

Genome of *Leptospira interrogans* serovar Copenhageni: identification of potential targets for diagnosis and vaccines

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**International Workshop of the Oswaldo Cruz Institute/FIOCRUZ for Leptospirosis Research Based on
Country Needs**

5th Global Leptospirosis Environmental Action Network (GLEAN) Meeting

10-12 November 2015
Rio de Janeiro, Brazil

ECONOMIC IMPACT

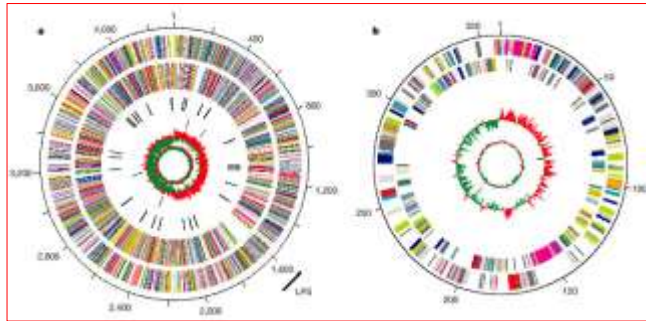
LEPTOSPIROSIS:

- is a public health concern: patient treatment requires hospitalization and not infrequently renal dialysis.
- represents a major economic burden in livestock; it produces abortion, stillbirth, infertility, failure to thrive, reduced milk production and death.

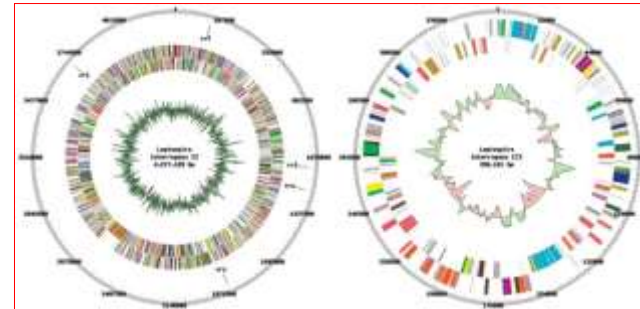
PREVENTION

- Currently veterinarian vaccines are based on inactivated whole cell leptospires; induce protective responses through induction of Abs against leptospiral LPS.
- However, these vaccines:
 - i) - fail to induce long-term protection against infection;
 - ii) - they do not provide cross-protective immunity against leptospiral serovars not included in the vaccine preparation.

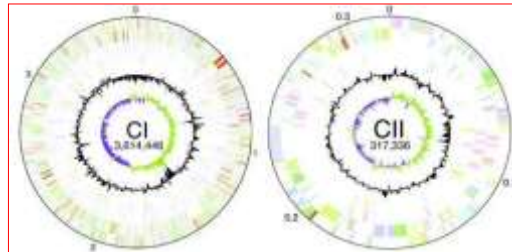
STRATEGY TO FIGHT LEPTOSPIROSIS



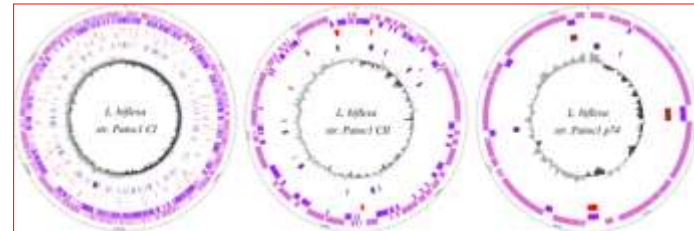
Ren et al., 2003, Nature, 422(6934):888-93



Nascimento et al., 2004a, Braz J Med Biol Res. 37(4):459-77;
Nascimento et al., 2004b, J. Bacteriol. 186(7) 2164-72



Bulach et al., 2006- PNAS USA 2006;103: 14560.



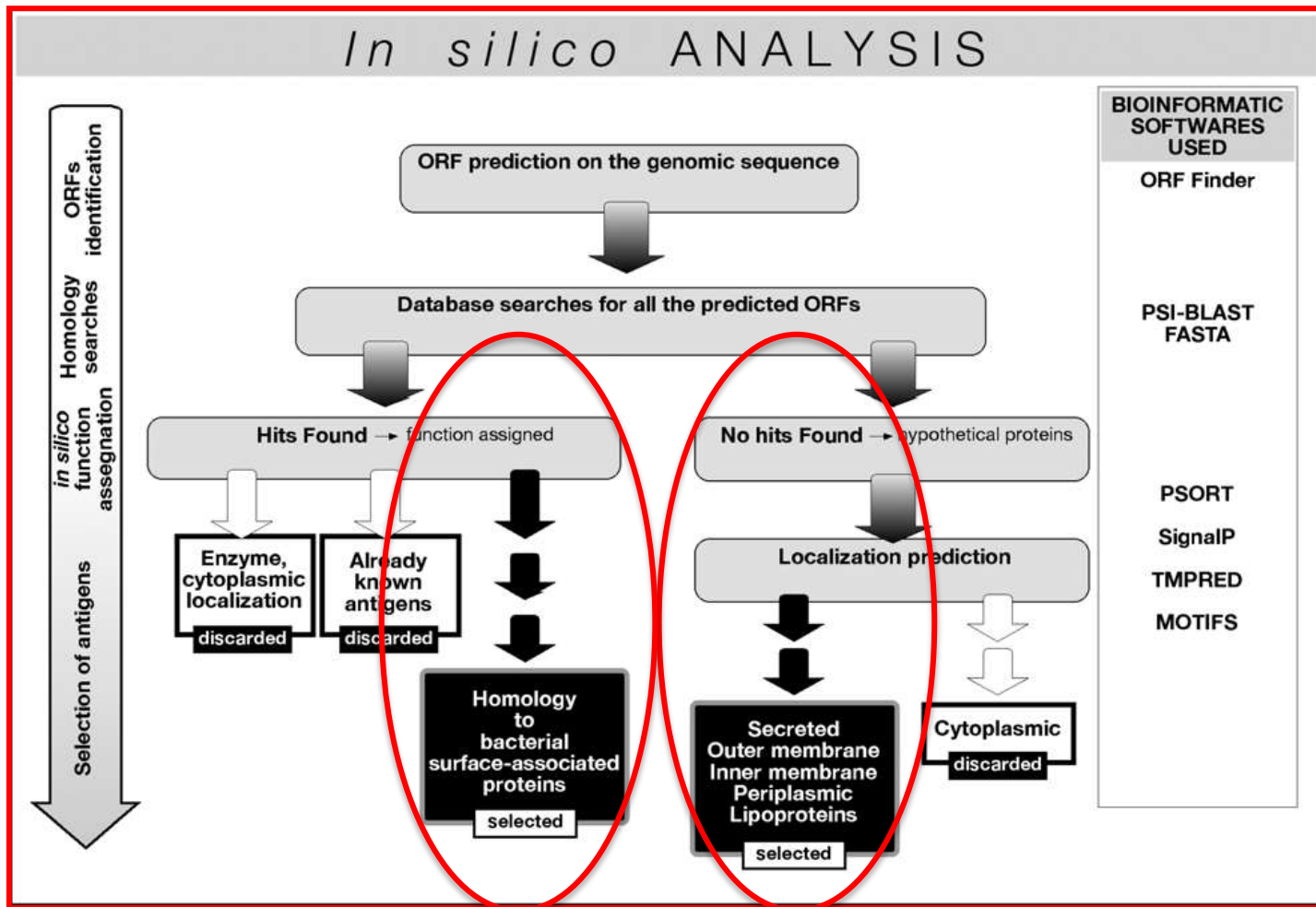
Picardeau et al., PLoS One. 2008):e1607

- **Project Title:** *Leptospira* Genomics with the proposal of sequencing more than 200 *Leptospira* isolates around the world;
- **Coordination:** Joseph M. Vinetz, (UC San Diego) and Derrick Fouts (J. Craig Venter Institute);
- **Participants** are several groups around the world;
- **These studies produced a manuscript:** Comparative Genomic Analysis of 20 *Leptospira* species, under revision

