OPEN ACCESS
Advances in Animal and Veterinary Sciences

Research Article

In Silico Identification of Diagnostic Candidates from Predicted Lipoproteome of *Mycoplasma mycoides* subsp. *capri*

Thachappully Remesh Arun, Rajneesh Rana*, Valsala Rekha, Thankappan Sabarinath

Division of Bacteriology and Mycology, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P., India.

Abstract | *Mycoplasma mycoides* subspecies *capri* (*Mmc*), the causative agent of caprine pleuro pneumonia/contagious agalactia is a major pathogen affecting goats worldwide. Development of specific immunodiagnostic assays for *Mmc* is often hampered by interspecies cross-reactivity with other caprine mycoplasmas and the intra species antigenic variability. The study presents a comparative and subtractive proteomic analysis to identify specific, conserved and immunogenic lipoproteins from *Mmc* proteome. Analysis of 896 proteins of *Mmc* strain 95010 predicted 72 lipoproteins by lipoP1.0 server. BLAST analysis revealed 17 putative non cross-reactive lipoproteins out of which seven were found to be conserved within the species. Further computational workflow employing *ExPASy protein* analysis tools and B cell epitope prediction softwares identified five lipoproteins suitable for development of immunodiagnostics.

Keywords | *Mycoplasma mycoides* subsp. *capri,* Contagious agalactia, Lipoprotein prediction

Editor | Kuldeep Dhama, Indian Veterinary Research Institute, Uttar Pradesh, India.
Received | June 12, 2017; **Accepted** | August 27, 2017; **Published** | October 08, 2017
***Correspondence** | Rajneesh Rana, Division of Bacteriology and Mycology, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P., India; **Email**: rajneeshrana01@yahoo.com
Citation | Arun TR, Rana R, Rekha V, Sabarinath T (2017). *In silico* identification of diagnostic candidates from predicted lipoproteome of *Mycoplasma mycoides* subsp. *capri.* Adv. Anim. Vet. Sci. 5(10): 419-424.
DOI | http://dx.doi.org/10.17582/journal.aavs/2017/5.10.419.424
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

Mycoplasma mycoides subspecies *capri* (*Mmc*) is one of the causative agents of contagious agalactia (CA) in goats. CA is a multi-etiological syndrome caused by four different species of *Mycoplasma*: the other three being *M. agalactiae* (*Ma*), *M. capricolum* subsp. *capricolum* (*Mcc*) and *M. putrefaciens* (*Mp*). The disease is characterized by mastitis, arthritis, kerato conjunctivitis, pneumonia, septicemia and high mortality in kids (Churchward et al., 2014).The disease caused by *Mmc* is highly prevalent in India and is described as caprine pleuropneumonia (CPP) (Manimaran et al., 2006).

Mmc belongs to *Mycoplasma mycoides* cluster, which consists of five closely related mycoplasmas that cause disease in ruminants. The other four species/subspecies belonging to the cluster are *M. capricolum* subsp. *capricolum* (*Mcc*), *M. capricolum* subsp. *capri pneumoniae* (*Mccp*), *M. mycoides* subsp. *mycoides* SC (*Mmm* SC) and *M. leachii* (*Ml*) (Manso-Silvanet al., 2009; Thiaucourt et al., 2011). *M. capricolum* is a closely related species of *Mmc* and the Genome-To-Genome Distance (GGD) value of the pair of species is even lower than the threshold values for species delimitation (Thompson et al., 2011). The *M. agalactiae* shares 18% of its genome with the *M. mycoides* cluster as a result of frequent horizontal gene transfer events (Sirand-Pugnet et al., 2007).

