of *Trichomonas foetus*

Abstract

Bovine Trichomonosis is the most neglected sexually transmitted disease in cattle. *Trichomonas foetus* is a flagellate protozoan known for causing simple symptoms like, vaginal discharge to as severe as abortion and infertility. Trichomonosis is very difficult to treat. Screening and isolations of infected animals is the only available strategy. Lack of simple, rapid, inexpensive point of care diagnostics kits is leading to rapid spread. All the published Transcriptomics, proteomics studies of *Trichomonas foetus* and comparative genomics based approach from *Trichomonas vaginalis,* a similar human pathogen were screened thoroughly to list set of highly expressed proteins. The FASTA Sequences of the proteins were analyzed by TMMHM and IEDB resource analysis for identification of Putative membrane proteins and antigenic domains respectively. Cysteine protease 8 was found to be consistently expressed extracellular secretary protein and Surface antigen BspA-like Protein, Chlamydia polymorphic membrane protein-like extracellular domain were found to be highly immunogenic membrane proteins.

Key words: Transcriptomics, proteomics,putative, immunogen, bovine, b-cell epitope prediction,TMMHM.

Introduction

India owns the world`s largest livestock population with 528 million of domestic animals. India ranks first for buffalo with 105.3 million population and second in cattle with 199 million. Livestock population is facing severe stress due to infectious disease outbreaks. (Compendium of Minimum standards of protocol, 2014)

With regard to cattle, only 28% percent of the breedable bovine population is under AI coverage. The rest of the eighty percent are covered through natural service with scrub bulls of unknown genetic and disease potential. The Conception rate of cattle is as low as only 35% in India. (National Dairy Support Project, 2012)

There has been little improvement in breeding by using high genetic potential of indigenous breeds over the years in organized farms. But the Bulls maintained by the farmers for natural service are not tested for genetic disorders and Sexually Transmitted Diseases (STDs). This is leading to rapid spread of STDs like Brucellosis, Infectious Bovine Rhinotracheitis (IBR), Bovine Tuberculosis,Trichomoniasis, Bovine Genital Campylobacteriosis, Foot and Mouth disease (FMD), Bovine Viral Diarrhoea (BVD) among bovine population in the country. Furthermore, bulls kept for natural service are not rotated after every three years, which is further leading to low productivity among bovine population. (Compendium of Minimum standards of protocol, 2012 & 2014)

Most of the diseases are zoonotic, pose public health issues. There are no epidemiological reports due to non- availability of simple, rapid and inexpensive point of care diagnostic kits. The burden is expected to be > 1Billion USD.

Venereal diseases of cattle- Proposed control strategies

India has the major challenges with regard to low productivity of livestock. Most of the cattle are in natural service with unknown genetic and disease potential. Inadequate coverage through artificial insemination, non-availability of elite males for natural service, poor hygienic conditions are the major causes of low conception, high morbidity & mortality. (National Dairy Support Project, 2012)

The Department of Animal Husbandry Dairying & Fisheries has formulated a Minimum Standard Protocol (MSP) and a Standard Operating Procedure (SOP) for production of bulls for natural service after consultation with experts from NDDB, ICAR and Veterinary Universities and special emphasis was given to improve the productivity of livestock through enhanced AI coverage. As per the impact analysis report submitted by NABARD, overall conception rate has increased from 20% to 35%. Few tests were prescribed and mandate to screen for Brucellosis, Bovine Tuberculosis, JD, Trichomonosis, Bovine Genital Campylobacteriosis, FMD , Infectious Bovine Rhinotracheitis (IBR),Bovine Viral Diarrhoea (BVD) to further improve the fertility in cattle. (Compendium of Minimum standards of protocol, 2012 & 2014)

Statement of the Problem

- Present gold standard protocol by OIE, Department of Animal Husbandry, Dairying & Fisheries for diagnosis of Trichomoniasis is Agent identification in Preputial washings and semen has several limitations.
- Agent detection by culturing and microscopy is not specific, cumbersome, time taking, expensive, requires technical expertise.
- Molecular detection by PCR is expensive, requires sophisticated lab set up, technical expertise and the services are available only at few research centres NDDB, CDDL, RDDL etc. compromising the application at resource limited stations at field level.
- Preputial washings and semen sample pose to get contaminated very easily, interfere with the test results.
- Trichomonosis is very difficult to treat. Screening and isolations of infected animals is the only available strategy. Lack of diagnostic kits is leading to rapid spread.

Hypothesis

- Transcriptomics and proteomics studies *Trichomonas foetus* of help us to identify the set of highly expressed genes and proteins respectively. Compative genomics based approach to identify putative antigenic proteins using proposed surface antigenic proteins of *Trichomonas vaginalis*, which is a widely studied human parasite causing infertility, would further enhance the process of identification of potential surface antigens of *T.foetus.*
- Present proposal addresses to identity putative antigenic proteins of *Trichomonas foetus* which further pave the way towards developing recombinant protein based diagnostic serological assays like ELISA and Lateral flow rapid kit for bulk screening of cattle in the field, organized farms, outbreaks, breeding stations as well as Individual in farms.
- Serological testing is simple, pose less contamination, rapid, inexpensive, sensitive, specific and easy to perform point of care diagnostics assays for field deployment.
- The potent surface antigenic proteins can also be further validated to develop Recombinant vaccine to control Trichomoniasis in cattle.

Review of literature

- In the National Livestock Policy 2013, the major challenges which India faces at present is low productivity of livestock was addressed on account of insufficient coverage through artificial insemination, low conception rates, non-availability of quality males for natural service, poor management practices and high mortality & morbidity losses due to diseases, amongst other factors.
- In pursuance of the National Livestock Policy, the Department of Animal Husbandry Dairying & Fisheries has formulated a Minimum Standard Protocol (MSP) and a Standard Operating Procedure (SOP) for production of bulls for natural service after consultation with experts from NDDB, ICAR and Veterinary Universities and accordingly lays special emphasis on improving the productivity of livestock through enhanced AI coverage. As per the impact analysis report submitted by NABARD, overall conception rate has increased from 20% to 35%.
- There is rapid spread of STDs like Brucellosis, Infectious Bovine Rhinotracheitis (IBR), Bovine Tuberculosis, Trichomonosis, Bovine Genital Campylobacteriosis, Foot and Mouth disease (FMD), Bovine Viral Diarrhoea (BVD) among bovine population in the country. Several point of care diagnostics like ELISA and Lateral flow assay rapid kit are developed for screening Brucellosis, Infectious Bovine Rhinotracheitis (IBR), Bovine Tuberculosis, Bovine Genital Campylobacteriosis, Foot and Mouth disease (FMD), Bovine Viral Diarrhoea (BVD) except for Trichomonosis.
- In India, only from the last decade, few point of care diagnostics like ELISA and Lateral flow assay rapid kit are developed and lab screening facility is available for Brucellosis, Bovine Tuberculosis, Para Tuberculosis (Rudrama, 2019), (Mallikarjuna 2017), (Manasa,2019}. Bovine trichomonosis is the most neglected cattle disease, which is responsible for drastic drop in fertility rate in cattle. Hence this work screen for potential antigenic genes of *Trichomonas foetus*, a protozoan parasite responsible to cause Trichomonosis in cattle.