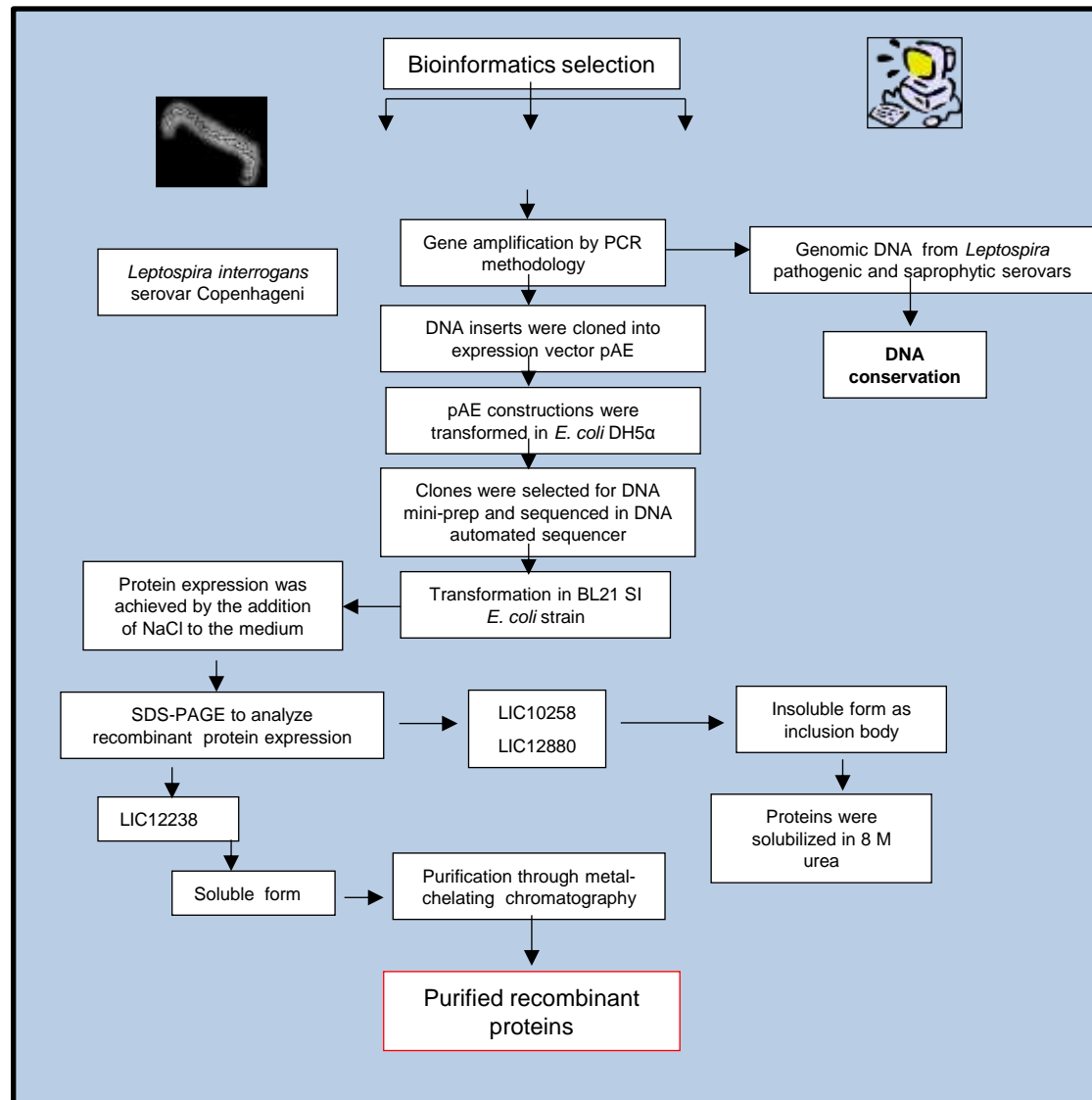
OUR GOAL

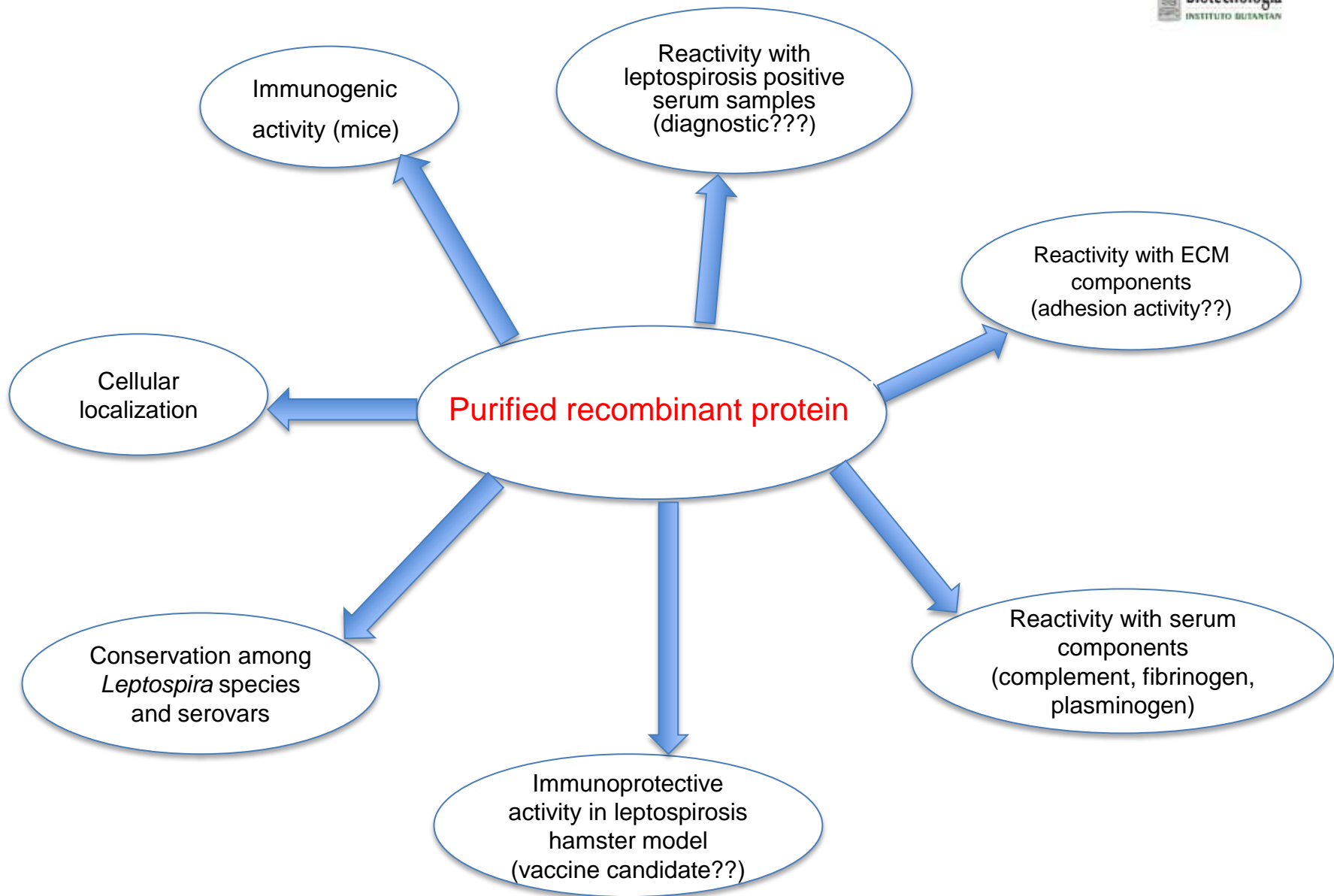
- **To explore the genome sequences of *L. interrogans* to search for new antigens in order to identify:**
 1. Vaccine candidates
 2. Diagnostic candidates
- **To gain knowledge on leptospiral pathogenesis**
 1. Identify mechanisms of adhesion/colonization
 2. Identify mechanisms of invasion/facilitator
 3. Identify virulence factors