Mmc strains also show considerable intra-species antigenic variability and the current *Mmc* serovar LC was even referred to as a separate species earlier *i.e. M. mycoides* subsp. *mycoides* Large Colony (*Mmm* LC) (Manso-Silvan et al., 2009; Vilei et al., 2006). The interspecies cross-reactions with other caprine mycoplasmas and intra-species variability frequently hamper the development of sensitive and specific immunodiagnostic assays (Vilei et al., 2006). Serological detection of antibodies against *Mmc* is generally

performed using in-house ELISAs based on whole cell antigen prepared from field isolates (Assuncao et al., 2004) and there are no specific diagnostic tests available till date. Immunoinformatics, a combination of immunology and informatics, has helped in developing methods which have been used to successfully identify antigenic epitopes in pathogens (Tomar and De, 2010). The current study employs an *in silico* analysis of the whole genome and proteome to identify novel diagnostic candidates from predicted lipoproteome of *Mmc* LC strain 95010.

MATERIALS AND METHODS

Genome/ Proteome Databases and Alignment Tools

The whole genome sequence of the *Mmc* strain 95010 was retrieved from the RefSeq database at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/) and the whole proteome was retrieved from Uniprot (Universal *Protein* Resource) database (http://www.uniprot.org/). All the individual nucleotide and protein sequences were retrieved and saved locally for further bioinformatics workflow as described in Figure 1.

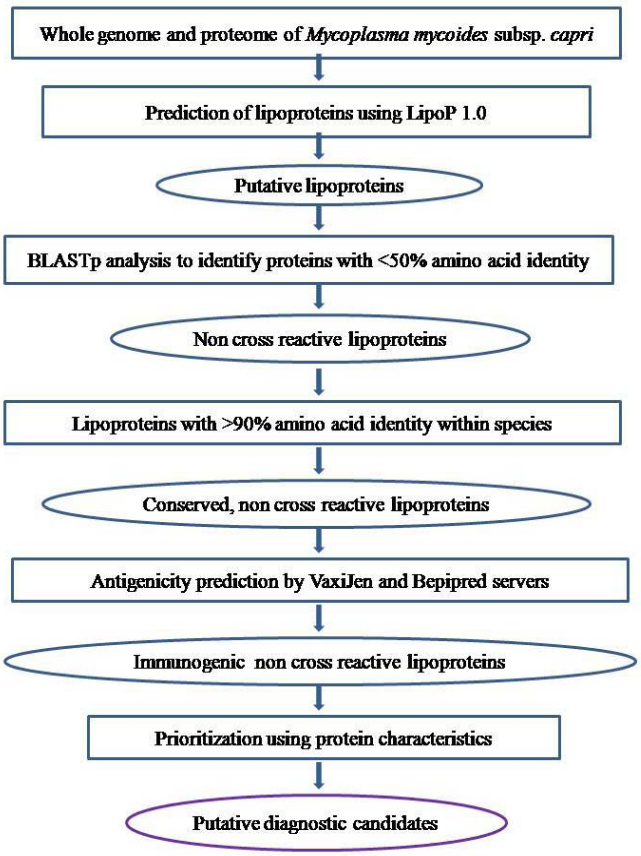

Figure 1: Illustration of comparative and subtractive analysis workflow to identify putative diagnostic candidates in *Mycoplasma mycoides* subsp. *capri*

Lipoprotein Prediction

Lipoproteins in *Mmc* proteome were predicted using LipoP 1.0 server (www.cbs.dtu.dk/services/LipoP/). Peptides with predicted N-terminal cleavage sites for signal peptidase II having log-odds score greater than zero are considered as potential lipoproteins. The LipoP 1.0 algorithm produces accurate predictions of lipoproteins and discriminates between lipoprotein signal peptides, other signal peptides and n-terminal membrane helices in bacteria (Rahman et al., 2008).

Selection of Putative Non Cross Reactive Conserved Lipoproteins

All the 72 predicted lipoproteins of *Mmc* LC 95010 genome were subjected to protein homology searches by using the Basic Local Alignment Search Tool (BLAST) service of UniProt server (http://www.uniprot.org). BLASTp analysis was performed against the UniprotKB database using BLOSM-62 matrix with an expectation value (E) threshold of 0.0001 and filtering was applied to avoid the low complexity regions. The results were saved locally to analyze amino acid identity with other organisms and to identify the intra-species variability among the *Mmc* strains.