With the recommendations of OIE , Ministry of Agriculture Department of Animal Husbandry, Dairying & Fisheries, Government of India on 22nd April 2014 published Compendium of Minimum standards of protocol & Standard operating procedures for Bovine breeding the proposed the s the list of diseases to be screened at Breeding stations

Disease	Test	Sample	Tested by officers of
Brucellosis	ELISA	Serum	CDDL/RDDL/ NDDB/PD_AD MAS
Bovine Tuberculosis	Bovine tuberculin PPD	Intra-dermal on the bull	Semen station/ CDDL/RDDL/ NDDB IVRI, Izatnagar
Trichomonosis	Agent identification	Preputial washings /semen	CDDL/RDDL/ NDDB
Bovine Genital Campylobacteriosis	Agent identification	Preputial washings	CDDL/RDDL/ NDDB
FMD	ELISA	Serum	PD-FMD,Mukteshwar and its laboratories/ NDDB
Infectious Bovine Rhinotracheitis (IBR)	Enzyme Linked Immunosorbent Assay (ELISA), Real-time PCR	Serum for ELISA, semen for real-time PCR	PD_AD MAS
Bovine Viral Diarrhoea (BVD)	Enzyme Linked Immunosorbent Assay (ELISA) for antibody detection (Ab-ELISA) for detection of antigen (Ag-ELISA).	Serum	--

Pathophsiology of Trichomonosis

Tritrichomonas foetus the causative agent of Trichomonosis in cattle has a pyriform body, with three anterior and one posterior flagellae, and an undulating membrane. Its typical jerky, rolling motion are detected by light and advanced microscopy (Warton, 1979). Initially only the trophozoite stage was reported, but few findings also report the psuedocyst form (Mariante,2003). The 'belfast' strain, is predominant in Europe, Africa and the USA. (Gregory,1990) The strain 'brisbane' is prevalent in Australia. (Elder,1964) and the strain

'manley' was rarely reported (Skirrow 1988). Apart from cattle, Tri*trichomonas foetus* infection were reported in has been reported in cats, pigs. (Šlapeta,2012), (Tachezy,2002)

There are few zoonoses reports associated with *Trichomonas foetus* found immune compromised and immune suppressed individuals (Yao C,2012).

Bulls exhibit no symptoms and preputial cavity is mostly infected permanently by as they grow older,(Bonduran,1997). Hence using bulls below 4 years old is the simple control strategy. (Yao C,2013) Infected cow, symptoms are vaginitis. In pregnant animals it spreads to the cervix and uterus. Which leads to placentitis causing early abortion (1–16 weeks) , exhibit irregular oestrous cycles, uterine discharge, pyometra, or early abortion. Cows usually clear their infection within 90 days and acquire a short-lived immune protection to T. foetus for a period of at least a year and in some cases up to three years. (Trichomoniasis. OIE Reference manual,2019)

Diagnostics methods for detection of *Trichomonas foetus*

Disease can be suspected as a cause of reproductive failure based on symptoms like, irregularity in oestrous, early abortion, requirement of repeated matigs. It was reported best to screen for the agent in preputial and vaginal washings or scrapings of the infected herd. (Buller,2013) Direct detection can be done by microscopy. Culturing using Modified Diamond's medium further enhances the chances of direct detection. (Bryan,1999) Rapid, Giemsa-based staining techniques can also be used. {Lun,1999) For identification of T. foetus in tissues like aborted foetus, fetal lungs and placenta were formalin fixed and Immunohistochemical methods using monoclonal antibodies were also described (Rhyan,1995) Nucleic acid detection methods are more sensitive. Conventional PCR and real-time PCR methods were developed ,where the highly conserved sequences of the 5.8S ribosomal RNA gene and the flanking internal transcribed spacer regions (ITS) were targeted. (Felleisen,1998), (Grahn, 2005), (Mcmillen,2006), (Hayes,2003)

A more suitable point of care assay for nucleic acids is isothermal amplification assay. A loop mediated (LAMP) targeting T. foetus 5.8S was developed and tested at the laboratory level. (Oyhenart, 2013). This requires minimal expertise but highly sensitive assay. Such studies have to be further improved and tested at field level. As there is no systemic infection normally, immune responses to *T. foetus* was not reported in Bulls. Few tests were however developed like, mucus agglutination test and intradermal test (Rhyan,1995) and a most recently an antigen-

capture enzyme-linked immunosorbent assay was developed. IgG1 and IgG2 antibodies were reported in the vaginal discharge and in serum in the infected cows, but these are not exploited for diagnostic purposes. (Bondurant,1997)

Limitations of the available diagnostics methods

- As per the OIE guidelines, Agent detection by culturing and microscopy is not specific, cumbersome, time taking, expensive, requires technical expertise.
- Molecular detection by PCR is expensive, requires sophisticated lab set up, technical expertise and the services are available only at few research centres NDDB, CDDL, RDDL etc. compromising the application at resource limited stations at field level.
- Preputial washings and semen sample pose to get contaminated very easily, interfere with the test results.

Purpose and Novelty

Till now, recombinant protein based diagnostics like ELISA and Lateral flow assays for screening Trichomonosis in cattle were not developed not only in INDIA and across the entire globe.

Hence, it is a novel to screen transcriptomics and proteomics data and comparative genomics with the closely related species to identify and propose list of potential antigenic proteins of *Trichomonas foetus* for enabling researchers to develop recombinant protein based point of care diagnostics.