STRATEGY FOR PROTEIN SELECTION



PIPELINE TO OBTAIN RECOMBINANT PROTEINS

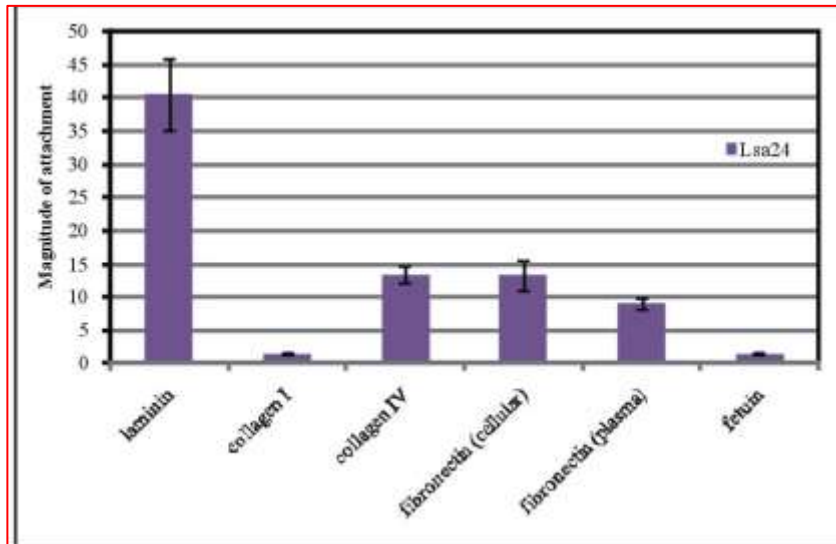




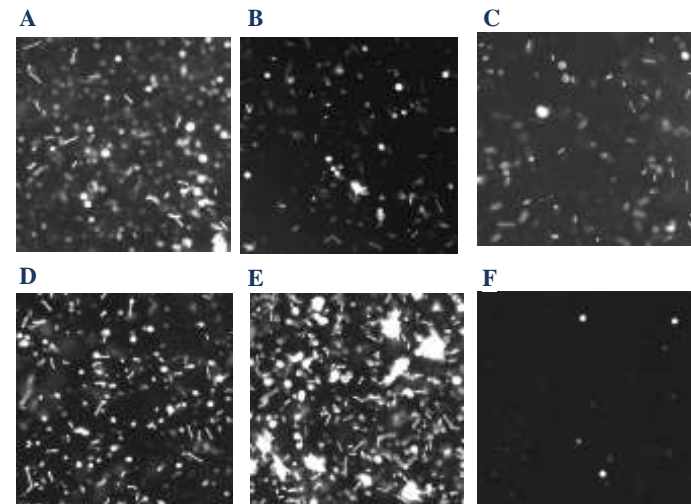
Interactions of recombinant proteins with human host components: ECM and plasma

IDENTIFICATION OF NOVEL EXTRACELLULAR MEMBRANE (ECM)- BINDING PROTEINS – ROLE IN ADHESION / PATHOGENESIS?

1. The first laminin-binding protein- Lsa24



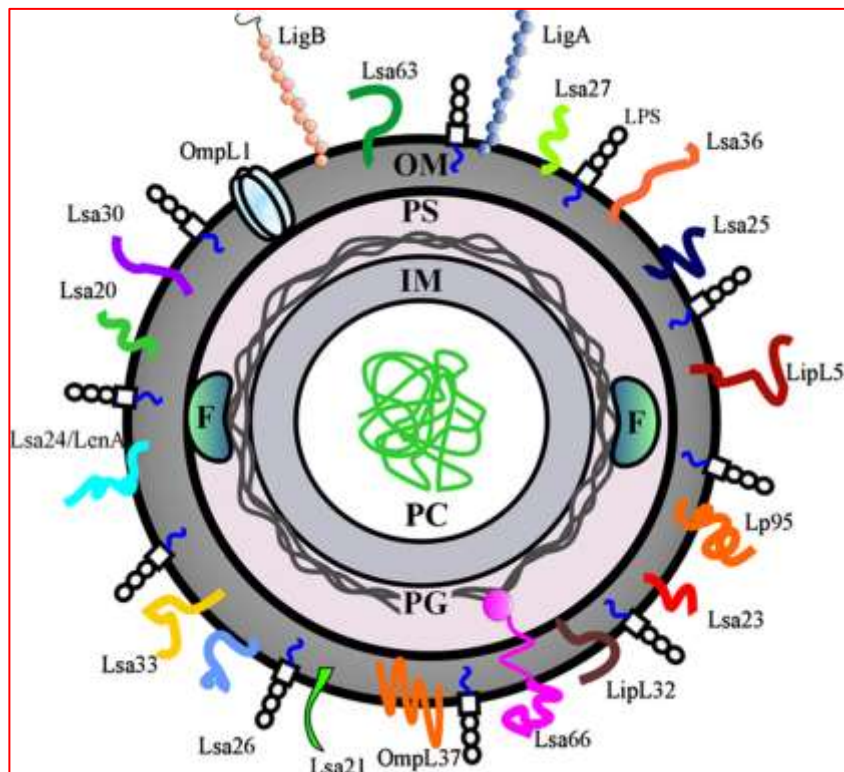
2. *L. interrogans* attachment to ECM macromolecules



laminin (A), collagen I (B), collagen IV (C), cellular fibronectin (D),
plasma fibronectin (E) or BSA (F)

SCHEMATIC REPRESENTATION OF THE *LEPTOSPIRA* CELL MEMBRANE

(OM)=outer membrane, (IM) inner membrane and (LPS) lipopolysaccharide. (PS), In the periplasmic space, the peptidoglycan (PG) is found strongly associated with the (IM). In this diagram, the protoplasmic cylinder (PC) is also shown, containing the nucleoid. The endoflagela (F) are located in the (PS)..



Barbosa et al., Infect. Immun., 2006 – Lsa24
 Atzingen et al., BMC Microbiol., 2008 – Lsa21
 Longhi et al., J. Med. Microbiol., 2009 – Lsa27
 Atzingen et al., J. Infect., 2009 – Lp95
 Oliveira et al., Microb. Infect., 2010 – LipL53
 Vieira et al., J. Infect., 2010 – Lsa63
 Oliveira et al., PLoS ONE, 2011 – Lsa66
 Mendes et al., Infect. Immun., 2011 – Lsa20
 Domingos et al., BMC Microbiol., 2012 – Lsa33 and Lsa25
 Souza et al., Microbiol. Pathog., 2012- Lsa30
 Fernandes et al., Infect. Immun., 2012- OmpL1
 Siqueira et al., Am. J. Trop. Med. Hyg., 2013- Lsa23, Lsa26 and Lsa36
 Fernandes et al., Microbiology, 2014 - Lsa44 and Lsa45
 Domingos et al., Microbiology, 2015- Lsa32
 Teixeira et al., PLoS One, 2015- Lsa46 and Lsa77

MINIREVIEW

Leptospiral extracellular matrix adhesins as mediators of pathogen-host interactions

Monica L. Vieira¹, Luis G. Fernandes^{1,2}, Renan F. Domingos^{1,2}, Rosane Oliveira^{1,2},
 Gabriela H. Siqueira^{1,2}, Natalie M. Souza^{1,2}, Aline R.F. Teixeira^{1,2}, Marina V. Atzingen¹
 & Ana L.T.O. Nascimento^{1,2}

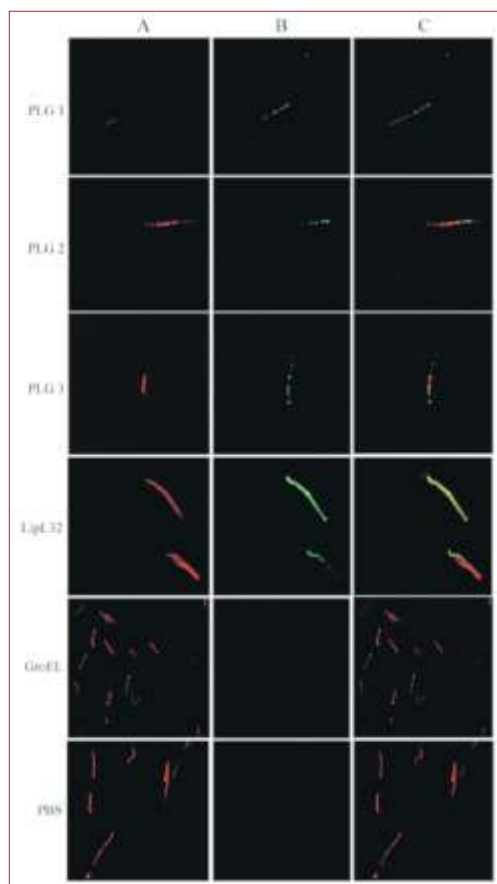
Lsa= Leptospiral surface adhesin

Plasminogen Acquisition and Activation at the Surface of *Leptospira* Species Lead to Fibronectin Degradation[▽]