Prioritization of Identified Candidates for Heterologous Expression

Physical and chemical parameters like molecular weight, isoelectric point (pI), *in vivo* half-life of protein in *E. coli* were estimated using the protparam tool of expasy server (http://web.expasy.org/protparam/). As the members of the genera Mycoplasma utilize TGA (opal stop) codons as tryptophan coding ones, genes containing TGA codons results in premature truncation during translation in normal *E. coli* host cells (Minion, 1998). All the sequences were analyzed for the presence of TGA codons to identify protein targets suitable for heterologous expression in *E.coli.*

B Cell Epitope Analysis and Antigenicity Prediction Using Vaxijen Server

Selected candidates were further analyzed by *in silico* antigenicity prediction server, VaxiJen (http://www.jenner.ac.uk/VaxiJen). It is the first server developed for alignment-independent prediction of protective antigens of bacterial, viral and tumour origin (Doytchinova et al., 2007). Immunogenicity of the proteins was also evaluated using Bepipred B cell epitope prediction software (http://tools.iedb.org/bcell/). The method combines the hidden Markov model with propensity scale methods for predicting linear B-cell epitopes (Larsen et al., 2006). The results were used to prioritize the selected proteins and those with significant B cell epitopes were selected so that they can be expressed as recombinant proteins in a heterologous ex pression system.

Table 1: Details of five putative lipoproteins identified as diagnostic candidates in *Mycoplasma mycoides* subsp *capri*

SI No.	Uniprot protein ID	Gene ID	Amino acid length	Number of TGA codons with position	pI value	Molecular weight (kDa)	Best BLASTp hit outside the species: Organism, percent identity	BLASTp within the species: Mmc strain, percent identity
1	F4MNX4	MLC_0780	466	No TGA codon	5.35	52.23	*Mcc*[1], 34%	*Mmc* strain PG3, 91%
2	F4MNX7	MLC_0810	563	4 (112, 265, 378, 489)	5.34	63.34	*Mcc*, 46%	*Mmc* strain PG3, 93%
3	F4MNX8	MLC_0820	790	5 (97, 165,166, 206, 382,483)	5.27	90.79	*Ma*[2], 28%	*Mmc* strain PG3, 94%
4	F4MPK9	MLC_3130	172	2 (113, 165)	8.88	18.19	*Mcc*, 42%	*Mmc* strain PG3, 95%
5	F4MR59	MLC_8630	247	5 (97, 130, 214, 231, 238)	5.07	25.39	*Mfer*[3], 40.4%	*Mmc* strain PG3, 91.5%

[1]*M. capricolum* subsp. *capricolum* (*Mcc*); [2]*M. agalactiae* (*Ma*); [3]*M. ferriruminatoris* (*Mfer*)

RESULTS

Sequences and Databases

The *Mmc* LC 95010 has a circular chromosome of 1,153,998 bp (GenBank accession number NC_015431) which consists of 922 putative CDS and a plasmid coding for 2 proteins (Thiaucourt et al., 2011). The whole proteome of *Mmc* (Proteome IDUP000010103) consists of 896 proteins which were mapped to 921 gene IDs. The putative CDS MLC_7780 was not found in the proteome as it was a pseudo gene and 19 proteins were identified to be coded by multiple genes.

Lipoprotein Prediction

Analysis of 896 proteins of *Mmc* LC 95010 using the LipoP 1.0 identified 72 proteins with predicted cleavage sites for signal peptidase II and 73 proteins with a predicted cleavage site for signal peptidase I. All the 72 lipoproteins with cleavage sites for signal peptidase II were selected for further bio informatic analysis and diagnostic target prioritization.