Objectives

1. Screen for Transcriptomics and proteomics data of *T.foetus* for highly expressed proteins
2. To screen highly expressed proteins of *T.vaginalis* and using comparative genomics based approach, identifying the related proteins in *T.foetus*
3. To screen for potential surface proteins
4. To analyze for the antigenic domains of the proteins
5. To propose the top 10 most suitable proteins as diagnostic markers of *T.foetus* infection

Methodology

- Extensive literature survey in Pubmed and Pubmed central was carried out to search for Transcriptomics and proteomics studies of *Trichomonas foetus* and *Trichomonas vaginalis* (NCBI-Pubmed)
- Screening and listing of highly expressed surface proteins, virulent proteins proposed to be involved in pathogenesis of Trichomonossis.
- Obtain the accession numbers for corresponding highly expressed transcripts, ESTs and proteins in *T.foetus and T.vaginalis* were from NCBI proteins data base. Downloaded the corresponding FASTA formats. (NCBI-Protein)
- Proposed potential virulent proteins of *T.vaginalis* are subjected to BLAST analysis to compare and identify the corresponding proteins of *T.foetus* (NCBI-BLAST)
- Confirmation of listed proteins as potential putative surface proteins was done using TMMHM. FASTA sequences of the proteins were submitted to TMHMM Server v. 2.0 for prediction of transmembrane helices in proteins. Out put were obtained with graphics (TMHMM)
- 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. B Cell Epitope Prediction Tools were used here are intended to predict regions of proteins that are likely to be recognized as epitopes in the context of a B cell response. The BepiPred-2.0 server were used to predict B-cell epitopes from the FASTA protein sequence (IEDB)

Results and Discussion

In a study of Functional profiling of the *Trichomonas foetus* transcriptome and proteome, which was a first ever large scale the first large-scale T. foetus expressed sequence tag (TfEST) project. When there was no complete geneome sequencing data was available, sequencing ESTs gave the clue regarding the pathogenesis. Table 1.1 shows the list of ESTs coding for highly antigenic proteins. (Huang, 2013)

Table 1.1 Among the TOP 50 most-sequenced ESTs in the T. foetus cDNA library

Contig No.	Highly antigenic transcripts
TfEST Contig0742 12	Adhesin protein AP51-3
TfEST Contig0752 15	Adhesin protein AP65-1
TfEST Contig0773 26	Adhesin protein AP65-1
TfEST Contig0781 36	Cysteine proteinase
TfEST Contig0764 20	Cysteine protease
TfEST Contig0776-2 23	Hypothetical protein1
TfEST Contig0748 14	Hypothetical protein1

Out of 15 Cysteine proteases expressed, 12 of these TfCPs were reported for the first time in this study. Comparison of Differential expression levels of the TfCPs clearly demonstrates that Trichomonas produces large amount of CP8, by CP16 and CP13. In the 2-DE reference map and protein identification of T. foetus, among the top highly abundantly expressed spots, Cysteine protease are found to be major antigenic proteins T. foetus trophozoite . Table 1.2 shows Putative antigenic proteins with transmembrane in th*e T. foetus* transcriptome when analysed by TMHMM

Table 1.2 Putative antigenic proteins with transmembrane in th*e T. foetus* transcriptome analyzed by TMHMM

TfEST ID	**TfEST Annotation**
TfEST017A04	BspA-like leucine rich repeat Surface antigen
TfEST006B04	Circumsporozoite protein precursor, putative
TfEST037G07	PPase: Ser/Thr Protein Phosphatase, PP2A catalytic subunit
TfEST024F05	Similar to T. vaginalis hypothetical protein TVAG_235360
TfEST033A06	Calcium motive P-type ATPase, PMCA-type
TfEST073F08	PPase: Clan SB, family S8, subtilisin-like serine peptidase
TfEST047E12	PPase: Ser/Thr Protein Phosphatase, Metallo-phosphoesterase
TfEST027H02	Protein SEY1, putative
TfEST_Contig0170	Tetraspanin
TfEST_Contig0461	(XP_001317322.1)
TfEST039E02	Similar to T. vaginalis hypothetical protein TVAG_247370

- In an interesting study of Comparative proteomic analysis of two pathogenic *Trichomonas foetus* genotypes, their findings highlight the importance of CPs as predominant factors of virulence and host-parasite interaction was reported. It was also

proposed to exploit CPs for diagnostic markers , vaccines as well as drugs development against *T. foetus*. (Stroud, 2017)

- In another study of Comparative transcriptomics to understand the relatedness between the bovine and feline genotypes of *Trichomonas foetus* was conducted to understand the mechanism of adaption of in specific host virulence. (Morin-Adeline, 2014)

  CP8 is the most preferred transcript among all CPs. Apart from cysteine proteases, protein phosphatase 2C ,Protein serine/threonine phosphatase activity , cathepsin L-like cysteine peptidase, Myb-like DNA-binding domain containing protein, pyruvate:ferredoxin oxidoreductase also were also among the top 100 highly expressed proteins in the bovine genotype. They have also reported several druggable domains, putative membrane proteins are listed in table 2.1.

2.1 Druggable domains of *Trichomonas foetus*

Transcript ID	Transcript length	BlastX transcript identifier
Bc12_comp9993_c0_seq1	3564	Pyruvate ferredoxin flavodoxin oxidoreductase
Bc12_comp9915_c0_seq1	314	Clan family phytocystatin-like peptidase inhibitor
Bc12_comp5305_c0_seq1	2198	gp63-like protein
Bc12_comp11217_c0_seq1	921	Clan family metacaspase-like cysteine peptidase
Bc12_comp10022_c0_seq1	973	Thioredoxin reductase

The Costa is the protein rich undulating membrane present in Trichomonas was analyzed as the enriched fraction. Several unique proteins were identified to be involved in pathology are listed in the table 3.1. (Ivone,2017)

3.1 Unique pathogenic proteins identified in Costa fraction

NCBI Entries	Entries Proteins	TrichDB Entries	Proteins	% Identities between T.vaginalis and T. foetus
KX579561 KX579561	Adhesin AP65 1 precursor EC 1 1 1 40	KX579662	Malic enzyme	69%
KX579602	CA family C19 ubiquitin hydrolase like cysteine peptidase EC 3 1 2 15	TVAG_475320	Clan CA, family C19, ubiquitin hydrolase-like cysteine peptidase	42%
KX579645	pyruvate:ferredoxin oxidoreductase A	TVAG_198110	pyruvate-flavodoxin oxidoreductase, putative 80	66%
KX579637	Clan CA family C1 cathepsin L like cysteine peptidase	TVAG_2980	Clan CA, family C1, cathepsin L-like cysteine peptidase	64%

Trichomonas vaginalis is closely related protozoan to T.foetus and exhibit similar pathology in human, more over since it is a human parasite, a lot of research work has been already done. Here we made an attempt to screen highly expressed antigenic proteins of T.vaginalis and with the idea of using comparative genomics based approach, identifying the related proteins in T.foetus. (Kuo, 2013) In a review of *Trichomonas vaginalis* virulence factors, Bacteroides surface protein A (BsPA) -like extracellular domain and Chlamydia polymorphic membrane protein-like extracellular domain were found to be potential proteins.(Hirt, 2013)

In a study to understand the mechanism behind tropism and survival of parasite in genital tract, surface proteome of six strains of *Trichomonas vaginalis* with differing adherence capacities analyzed(de Miguel, 2010)

Table 4.1 shows the list of Proteins highly expressed, > 2 fold increase in more adherent strains with high copy number.