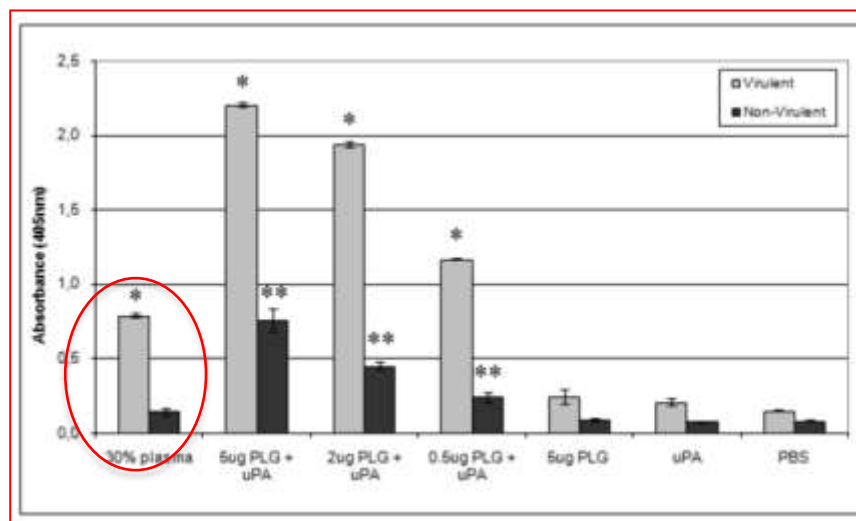
Monica L. Vieira,^{1,2} Silvio A. Vasconcellos,³ Amane P. Gonçalves,³
Zenaide M. de Moraes,³ and Ana L. T. O. Nascimento^{1,2*}



1. PLG binds at the bacterial surface

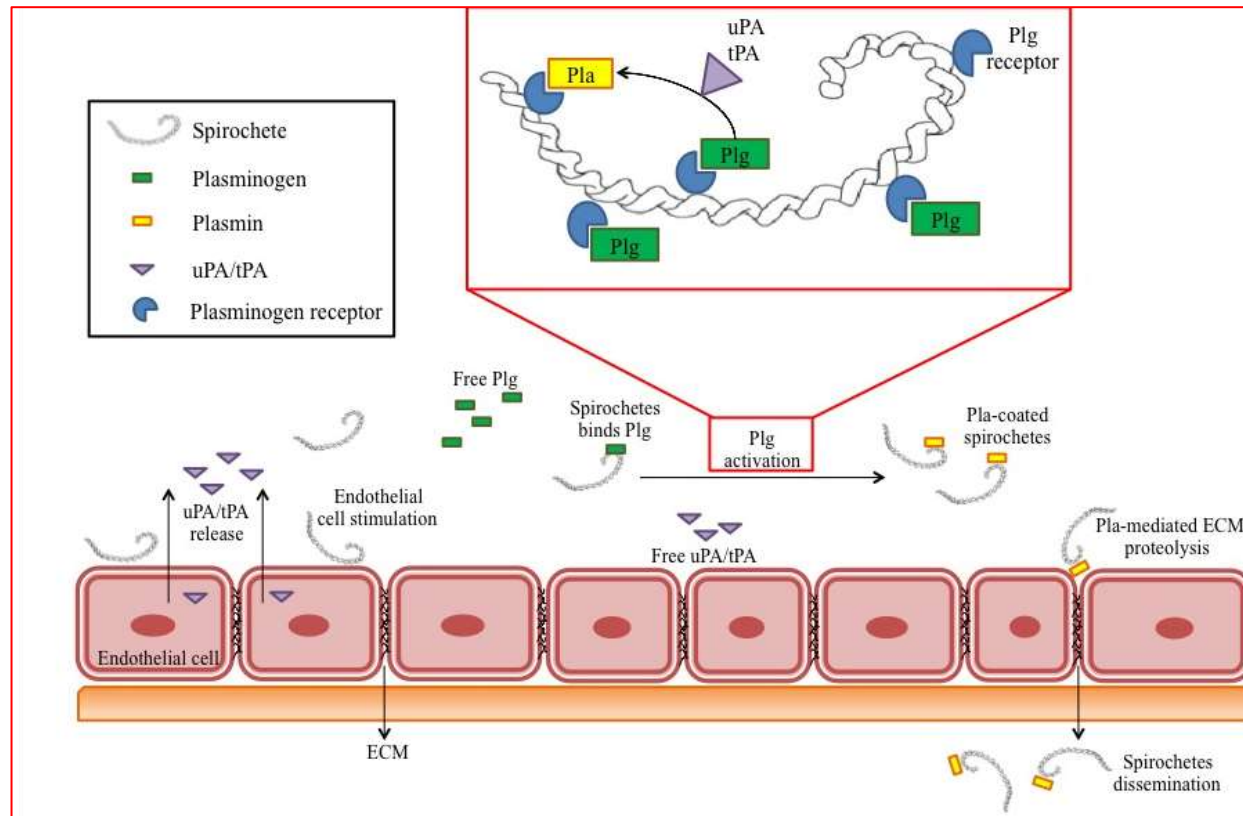


2. Plasmin is generated when uPA is available



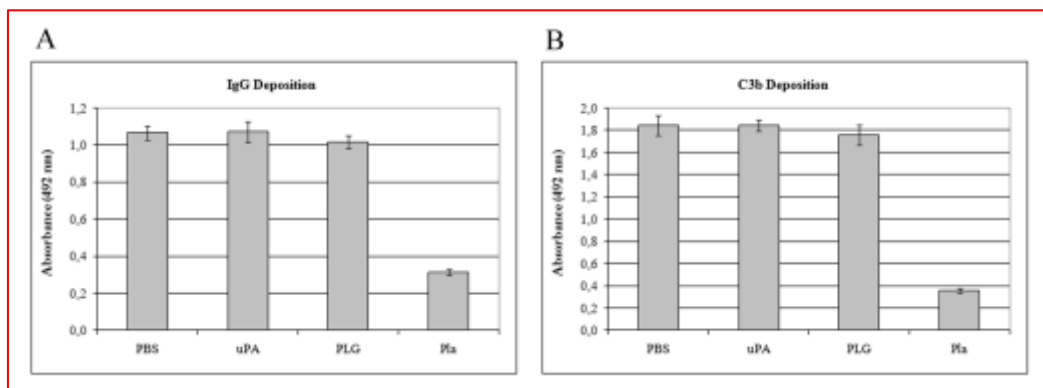
Miriam I. Flores,^{1,2} Martina F. Allington,² Roscoe W. Waring,^{1,2} Brenda S. Knicker,²
 Susan E. Donohue,^{1,2} Håkan A. Yvonneff,² and Jan L. T. P. Moolenaar^{1,2}

Schematic model of spirochetal-host interactions when the bacteria are endowed with Pla activity and anticipated pathophysiological consequences.



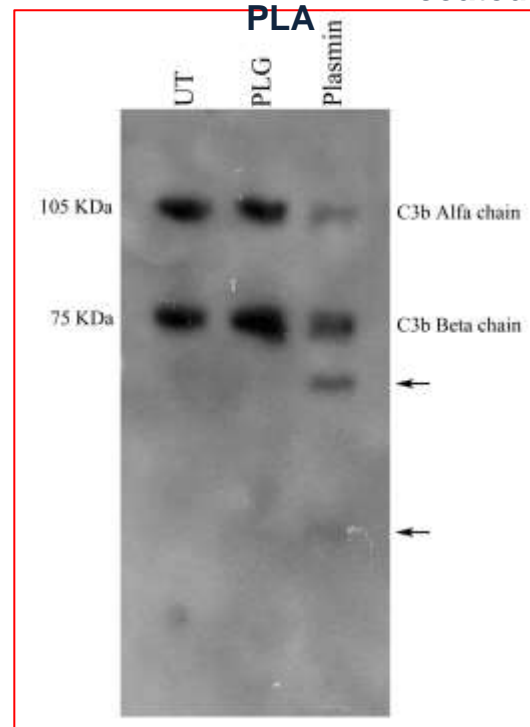


PLA- *Leptospira* decreases IgG Fc regions and C3b depositions at the bacterial surface



As a consequence, there is a decrease in opsonophagocytosis helping the bacteria to escape the host immune system.