Blast Analysis

Lipoproteins which showed less than 50% identity with the other organisms were considered as putative non cross reactive proteins of *Mmc*. BLAST analysis revealed 17 putative lipoproteins which qualified the selection criterion. The non-cross reactive proteins were further analyzed for intra-species variation. Out of 17 putative lipoproteins, only 7 candidates were having more than 90% identity with the other *Mmc* strains PG3 and GM12. The absence of a hit against *Mmc* strain GM12 was not considered as a criterion for exclusion, since only the partial proteome (372 proteins) is available for *Mmc* strain GM12.A complete proteome with 779 protein entries was available for *Mmc* type strain PG3 and all the lipoproteins having a homolog with more than 90% identity in the strain were considered to be conserved.

Antigenicity Prediction Using Vaxijen Server and Bepipred Epitope Prediction Tool

For antigenicity prediction using the VaxiJen server, a threshold value of 0.5 was selected and the candidates which gave a value above cut-off were considered as predicted antigens. Analysis of 7 selected putative lipoprotein predicted five to be antigenic, whereas two proteins -Uniprot IDsF4MPK7 (MLC_3110) and F4MPS5 (MLC_3800) were predicted to be non antigenic. Bepitope linear B cell epitope prediction server identified epitopes in all the five selected lipoproteins - F4MNX4 (MLC_0780), F4MNX7 (MLC_0810), F4MNX8 (MLC_0820), F4MPK9 (MLC_3130) and F4MR59 (MLC_8630) as given in Figure 2.

Prioritization of Diagnostic Candidates Based on Protein Parameters

Presence of TGA stop codons was identified and protein parameters- molecular weight, isoelectric point (pI) and *in vivo* half life of protein in *E. coli* were estimated for the selected five conserved, non cross reactive lipoproteins. There was only one lipoprotein (Uniprot IDs F4MNX4 without any TGA codons in the entire length of the gene sequence. The other candidates have immunogenic regions without TGA codons that can be expressed in prokaryotic expression system for the development of sero diagnostics. The details of bio informatic analysis of selected diagnostic candidates are given in Table 1.

There was an additional set of four candidates (F4MP11, F4MQW4, F4MQW9 and F4MRA1) which have cross reactivity only with the closely related bovine pathogens *M. mycoides* subsp. *mycoides* SC, *M. leachii* and wildlife pathogen *M. ferriruminatoris*. These can also be evaluated in diagnosis of *Mmc* as the above mentioned organisms are