4.1 Putative membrane proteins sorted into functional groups according to BLAST analysis and genome annotation from Adherent strains of *T.vaginalis*

Name of the protein	No. of proteins identified In the study	Copy No. genes in genome
GP63-like _	16	77
Surface antigen BspA-like	11	658
Chlamydial polymorphic outer membrane protein	5	27
Tetraspanin family protein	3	9
P270-related protein	9	25
Immunodominant variable surface antigen-like	2	15
Subtilase family protens	7	27
Clan CA, family C2, calpain-like cysteine peptidase	2	10
Clan S, family S54, rhomboid like□serine peptidase	1	9
Nicastrin precursor, putative	1	2

Results- Sample insilico analysis results for few proteins

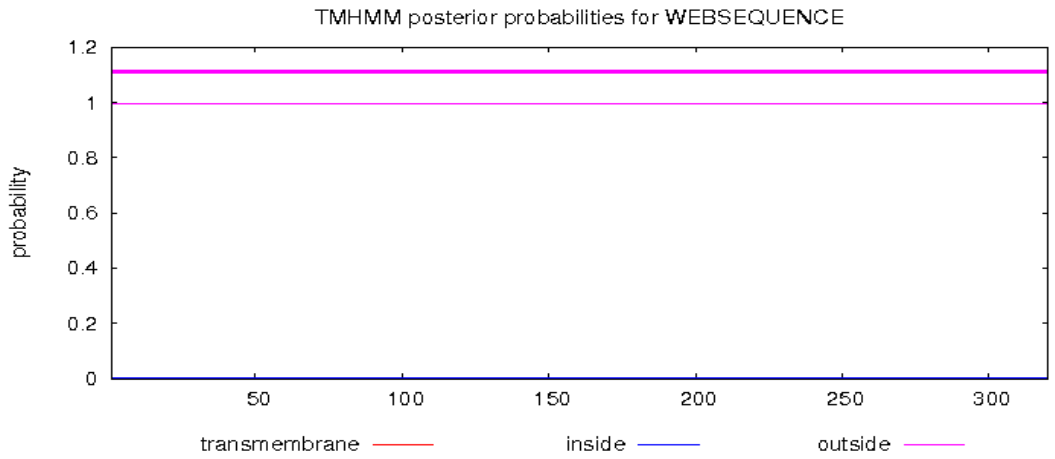
- **TMHMM analysis of Cysteine protease**

  Cysteine protease is an extracellular secreted protein with no transmembrane domain.

TMHMM result

HELP with output formats

```
# WEBSEQUENCE Length: 320
# WEBSEQUENCE Number of predicted TMHs:  0
# WEBSEQUENCE Exp number of AAs in TMHs: 0.0834
# WEBSEQUENCE Exp number, first 60 AAs:  0.01503
# WEBSEQUENCE Total prob of N-in:        0.00384
WEBSEQUENCE     TMHMM2.0        outside      1   320
```

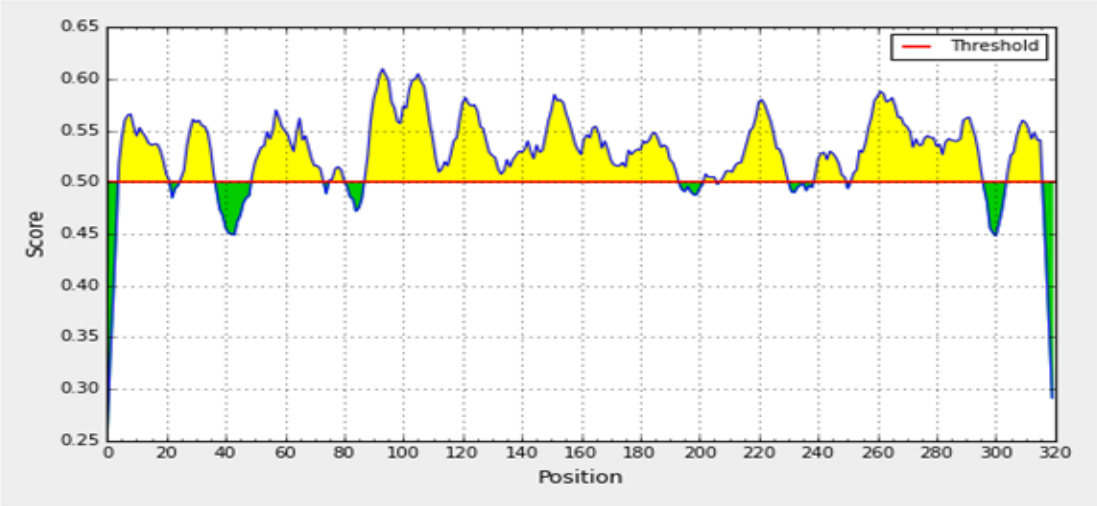


- **IEDB resource analysis shows large epitopic domains**

  - Bepipred Linear Epitope Prediction 2.0 Results

    Center position: 4 Threshold: 0.500

    Average: 0.528 **Minimum:** 0.253 **Maximum:** 0.610



Predicted peptides: Putative Antigenic domains

No.	Start	End	Peptide	
1	5	22	FAAFSSAAALYQLHEQKA	18
2	26	37	WMRSTNQFYTGD	12
3	50	74	KRFVESHNANPANSYKVTLNNFAAL	25
4	76	81	PSEYKS	6
5	88	194	AFNRNSQHAKITKAQKTNTESVDWREKGVVNDVRNQYMCGSCWAFSAIQAIESVYAIGT GTLLSLSEQNLVDCVDTCEGCNGGLMDAAYDYVIEKQNGQFNTEASYW	107
6	203	206	MFDK	4
7	208	230	EKAGSISGYYNVAASSEDDLAAK	23
8	240	250	AIDASAVGFQL	11
9	253	296	GGIYDNSGCSSVMLDHGVGCVGFGVEGGTQYWIVRNSWGSWWGE	44
10	305	316	KDNQCGIATMSC	12