C3b is cleaved by leptospire-coated PLA

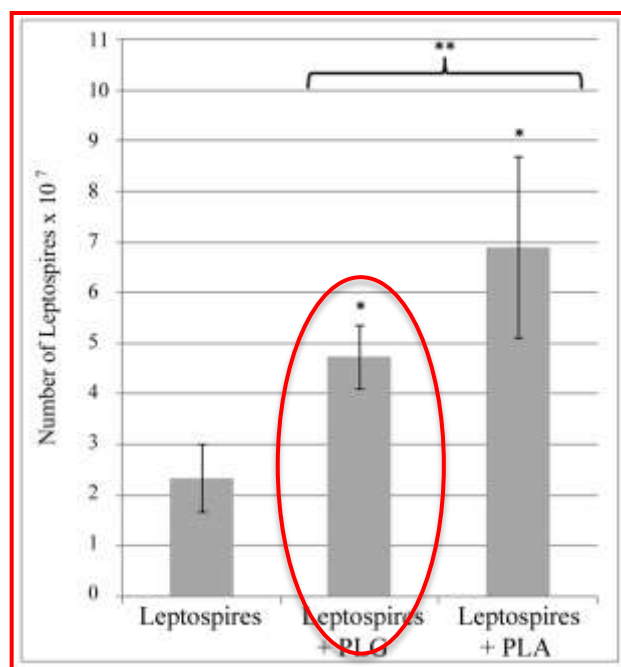




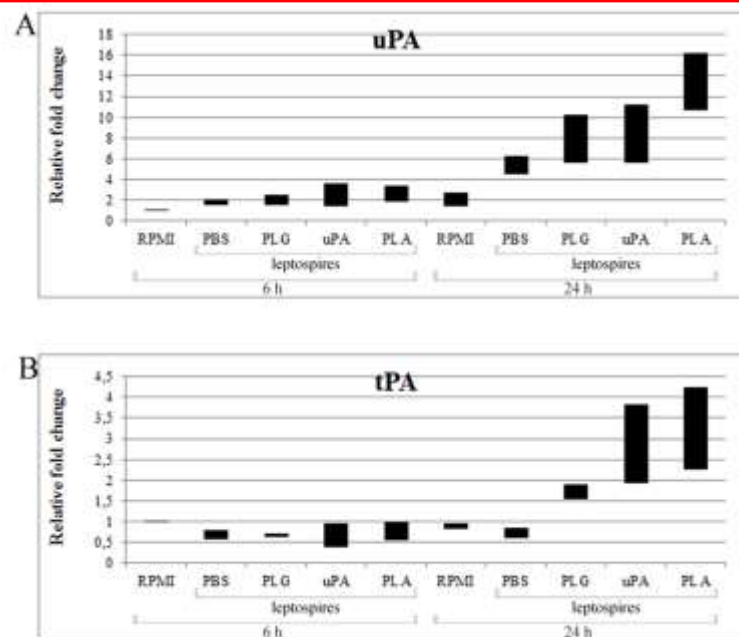
Interaction of *Leptospira interrogans* with Human Proteolytic Systems Enhances Dissemination through Endothelial Cells and Protease Levels

Monica L. Vieira,^{a,b} Miryam P. Alvarez-Flores,^c Karin Kirchgatter,^d Eliete C. Romero,^e Ivy J. Alves,^a Zenaide M. de Moraes,^f Silvio A. Vasconcellos,^g Ana M. Chudzinski-Tavassi,^{c,g} Ana L. T. O. Nascimento^{a,b}

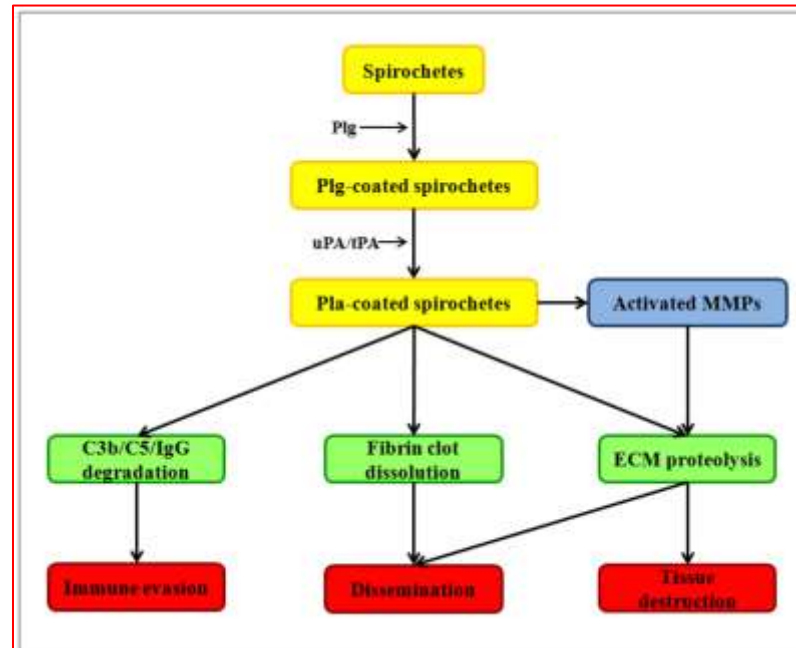
1- Penetration of HUVECs monolayers is increased by plasmin-coated leptospires.



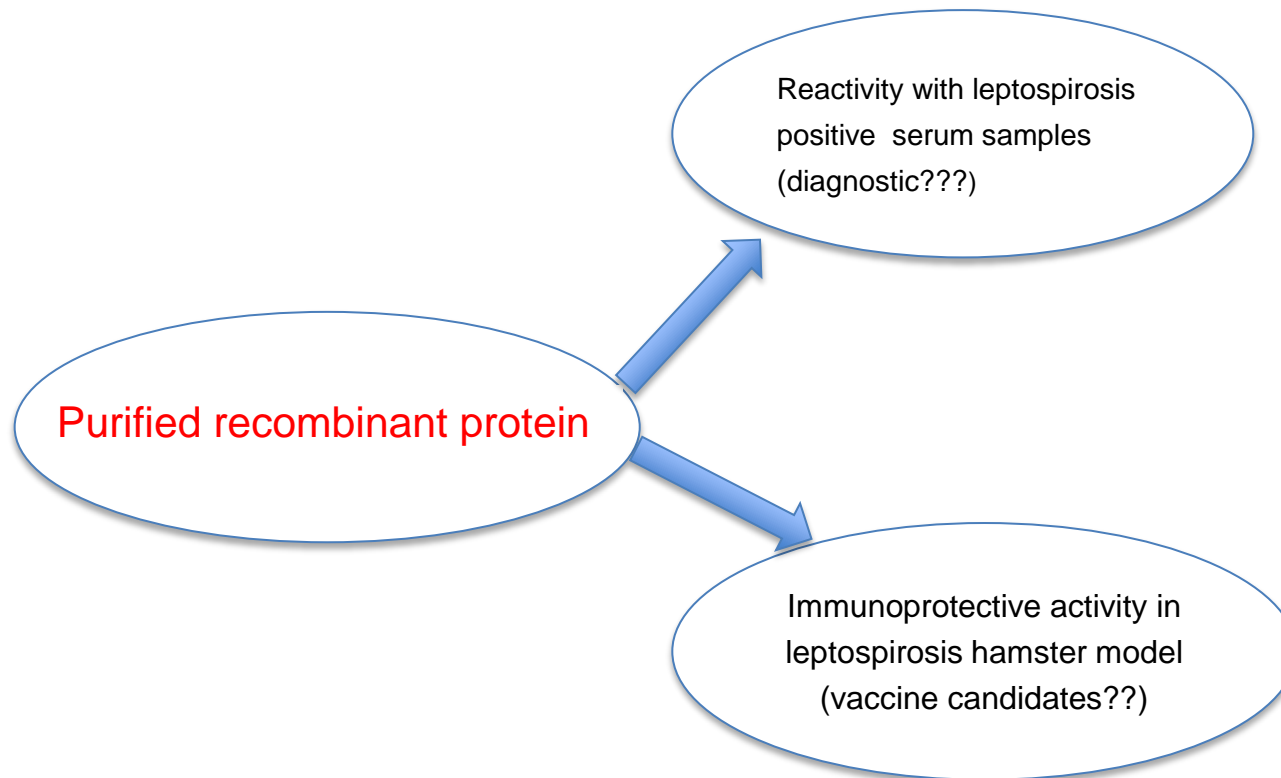
2- *L. interrogans*-PLA upregulate the transcription of PLG activators from HUVECs.