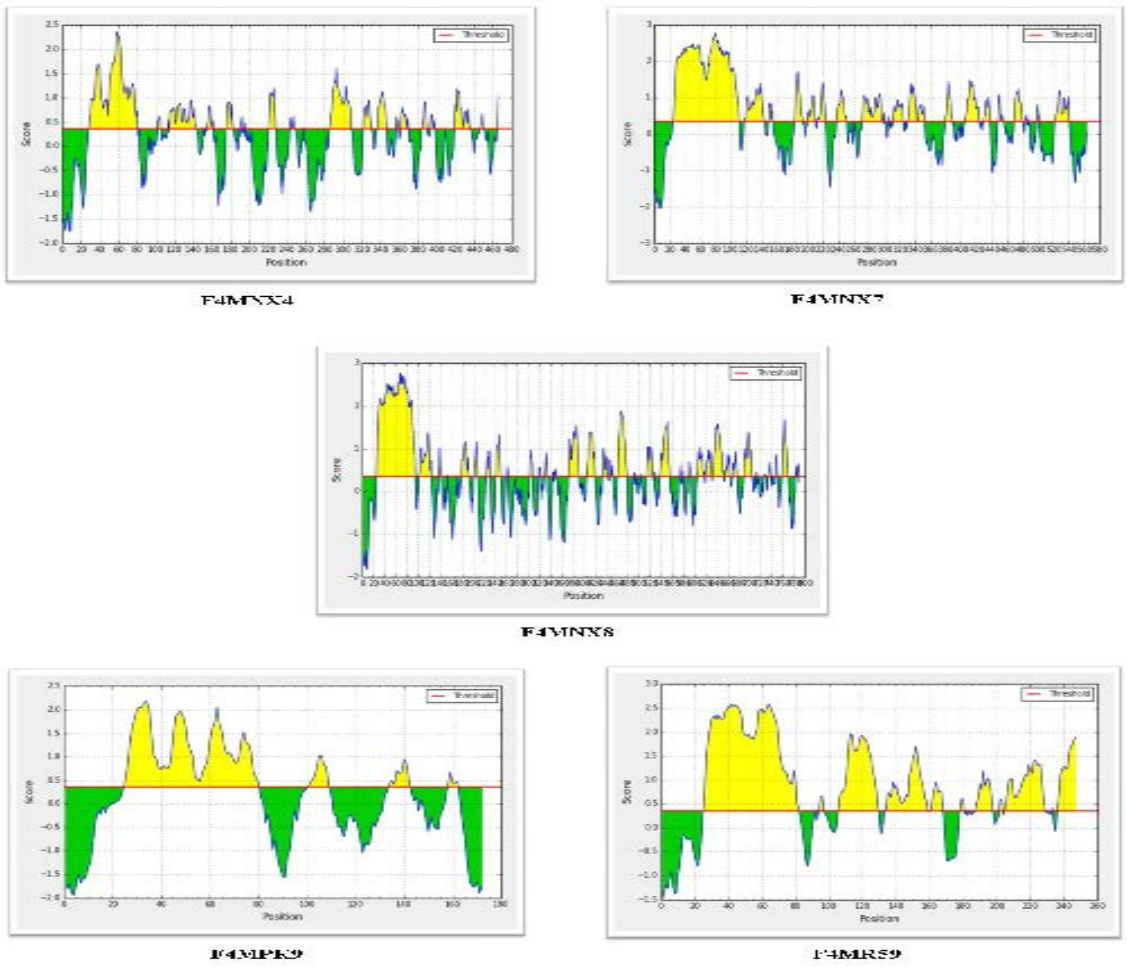

Figure 2: Prediction of linear B cell epitopes in the five selected lipoproteins using Bepipred server. The regions above the threshold (0.35) are antigenic, shown in yellow while, green color reflects the polypeptide regions that could not satisfy the threshold margin

not caprine pathogens.

DISCUSSION

Mycoplasmal lipoproteins are excellent immunogens, which have been used for sero diagnosis of various *Mycoplasma spp*. (Bruderer et al., 2002; Fusco et al., 2007; Alberti et al., 2008). Hence, the identification of conserved immunogenic lipoproteins in *Mmc* is an important step towards development of sensitive and specific immunodiagnostics. Churchward et al. (2014) performed immuno proteomic characterization of *Mmc* by mass spectrometry analysis two-dimensional (2D) electrophoresis spots and western blot. The proteins identified in these studies were mostly metabolic enzymes and other cytoplasmic proteins. Although the identified immunogens can be good vaccine candidates, their utility as diagnostic candidates was limited because of the high similarity with closely related mycoplasmas. Even the 2D electrophoresis and western blot analysis of liposoluble proteome (Corona et al., 2013) could not identify any lipoproteins. The lack of lipoproteins and other membrane-associated proteins identified in these studies is probably due to their low abundance in comparison to other cellular proteins and lack of solubility when preparing the samples for isoelectric focusing (Churchward et al., 2014).

In the current study, putative diagnostic candidates were identified using a bio informatic workflow employing lipoprotein sequence prediction, BLAST analysis, Vaxigen antigenicity prediction server, Bepipred linear epitope prediction tool and protein parameters including presence of TGA codons. Our study identified 72 putative lipoproteins using LipoP 1.0 server which has been used earlier for lipoprotein identification from other *Mycoplasma spp.* including the closely related *M. mycoides* subsp. *mycoides* SC (Heller et al., 2016).