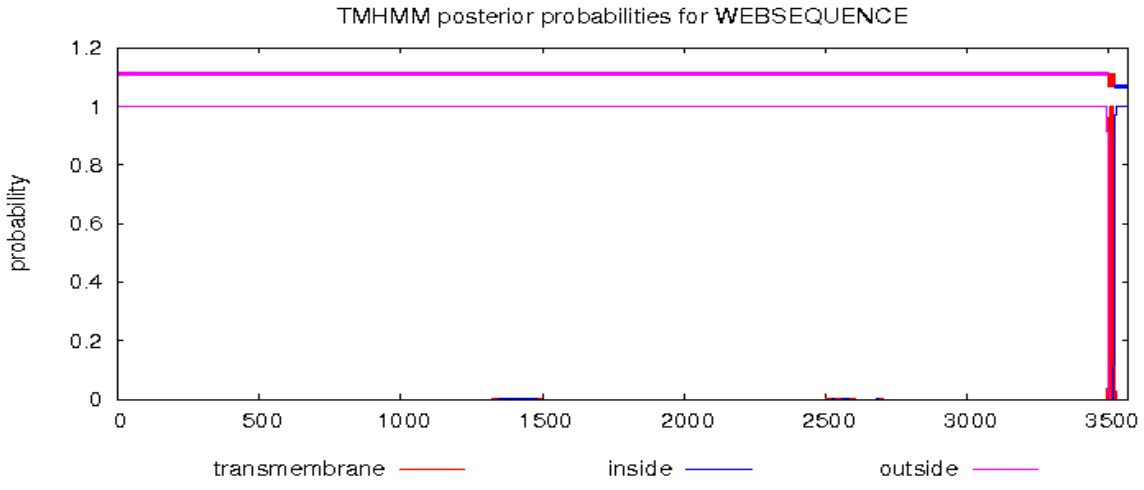
- **BspA like protein**

  It has a huge extracellular domain and a small transmembrane domain

TMHMM result

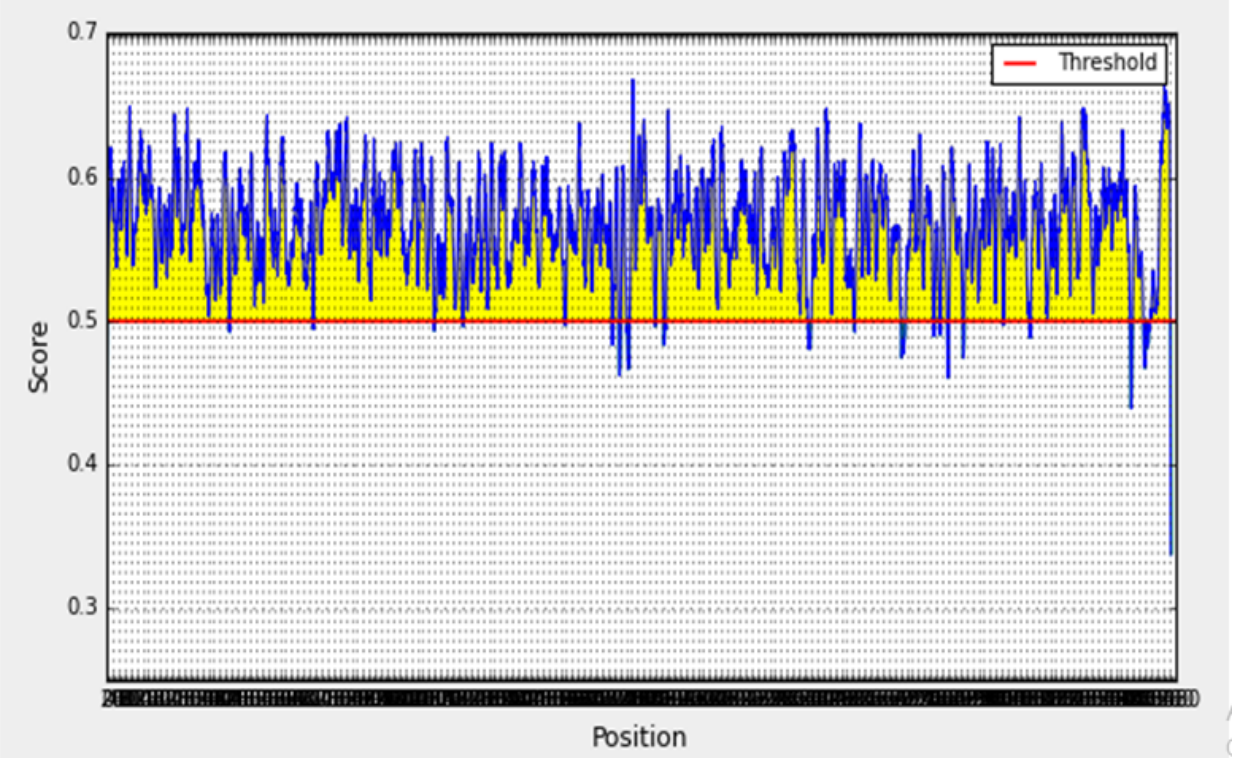
HELP with output formats

```
# WEBSEQUENCE Length: 3567
# WEBSEQUENCE Number of predicted TMHs:  1
# WEBSEQUENCE Exp number of AAs in TMHs: 23.2005
# WEBSEQUENCE Exp number, first 60 AAs:  0
# WEBSEQUENCE Total prob of N-in:        0.00002
WEBSEQUENCE     TMHMM2.0        outside      1  3498
WEBSEQUENCE     TMHMM2.0        TMhelix   3499  3521
WEBSEQUENCE     TMHMM2.0        inside    3522  3567
```



- **IEDB resource analysis**

  BspA like protein has very large epitopic domain



Predicted peptides:

Start	End	Peptide	
5	412	EEDLIPSKSFENHNNIETVVLESISEIGEYAFNNCENLQTLNIEKVNIIGRNSFSNCVNLHRISIPLVRTL SQNIFSNSGISEIRIDKVLTIEESAFENCLMLSSFQCQTCEQLSGNSIFKECQNLESISFPNLRMVDVTS ANLFYNCNKLTKVSFGNHPPKVFNRQVFANSFSNKIKLNLPNDEAYYTYDNDIAIEGDVIGDSKWC GLSLYISIVCKINDLQEISSSSIRQCILNSQINDFNLVTKIEVISGVLKESDFDFISQNLQNLNHLELIDN DNVPSNCLTNNFKLKNVSLGWATSINSRAFSNLNELTEVRGDHISAIFSEAFINCPLLKNIHFPELINI YSSAFENCINLEKISFPKVLTINNKGFFGCLKLLEIDLPNVSTVKESAFSECTSINSVILP	408
415	693	KIIEKQAFSKCSELRKISFPIIETIQQNCFEKCQKLSDINLPKLNTLESYVFLECSGLLTITLPKLIALST GTFSKCSVLESIFIPEVTVIPNSCFSYCEKLIIDFSNFTRIGEFAFQQCNSLTVLDSSSITKLYTGSFKEC GNLIRINLPNINTVPEEAFISCVKLEEVSLKNVESLSKSCFSKCESLQRIEFNQILAIPESAFSYCIRLEN ISFERATSINRYSFANCISLEIINLTTVKSIGYNCFENCYKLRNIDFPSLENIGESCFKNCSSIEE	279
696	1098	FLRLQTLSSNVFEKCTSLVNVYLPQLLTLESEAFKDCQSLLSIEIESLTTINGNGQFKGCQQLKNVSFI SLRTISDSNSDIFENCQSLNSIQLGSEIPKTFNKNTFINRNSAEVMTLLLPGYQSYLNYDASTFIEGDIA NDQKWCSIELVQLLTKYRFNQNPSKMIYASNINEAISKVNIPVTEILQLEILEGYVDIEQMSNIKTQL TELEYLFISNEVFNISNKIPDNFFSGHQSIKEIHINTFLELGESSFGNCPSLETFTSSTITTITSNAFSESIN LKTIEFNSLTEVSNDCFKDFVNLQEINLPKVETLYENCFFGCSKLETIDLPSLKSIKGKSQFSNCENLK KVVFKALQSIESTNSDIFDSCQSLEQIELSPTVPKIFHKDTFVNSQSKNRIQLI	403
1102	1195	YDDYVRYDKDTSIDGDIPDDAKWCSLELRPLQVVVKINENKESVGGTLEGAITFSNVEPENILTIDII EGKLDKKDFCLNNEQDKSLLIKYPNL	94
1197	1534	EFIIRDNIQLNYDIIPNNFFSNHKNLETIIIQPDCQIIFSDTFLNCEKIQKVSLNCTKIPTSLFKDLKSLAE VEINSVDEIPEELFYNCQKLQKVTATNATLIKKDAFYHCIALIEIEFESVQTIQIAQFSGLLELTTVHL NALISIPKDSFKDCVKLETLIIEKVENLEDSCFSNCKSLKILNCPNLEKISTNTVFENCVSLEEVNFDR LTNLPSNTFENLLYLKNVNIPQLKTTGSFTFRNCSSLENITFFELEEIGSEVFCKCTSLELVNLPSLSK MNERCFIGCISLVTISLPSLFVIPSESFKELPNLTTIQFEKATEIQESAFQNCYKLYDI	338
1536	1537	LP	2
1541	1694	LIGDNAFMNCISIQDIVLNSLIKISDYIFSNCSHLQKIEINNLNSTGVNCFEHCTSLKEISIPKLITLEKET LFKCSDLESVNFPQVEIVKTRAFSNCISIKSIKLSELHTIEGEEIFQECISLGSSSFPKLTYANKDDPNLF ENCKNMASIEL	154

Part-2 of antigenic domains of BspA like protein

1700	1716	KVFHHDVFKNQHSTDPI	17
1725	1743	DYWTYDNNNEIQGDEIGDC	19
1754	1838	VTEVIVNQYNTNQKNINDNKLRLTKERKSVIGNSLSMAVWRSGCKQNTDLSFPTVSSIEIVGGKINS TDFVDKLDNNMAKYYPNL	85
1841	1867	FIIESGVKLNESELPSYSFKDHSKVKL	27
1875	2350	FLRTESLYSSSLETLVLHNVPKIHFSDFQGCLSLTTLTVDIVTEFESHLFINHSSIAEIIAPQLQTIGDQC FENSSIKTVQCPLLTSLGYAAFRNTYEFTKFTNLGTITIIPEECFYNSNIQEIDSTSIIHLKKGCFMNSN VHRVILPNLQSLSEAVFKNTNHLESIDPLTNIYTIPIECFYNSSLTQIELMNCNTIETKAFEKSHIEIVIG TKLEIIESYAFCNCQKLKEISFATVIKIGESTFELSNIKTVESETLIQIGKASFRSSCIQAFNCPNIENILE SSFENCQFLETISELNNIHLIPLKCFYNCSIEIISSTSVEIIGESAFEKSDIQKIDFQTVKLIEKNGFKDCS YLQITNAPVLETIQGYSFYNCTNLNYILLPKVKTVGEYSFAYSNLQTFNKEDDTETLMINSFAFMNS SIESITVSSEITMGESIFKNCVELFKIQFDKIKEIASSMCANSLKLEEVIIPK	476
2362	2506	CIKLQKIDFTSIKVIKAYAFSSTSLVTITSTKIEYIGSHAFEFCLELTNVNLMSPSNNLIIGSSCFLECKK LKEAMIHGTKAMIEAEAFASSQLERIEISSLLEIESTAFKSCSLLLYVKFDNIVEIPSSLFRGLTKLAEV IIPK	145
2509	2662	IIGNYSFFGCSSLEHINMQSIQIINWYAFGQTPLTTLSFTTIQFIGPYSFSNCTKLTTVEIVNSMPSLVISI DDYCFSFTSLKTITIICQSIIIKDSAFKNSTINEIAIDSELVLYPSVFENCIELNKVQLALIKEINASIFKN CVKLEDVVIP	154
2677	2770	LLKQIDFESIKVIESFAFSFTALVTINSYTIESLGSHAFEHCLELTTINLNSPTSNFVVGSYCFSECSKLQ YITVNAKTIESYAFNQCSAISIL	94
2775	2792	LKILEGSGHFCQCKKLET	18
2795	2816	LPLLEEVSRYNNDLFEGCSLMR	22
2825	2868	PKTFSNQAFSDISKENCRIVLNSLAEYVEYDDDTSINGDEFNDC	44
2875	3005	FSDIYHSIIIYGNEYHGNSLEKAIQYSPFPSTRSVIINAGHLYSDELPFYQRTIESLIIFSEVVIHGTLPVD SFSQWSSLEEVELDVFTSLTYSIFQDSSIKIFKGKYLQEISADTFKDCSLSLESIELDV	131
3007	3093	QKIPNNAFQAFSRLETIILPNLQTIPHQAFKDCTSIQTIRFDNVVIIEGNEQFSGCSSLTNIYLPQLQTID SSNEYIFAECYELIGI	87
3097	3429	STPPNTFHRNVFRSLSQEIELTTPNIRDLYLYDNSTDVEGDKKGDCRWCSLRLKPIEIEILINKEYTYF VNALSFIPNDITVTELSIVRGQIFQESFQFTINENNNDNGPMSVLFDSLESFEIQSETIFHSILPSKLFKN HSSIKNITLASTKGIGRSCFEYCIRLEDITVSLSDNNLNLLNTTTTGETRLLSTKILEGEETEFQICERA FYGCTKLKKVSIELIDKIESSAFENCISLNEIKHTNLKNIEDSAFKGCSSLISFDSTTLEILFDSAFKDCS SLQSVNIPYVSEMIGDSHFENCVSLSEIHLNSLAIVDYNSENIFQNCPNFES	333
3439	3474	TFNDNLFINTGANLSEIELILENDESYYYYADSEGE	36
3498	3564	FLILTGVLLFLVIVTGIFFLILGIHKGWFKCSQNHDRHLNLISQNSDQLETNSESENSDEINQTSEN	67