PLG/PLA ACTIVATION ON THE SURFACE OF SPIROCHETES AND MAIN FUNCTIONAL ROLES IN PATHOGENESIS

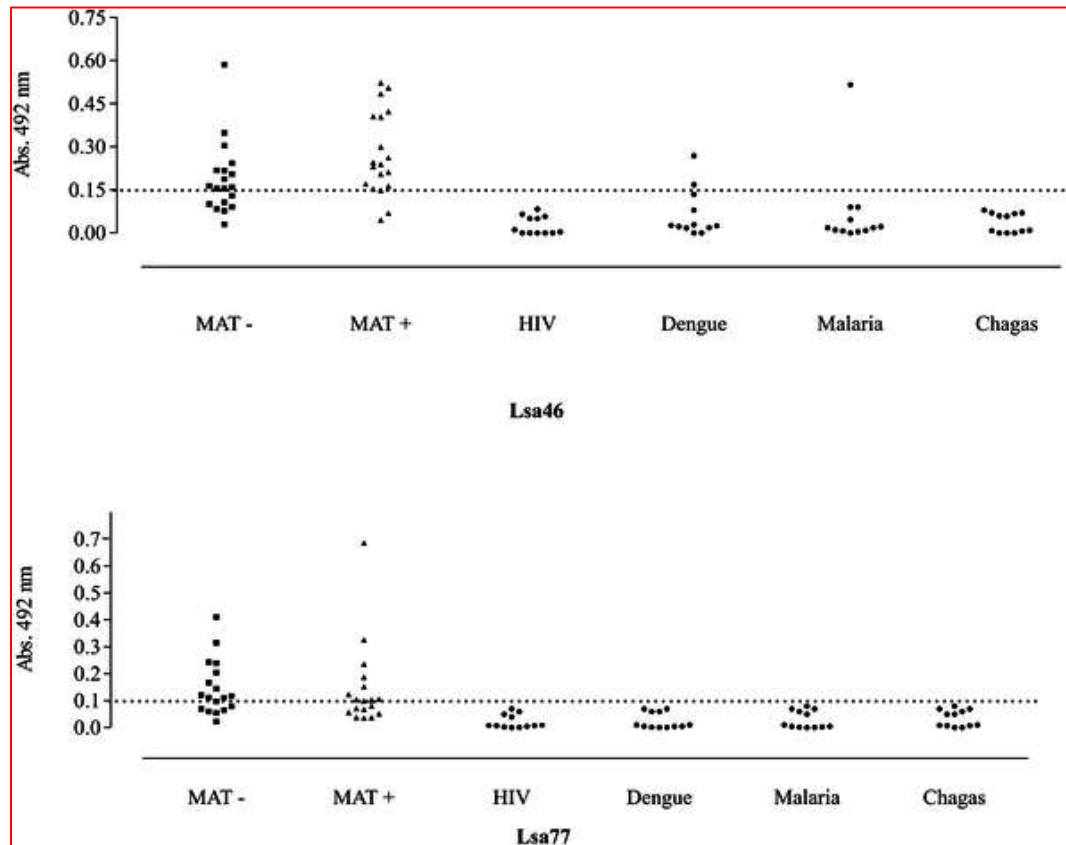


- Immune evasion on acting on IgG and complement system,
- Dissemination through fibrinolysis, and
- Tissue destruction through ECM proteolysis.



Features of Two New Proteins with OmpA-Like Domains Identified in the Genome Sequences of *Leptospira interrogans*.

Teixeira AF, de Moraes ZM, Kirchgatter K, Romero EC, Vasconcellos SA, et al. (2015)

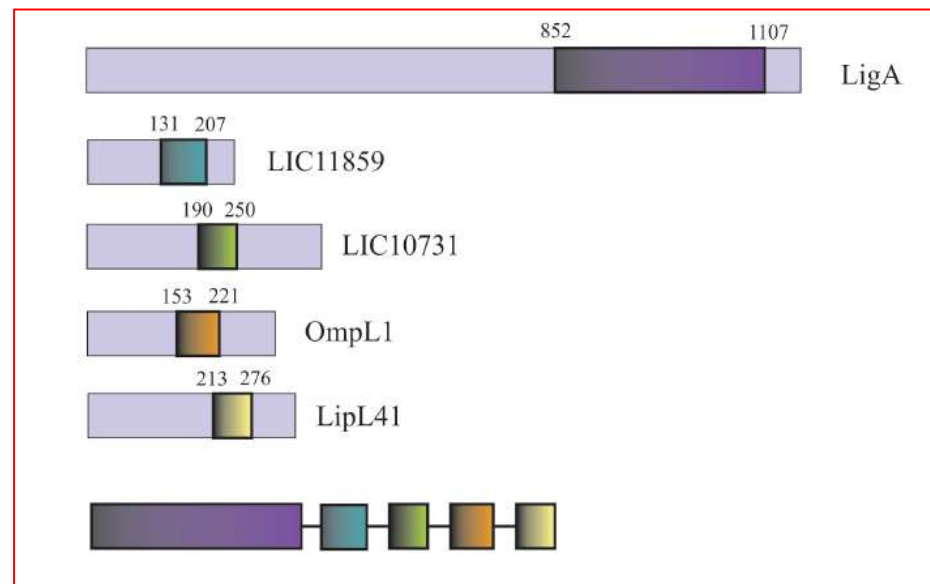


Detection of antibodies against recombinant proteins in human leptospirosis and in unrelated febrile diseases serum samples.

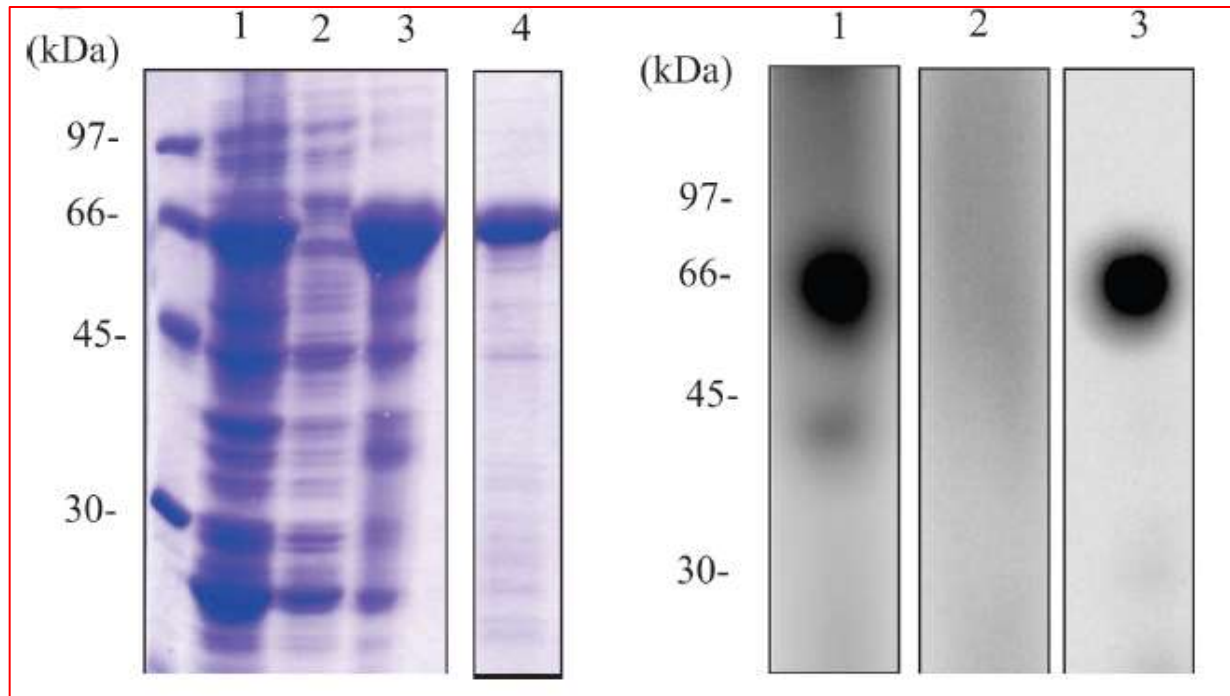
Construction of a chimeric protein based on B and T cell epitopes and known protective domains