BLASTp analysis was performed to identify the putative non cross reactive lipoproteins in *Mmc* proteome. Due to the high genetic similarity within mycoides cluster, there were no proteins with "no hits" in the BLASTp analysis. Most investigators describe protein similarity in terms of "percent identity" of amino acids whereas E-values and bit-scores are also useful for inferring homology. Non-cross-reacting domains usually show less than 70% sequence identity, emphasizing the genetic basis for immunological specificity (Maeland et al., 2015). McNulty et al. (2015) adopted the criterion of 70% amino acid sequence identity over more than 70% of the total protein length for identification of putative diagnostic antigens from *Onchocerca volvulus*. Their study identified 60 diagnostic candidates, which satisfy the criterion from a total of 241 immunoreactive proteins analyzed. Here, we adopted a more stringent criterion of less than 50% identity over the entire length of protein to identify 17 non cross-reactive ones out of 72 lipoproteins. When two protein sequences have less than 50% identity, the risk of cross reactivity is expected to be rare (Silvanovich et al., 2006). Although it is difficult to predict antibody cross-reactivity based on global sequence similarity, this level of conservation makes these proteins more attractive immunodiagnostic candidates than those having orthologues in related species.

Significant protein variability within the species is well documented for various *Mycoplasma spp*. (Calus et al., 2007; Salam et al., 2013). Fischer et al. (2012) conducted a multi locus sequence typing (MLST) analysis of 33 *Mmc* isolates and identified a very high genetic diversity within the species. The putative diagnostic candidate for *Mmc* should also address the high intra-species variability within *Mmc*. In our study, seven out of 17 non cross reactive lipoproteins showing 90% amino acid identity over the entire length of protein were selected for further evaluation.

VaxiJen is the first server for alignment-independent prediction of protective antigens. It allows antigen classification based on the physicochemical properties of proteins without depending on the sequence alignment. It has been used for prediction of vaccine candidates in several bacterial species including *M. agalactiae* (Forouharmehr and Nassiry, 2015). Five candidates which were identified to be antigenic by VaxiJen server can also be used as potential vaccine candidates for *Mmc*. All the identified targets MLC_0780, MLC_0810, MLC_0820, MLC_3130 and MLC_8630 are uncharacterized proteins. Recently, an *in silico* analysis combined with 2D electrophoresis and western blot predicted eight novel uncharacterized antigens to have high immunological value and Mbov_0579 was found to be the best antigenic target for sero diagnosis of *M. bovis* (Khan et al., 2016).

This study identified novel diagnostic candidates, which can be utilized in the development of sensitive and specific diagnostic tests and recombinant vaccines against *Mmc*. Our selection procedure theoretically guarantees that the identified lipoproteins have the potential to achieve an adequate degree of specificity and sensitivity, minimizing the likelihood of false positives associated with current diagnostic tests. The predicted lipoproteins need to be expressed in suitable prokaryotic expression system and validated for the development of immunodiagnostics.

ACKNOWLEDGEMENTS

We thank Director, Indian Veterinary Research Institute for funding the necessary facilities for the research programme under which the current study is conducted. The first author is thankful to Department of Science and Technology (DST), India for providing financial support (Inspire fellowship) during the period this research work.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Thachappully Remesh Arun and Valsala Rekha performed the in silico analysis work. Rajneesh Rana supervised the work and aided in writing the manuscript. Thankappan Sabarinath aided in writing the manuscript.