- BspA like protein huge antigenic domains as large as 400 aa. Cloning and expressing such single domains alone will be most suitable to develop diagnostics and therapeutics.

- Thorough analysis, reveals few consistently, highly expressed in *T.foetus* and *T.vaginalis* transcriptomics and proteomics were identified and shortlisted as Putative immunogenic proteins in Table 5.

Table 5. List of Putative Immunogenic proteins of *T.foetus*

Name of the protein	Accession No T.foetus	No. Of Amino Acids	Size Of Extracellular Domain	Size of the largest Antigenic Domain
Cysteine protease 8	OHT13704.1	320	320	107
Surface antigen BspA-like Protein	OHS99292.1	3567	3498	476
Clan SB, family S8, subtilisin-like serine peptidase	OHT04136.1	866	819	69
Chlamydia polymorphic membrane protein-like extracellular domain	OHS93232.1	726	640	163
hypothetical protein TRFO_10391	OHS95735.1	870	837	216
Circumsporozoite protein precursor	OHT08051.1	241	241	25
Immuno-dominant variable surface antigen-like protein	OHT11175.1	270	270	30
Ser/Thr protein phosphatase	OHT00560.1	506	419	27
Tetraspaninfamily protein	OHS99351.1	208	192	113
Adhesin AP65	ARM19795.1	575	575	65
GP63-like protein	OHS97275.1	620	561	63

Cysteine proteinases are the highly expressed extracellular protease, plays a vital role in host–parasite interactions such as virulence, adherence, nutrition acquisition and inflammation**.** CPs shown to induce apoptosis in cultured bovine vaginal and uterine epithelial cells. Which clearly indicates the role of CPs in pathogenic symptoms like, vaginitis and early abortion in cows. Among the identified 15 different CPs, the Differential expression levels of the TfCPs in the transcriptome, CP8 was expressed at the highest level, followed by TfCP16 and TfCP13, implying that these are the major CPs expressed in T. foetus trophozoite (Kuo, 2013). Hence it can be exploited to develop recombinant protein based diagnostics and as well as drug target.

BspA like and Pmp like proteins are similar to transmembrane surface adhesion proteins of Bacteroidales/Spirochaetales and Chlamydiales respectively were found in *T.vaginalis and T.foetus*. These surface proteins mediate host-pathogen interactions and promote cell aggregations pointing to a pivotal role in virulence. Pmp like proteins were found to be mediating the initial binding of the obligate intracellular pathogen and eventual invasion into the host cell (Handrich,2019).

With this understanding and Insilco analysis we propose BspA and Pmps can be exploited to develop recombinant protein based diagnostics, drug targets as well as subunit vaccines

Conclusions and future prospects:

- Insilico approaches to identify putative antigenic proteins are no cost, and eco friendly pave the way towards developing economically viable solutions for social needs in the form of development of diagnostics and therapeutics. Proposed Recombinant protein based approach has immense potential to meet the global demand. Diagnostics serological assays like ELISA and Lateral flow rapid kit can be developed for bulk screening of cattle in the field, organized farms, outbreaks, breeding stations as well as Individual in farms.

- The potent surface antigenic proteins can also be further evaluated and validated to develop Recombinant vaccine to control Bovine Trichomonosis. Protein structure elucidation by X-ray crystallography and drug designing can be made possible. Such studies need further analysis and validation.

Highlights

- Insilico approach are no cost, and eco friendly.
- Proposed Recombinant protein based approach meets the demand of the large scale screening
- economically viable solutions for social needs in the form of diagnostics and therapeutics

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. Rudrama, D. P., Prudhvi, C. M., Revathi, P., Mukta, J., Soumendra, N. M., Jagdip, S. S., Sudhakar, P., Kavi, K. PB., and Rathnagiri, P. (2019) Development and Validation of Rapid, Sensitive and Inexpensive Protein G-based Point of Care Diagnostic Assay for Serodiagnosis of Paratuberculosis at Resource-Limited Areas. *Current Trends in Biotechnology and Pharmacy*. 13 (3), 232-242
5. Mallikarjuna, R. C., Anumolu, V.K., Prudhvi, C. M., Revathi, P., Manasa, M., Rathnagiri, P., Vijayaraghavan, R. (2017) Sero Prevalence And Validation Of In-House IgM Elisa Kit For The Detection Of Brucella Antibody In Human Serum Samples *Asian Jr. of Microbiol. Biotech. Env. Sc*. 19 (4), 975-980
6. Manasa, M. , Revathi, P., Prudhvi, C. M., Maroudam, V., Navaneetha, P., Dhinakar, Raj,G., Kavi, K. PB., Rathnagiri, P. (2019) Protein-G-based lateral flow assay for rapid serodiagnosis of brucellosis in domesticated animals, *Journal of Immunoassay and Immunochemistry*, 40:2, 232-242
7. Warton, A., Honigberg, B,M. (1979) Structure of trichomonads as revealed by scanningg electron microscopy. *J. Protozoot*., 26, 56–6
8. Mariante, R.M., Lopes, L.C., Benchimol, M. (2003) Tritrichomonas foetus pseudocysts adhere to vaginal epithelial cells in a contact-dependent manner. *Parasitology Research, 92*, 303-312
9. Gregory M.W., Ellis B., Redwood D.W. (1990) *Comparison of sampling methods for the detection of Trichomonas foetus*. Vet. Rec., 127, 16.
10. Elder, J,K. (1964) Examination of twelve strains of Trichomonas foetus (Reidmuller) isolated in Queensland and the description of a new serotype, T. foetus var. brisbane. Queensl. *J. Agric. Sci*., 21, 193–203.
11. Skirrow, S,Z.,Bondurrant, R,H. (1988) Bovine trichomoniasis. *Vet. Bull*., 58, 591–603.