Chimeric protein-1 (Chi-1)



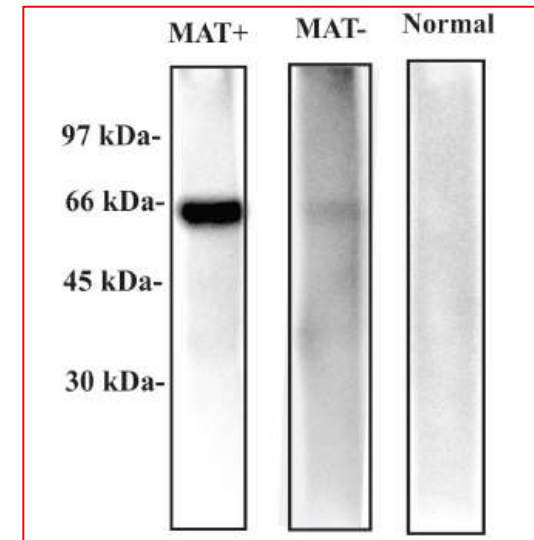
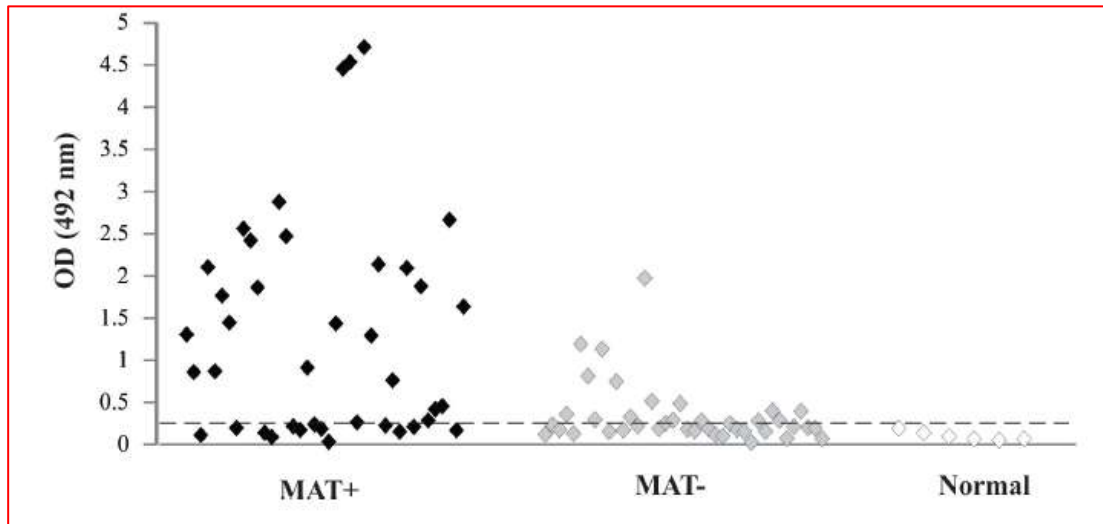
Chi-1 recombinant protein expression and recognition by antibodies



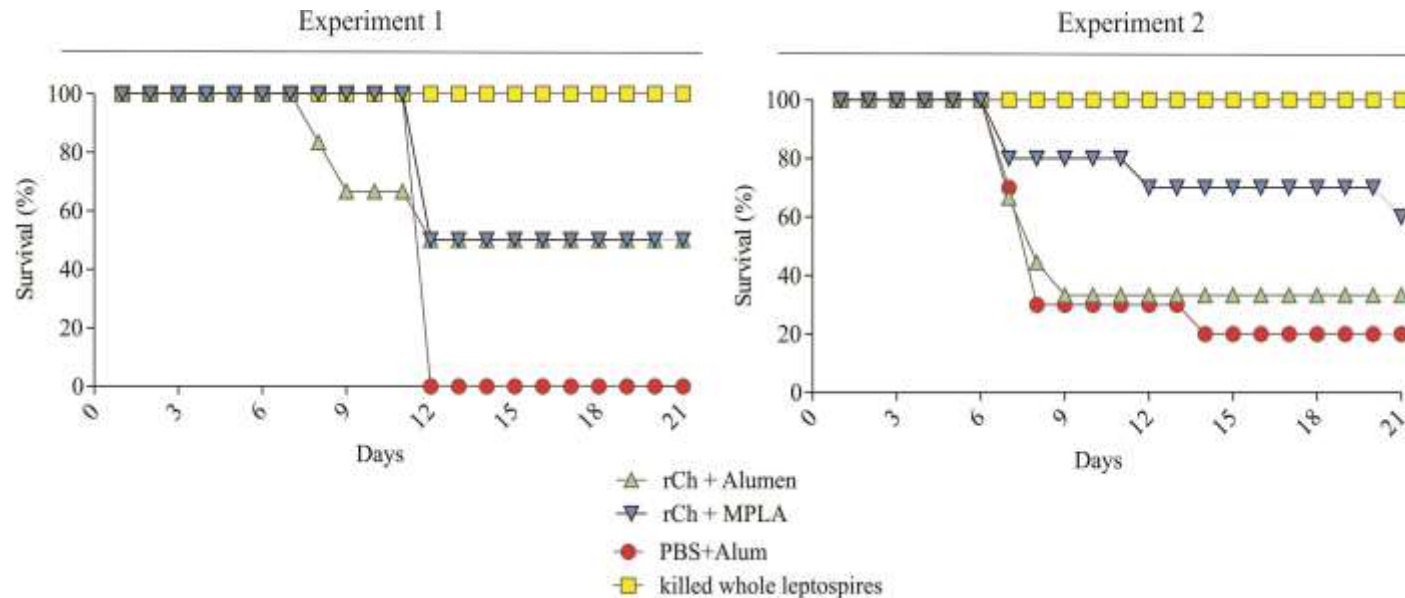
- Left panel- Coomassie blue stained SDS-PAGE of recombinant chimeric protein (Chi-1) expression (lanes 1 to 3) and after purification (lane 4); C. Western blotting.

- Right panel- Western blotting of Chi-1 probed with antiserum produced in mice (1); non-immune serum (2) and mAbs anti-His (3).

Reactivity of Chi-1 recombinant protein with confirmed (MAT+) and non-confirmed (MAT-) leptospirosis serum samples



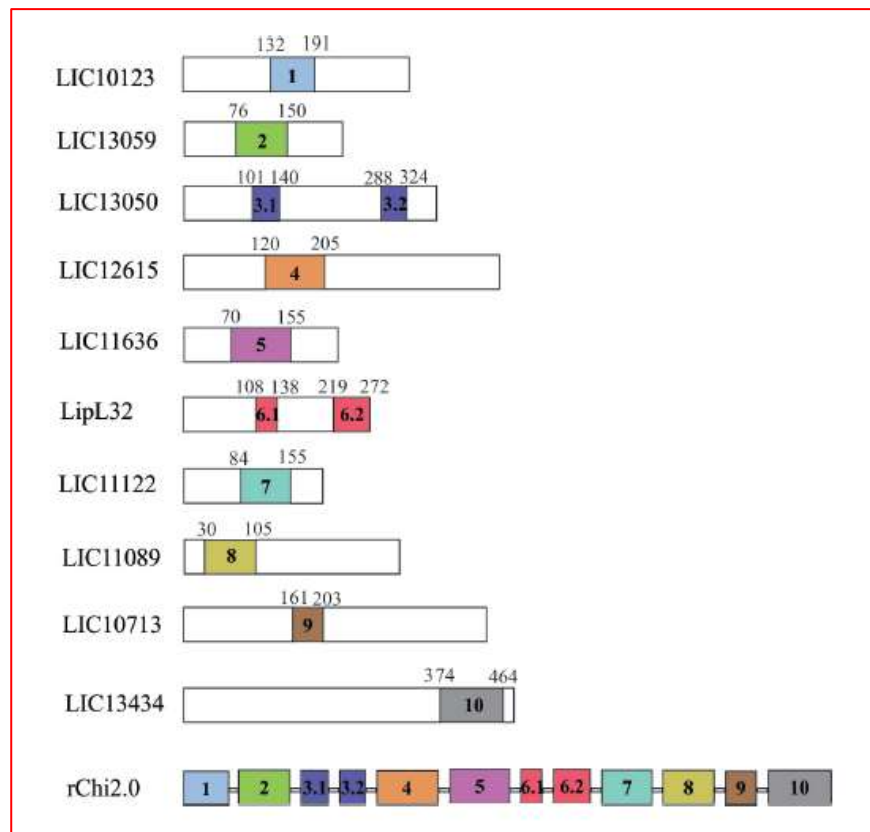
Evaluation of protective immunity of chimeric protein-1 (Chi-1) against lethal infection in the hamster model of leptospirosis.