REFERENCES

- Alberti A, Robino P, Chessa B, Rosati S, Addis MF, Mercier P, Mannelli A, Cubeddu T, Profiti M, Bandino E, Thiery R (2008). Characterisation of *Mycoplasma capricolum*P60 surface lipoprotein and its evaluation in a recombinant ELISA. Vet. Microbiol. 128(1): 81-89. https://doi.org/10.1016/j.vetmic.2007.09.020
- Assunçao P, De la Fe C, Ramirez AS, Andrada M, Poveda JB (2004). Serological study of contagious agalactia in herds of goats in the Canary Islands. Vet. Rec. 154(22): 684-687. https://doi.org/10.1136/vr.154.22.684
- Bruderer U, Regalla J, Abdo EM, Huebschle OJ, Frey J (2002). Serodiagnosis and monitoring of contagious bovine pleuropneumonia (CBPP) with an indirect ELISA based on the specific lipoprotein LppQ of *Mycoplasma mycoides* subsp. *mycoides* SC. Vet. Microbiol. 84(3): 195-205. https://doi.org/10.1016/S0378-1135(01)00466-7
- Calus D, Baele M, Meyns T, de Kruif A, Butaye P, Decostere A, Haesebrouck F, Maes D (2007). Protein variability among *Mycoplasma hyopneumoniae* isolates. Vet. Microbiol. 120(3): 284-291. https://doi.org/10.1016/j.vetmic.2006.10.040
- Churchward CP, Rosales RS, Gielbert A, Domínguez M, Nicholas RA, Ayling RD (2015). Immunoproteomic characterisation of *Mycoplasma mycoides* subspecies *capri* by mass spectrometry analysis of two-dimensional electrophoresis spots and western blot. J. Pharm. Pharmacol. 67(3): 364-371. https://doi.org/10.1111/jphp.12344