12. Šlapeta, J., Müller, N., Stack, C.M., Walker, G., Lew,Tabor A., Tachezy, J. (2012) Comparative analysis of Tritrichomonas foetus (Riedmüller, 1928) cattle genotype and Tritrichomonas suis (Davaine, 1875) at 10 DNA loci. *Int. J. Parasitol*., 42, 1143–1149.
13. Tachezy, J., Tachezy, R., Hampl, V., Se,Dinova, M., Vanacova, S., Vrlik, M., Van, Ranst, M., Flegr J. Kuldaa J. (2002) 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.
14. Yao C. (2012) Opportunistic human infections caused by Tritrichomonas species: a mini-review. *Clin. Microbiol*. News, 34, 127–131.
15. Bondurant R.H. (1997) Pathogenesis, diagnosis and management of trichomoniasis in cattle. *Vet. Clin. North Am. Food Anim. Pract*., 13, 345–361
16. Yao C. (2013) Diagnosis of Tritrichomonas foetus-infected bulls, an ultimate approach to eradicate bovine trichomoniasis in US cattle? *J. Med. Microbiol*., 62, 1–9.
17. Trichomoniasis. *OIE Reference manual* - Chapter 3.04.15- Accessed on May 2019.
18. Buller, N., Corney, B. (2013) Bovine Trichomonosis. *Australian New Zealand Standard DiagnosticProcedure*.http://www.agriculture.gov.au/SiteCollectionDocuments/animal/ahl/ANZSDP-Bovine-trichomoniasis.pdf (assessed 1 August 2017).
19. Bryan L.A., Cambell J.R., Gajadhar, A,A. (1999) Effects of temperature on the survival of Tritrichomonas foetus in transport, Diamond's and InPouch™ TF media. Vet. Rec., 144, 227–232. Lun, Z, R., Gajadhar, A,A. (1999) A simple and rapid method for staining Tritrichomonas foetus and Trichomonas vaginalis. *J. Vet. Diagn. Invest*., 11, 471–474.
20. Rhyan J,C., Wilson K,L., Bengess D,E., Staokhouse L,L.,Quinn, W,J. (1995) The immunohistochemical detection of Tritrichomonas foetus in formalin-fixed paraffin-embedded sections of bovine placenta and fetal lung. *J. Vet. Diagn. Invest*. 7, 98–101.
21. Felleisen, R.S.J., Lambelet, N., Bachmann P., Nicolet J., Muller, N., Gottstein, B., (1998) Detection of Tritrichomonas foetus by PCR and DNA enzyme immunoassay based on rRNA gene unit sequences. *J. Clin. Microbiol.*, 36, 513–519.
22. Grahn, R,A., Bondurant, R,H., Hoosear, K,A., Walker, R,L., Lyon, L,A. (2005) An improved molecular assay for Tritrichomonas foetus. *Vet. Parasitol*., 127, 33–41.
23. Mcmillen, L., & Lew, A,E. (2006) Improved detection of Tritrichomonas foetus in bovine diagnostic specimens using a novel probe-based real time PCR assay. *Vet. Parasitol.*, 141, 204–215.

24. Hayes D,C., Anderson R,R., Walker, R,L. (2003) Identification of trichomonadid protozoa from the bovine preputial cavity by polymerase chain reaction and restriction fragment length polymorphism typing. *J. Vet. Diagn. Invest.,* 15, 390–394
25. Oyhenart, J., Martínez, F., Ramírez, R., Fort, M., Breccia, J,D. (2013) Loop mediated isothermal amplification of 5.8S rDNA for specific detection of Tritrichomonas foetus. *Vet. Parasitol.,* 193, 59–65.
26. Huang, K,Y., Shin, J,W., Huang, P,J., Ku,F,M., Lin, W,C., Lin, R., Hsu, W,M., Tang, P. (2013) Functional profiling of the Tritrichomonas foetus transcriptome and proteome. *Mol Biochem Parasitol*.187(1):60-71.
27. Stroud,L,J., Slapeta,J., PadulaM,P., Druery,D., Tsiotsioras,G., Coorssen,J,R.,, Stack,C,M. (2017) 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.
28. Morin-Adeline, V., Lomas, R., O'Meally, D., Stack, C., Conesa, A., Šlapeta, J. (2014) 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.
29. Ivone de, Andrade, Rosa, I., Caruso MB, de Oliveira Santos E, Gonzaga L, Zingali RB, de Vasconcelos ATR, de Souza W, Benchimol M. (2017) The costa of trichomonads: A complex macromolecular cytoskeleton structure made of uncommon proteins *Biol Cell*. 109(6):238-253.
30. Kuo,Yang, Huanga,b., Jyh,Wei, Shinc., Po,Jung, Huangd., Fu,Man, Kua., Wei,Chen, Lina., Rose, Lina., Wei,Min, Hsua,, Petrus, Tanga. (2013) Functional profiling of the Tritrichomonas foetus transcriptome and proteome *Molecular & Biochemical Parasitology* 187 , 60– 71.
31. Hirt R. P. (2013) Trichomonas vaginalis virulence factors: an integrative overview. *Sexually transmitted infections*, 89(6), 439–443.
32. de Miguel, N., Lustig, G., Twu, O., Chattopadhyay, A., Wohlschlegel, J. A., & Johnson, P. J. (2010) Proteome analysis of the surface of Trichomonas vaginalis reveals novel proteins and strain-dependent differential expression. *Molecular & cellular proteomics: MCP,* 9(7), 1554–1566.

33. Handrich, M.R., Garg, S.G., Sommerville, E.W. (2019) Characterization of the BspA and Pmp protein family of trichomonads. *Parasites Vectors* **12,** 406
34. *National Center for Biotechnology Information*, http://www.ncbi.nlm.nih.gov
35. *TMHMM Server v. 2.0* http://www.cbs.dtu.dk/services/TMHMM/
36. *Immune Epitope Database* https://www.iedb.org/