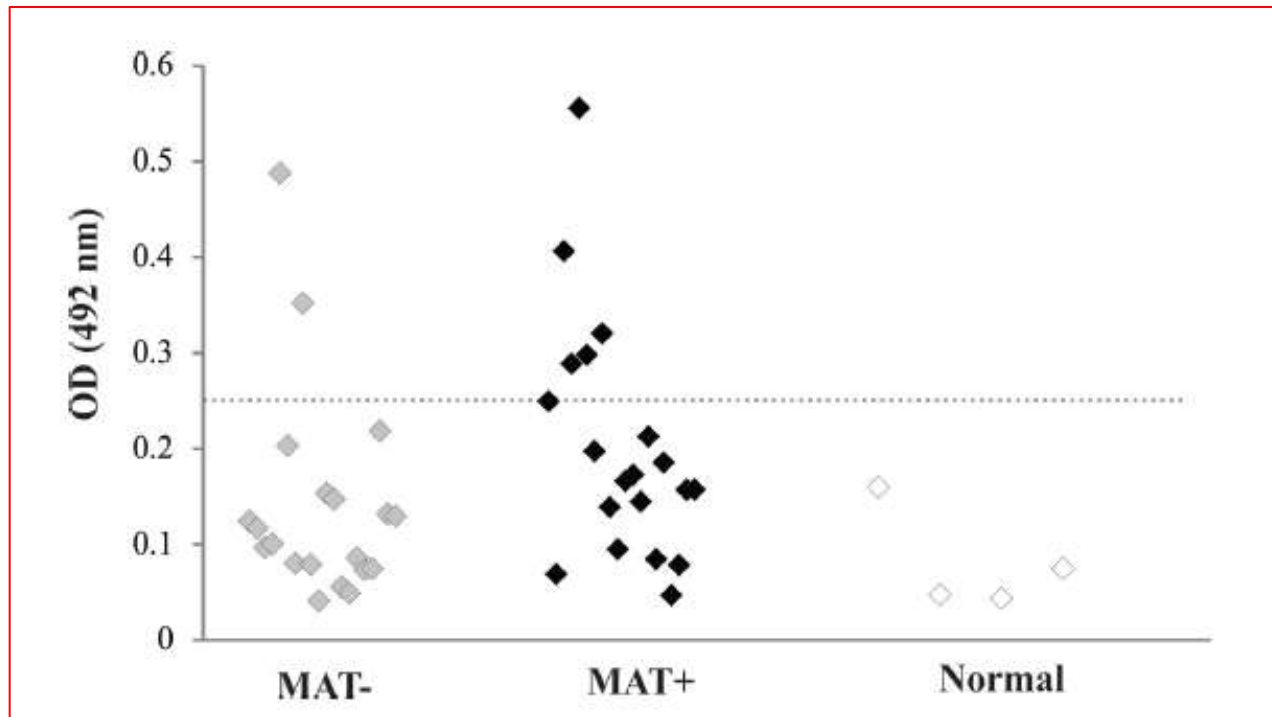
Groups	Experiment 1		Experiment 2	
	% de protection (n° surviving/total)	Culture (negative/total)	% de protection (n° surviving/total)	Culture (negative/total)
PBS + Alum	0 (6/6)	-	20 (2/10)	0/2
killed whole leptospire	100 (6/6)	6/6	100 (10/10)	10/10
rCh+Alum	50 (3/6)	2/3	33 (3/9)	1/3
rCh+MPLA	50 (3/6)	3/3	60 (6/10)	1/6

Construction of a chimeric protein based on B and T cell epitopes and known protective domains

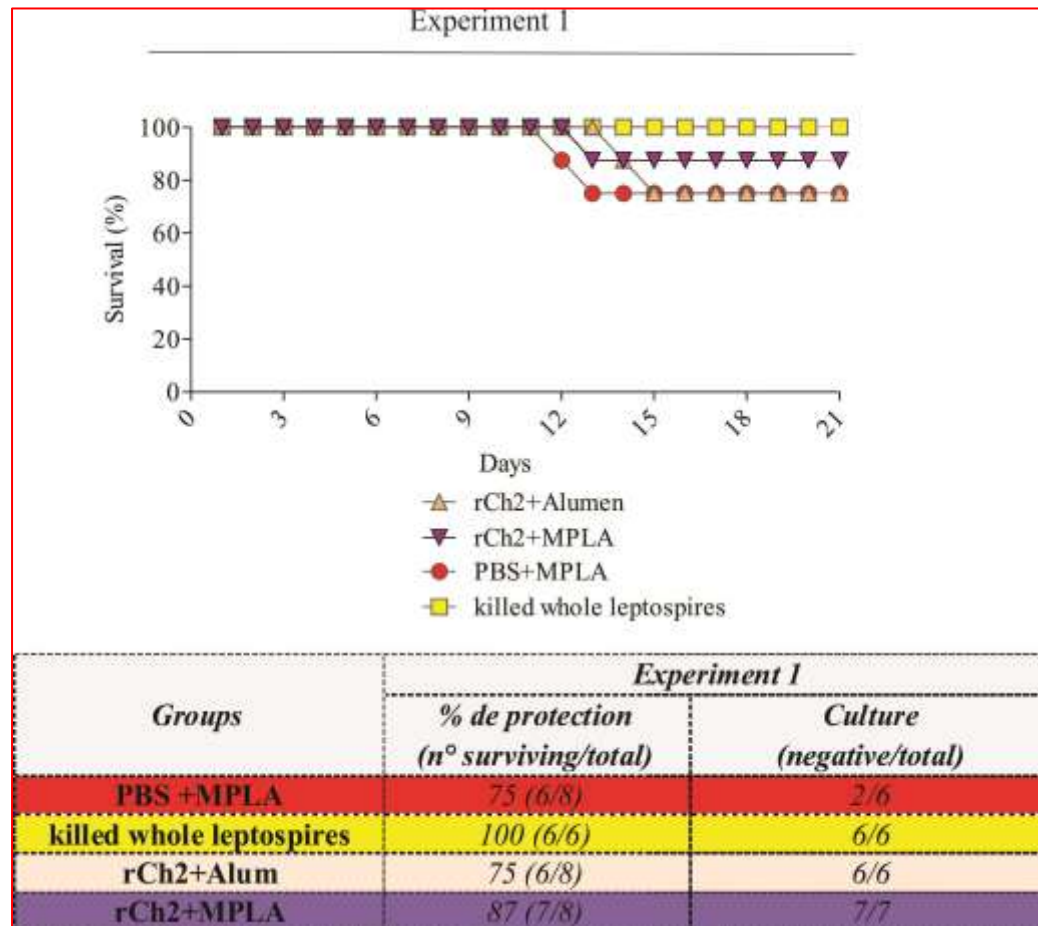
Chimeric protein-2 (Chi-2)



Reactivity of Chi-2 recombinant protein with confirmed (MAT+) and non-confirmed (MAT-) leptospirosis serum samples



Evaluation of protective immunity of chimeric protein-2 (Chi-2) against lethal infection in the hamster model of leptospirosis.



CONCLUDING REMARKS

- We have identified several ECM-binding proteins that are novel putative adhesins of *Leptospira*;
- We have established the generation of PLA on the surface of *Leptospira* spp. using the host urokinases;
- Leptospire have a redundant repertoire of adhesion molecules and PLG-binding proteins that are probably part of their invasion strategies ;
- The data obtained with PLG/PLA system associated to *Leptospira* triggering a cascade of events that enhance the proteolytic power of the bacteria, suggest a mechanism that the bacteria may employ to invade/penetrate the cells.
- The construction of chimeric proteins based on B/T lymphocyte epitopes or known immunoprotective regions is a promising strategy to achieve vaccine candidates;

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