- Corona L, Cillara G, Tola S (2013). Proteomic approach for identification of immunogenic proteins of *Mycoplasma mycoides* subsp. *capri*. Vet. Microbiol. 167(3): 434-439. https://doi.org/10.1016/j.vetmic.2013.08.024
- Doytchinova IA, Flower DR (2007). Vaxi Jen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics. 8(1): 4.
- Fischer A, Shapiro B, Muriuki C, Heller M, Schnee C, Bongcam-Rudloff E, Vilei EM, Frey J, Jores J (2012). The origin of the '*Mycoplasma mycoides* cluster' coincides with domestication of ruminants. PLoS One. 7(4): e36150. https://doi.org/10.1371/journal.pone.0036150
- Forouharmehr A, Nassiry MR (2015). B and T-Cell Epitopes Prediction of the P40 Antigen for Developing *Mycoplasma Agalactiae* Vaccine Using Bio informatic Tools. G3M. 13 (1): 3954-3961.
- Fusco M, Corona L, Onni T, Marras E, Longheu C, Idini G, Tola S (2007). Development of a sensitive and specific enzyme-linked immunosorbent assay based on recombinant antigens for rapid detection of antibodies against *Mycoplasma agalactiae* in sheep. Clin. Vaccine Immunol. 14(4): 420-425. https://doi.org/10.1128/CVI.00439-06
- Heller M, Gicheru N, Tjipura-Zaire G, Muriuki C, Yu M, Botelho A, Naessens J, Jores J, Liljander A (2016). Development of a novel cocktail ELISA and a field-applicable lateral flow rapid test for diagnosis of contagious bovine pleuro pneumonia. J. Clin. Microbiol. 54(6): 1557-1565. https://doi.org/10.1128/JCM.03259-15
- Khan FA, Faisal M, Chao J, Liu K, Chen X, Zhao G, Menghwar H, Zhang H, Zhu X, Rasheed MA, He C (2016). Immunoproteomic identification of MbovP579, a promising diagnostic biomarker for serological detection of *Mycoplasma bovis* infection.Oncotarget. 7(26): 39376. https://doi.org/10.18632/oncotarget.9799
- Larsen JE, Lund O, Nielsen M (2006). Improved method for predicting linear B-cell epitopes. Immunome Res. 2(1): 2. https://doi.org/10.1186/1745-7580-2-2
- Maeland JA, Afset JE, Lyng RV, Radtke A (2015). Survey of immunological features of the alpha-like proteins of *Streptococcus agalactiae*. Clin. Vaccine Immunol. 22(2): 153-159. https://doi.org/10.1128/CVI.00643-14
- Manimaran K, Singh VP, Ltu K, Das S, Kumar AA, Srivastava SK (2006). Immune responses against different inactivated vaccines of *Mycoplasma mycoides* subsp. *capri* in goats. J. Appl. Anim. Res. 29(2): 117-120. https://doi.org/10.1080/09712119.2006.9706584
- Manso-Silvan L, Vilei EM, Sachse K, Djordjevic SP, Thiaucourt F, Frey J. (2009). Mycoplasma leachii sp. nov.as a new species designation for *Mycoplasma sp.* Bovine group 7 of Leach, and reclassification of *Mycoplasma mycoides* subsp. *mycoides* LC as a serovar of *Mycoplasma mycoides* subsp. *capri*. Int. J. Syst. Evol. Microbiol. 59: 1353–1358. https://doi.org/10.1099/ijs.0.005546-0
- McNulty SN, Rosa BA, Fischer PU, Rumsey JM, Erdmann-Gilmore P, Curtis KC, Specht S, Townsend RR, Weil GJ, Mitreva M (2015). An Integrated Multiomics Approach to Identify Candidate Antigens for Sero diagnosis of Human Onchocerciasis. Mol. Cell Proteomics.14(12): 3224-3233. https://doi.org/10.1074/mcp.M115.051953
- Minion CF (1998). Mycoplasma gene expression in Escherichia coli. In: R.J. Miles and R.A.J. Nicholas (eds), Methods in Molecular Biology, Humana Press Inc., Totowa, NJ, Vol. 104: 259-265. https://doi.org/10.1385/0-89603-525-5:259
- Rahman O. Cummings SP, Harrington DJ, Sutcliffe IC (2008). Methods for the bio informatic identification of bacterial lipoproteins encoded in the genomes of Gram-positive bacteria World J. Microbiol. Biotechnol. 24(11): 2377-2382 https://doi.org/10.1007/s11274-008-9795-2.
- Salam HS, Hotzel H (2013).Investigation of phenotypic variations amongst *Mycoplasma bovis* field German isolates using SDS-PAGE.Beni-Suef J. Appl. Sci. 2(2):103-107.
- Silvanovich A, Nemeth MA, Song P, Herman R, Tagliani L, Bannon GA (2006). The value of short amino acid sequence matches for prediction of protein allergenicity. Toxicol. Sci. 90(1): 252-258. https://doi.org/10.1093/toxsci/kfj068
- Sirand-Pugnet P, Lartigue C, Marenda M, Jacob D, Barré A, Barbe V, Schenowitz C, Mangenot S, Couloux A, Segurens B, De Daruvar A (2007). Being pathogenic, plastic, and sexual while living with a nearly minimal bacterial genome. PLoS Genet. 3(5): e75. https://doi.org/10.1371/journal.pgen.0030075
- Thiaucourt F, Manso-Silvan L, Salah W, Barbe V, Vacherie B, Jacob D, Breton M, Dupuy V, Lomenech AM, Blanchard A, Sirand-Pugnet P (2011). *Mycoplasma mycoides*, from" mycoides Small Colony" to" capri". A micro evolutionary perspective. BMC Genomics. 12(1): 114. https://doi.org/10.1186/1471-2164-12-114
- Thompson CC, Vieira NM, Vicente ACP, Thompson FL (2011). Towards a genome based taxonomy of Mycoplasmas. Infect. Genet. Evol. 11(7): 1798-1804. https://doi.org/10.1016/j.meegid.2011.07.020
- Tomar N, De RK (2010). Immunoinformatics: an integrated scenario. Immunol. 131(2): 153-168. https://doi.org/10.1111/j.1365-2567.2010.03330.x
- Vilei EM, Korczak BM, Frey J (2006). *Mycoplasma mycoides* subsp. *capri* and *Mycoplasma mycoides* subsp. *mycoides* LC can be grouped into a single subspecies. Vet. Res. 37(6): 779-790 https://doi.org/10.1051/vetres:2006037.