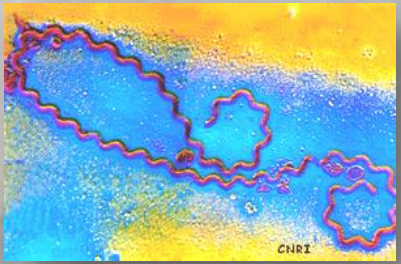
First Scientific Day

Of Institut Pasteur in New Caledonia

21st November 2013



Contribution of the experimental *in vivo* models in the comprehension of the leptospirosis pathophysiology

Apports des modèles expérimentaux *in vivo* à la compréhension de la physiopathologie de la leptospirose

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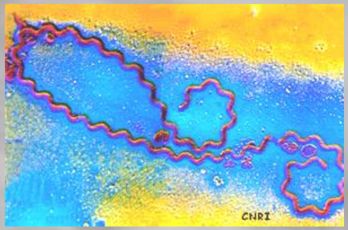
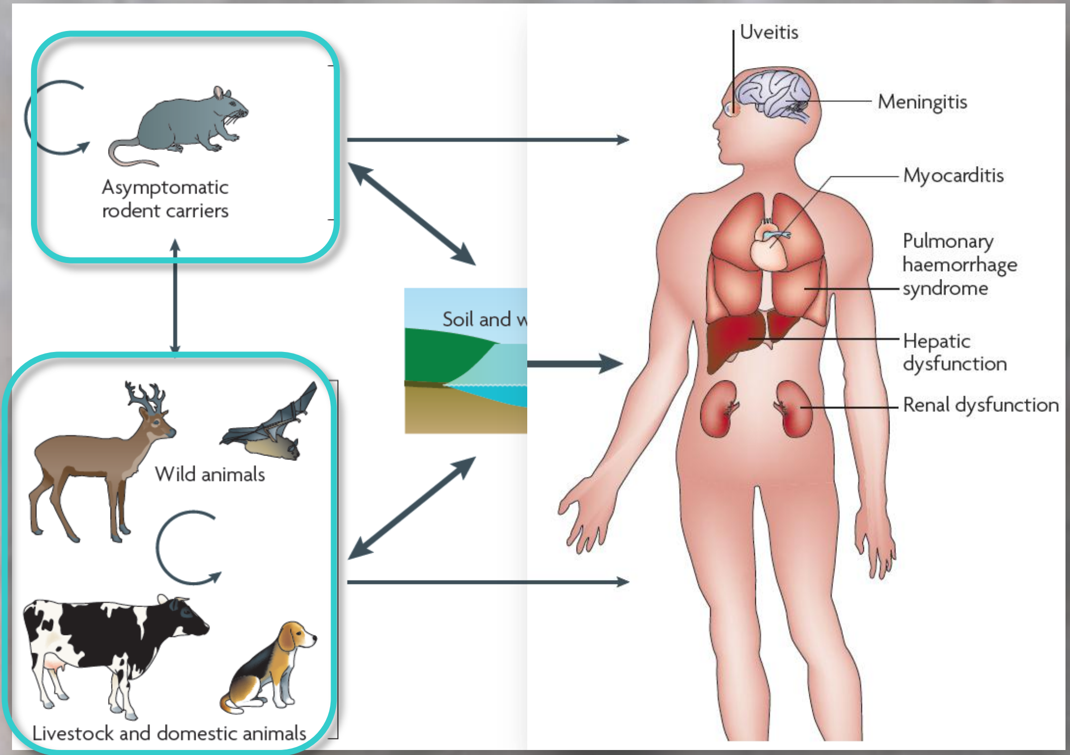


Leptospirosis, a complex zoonosis

Ko, Goarant & Picardeau, Nat Rev Microbiol 2009



Polymorphism in human

+/- symptomatic

Flu-like symptoms

Weil's syndrome (10%†)

Pulmonary hemorrhages SPHS (50%†)

Asymptomatic reservoirs

Especially rodents

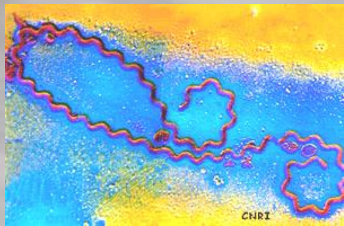
Rats and mice

Other (+/- susceptible) mammals

Domestic and wild animals

Depending on infecting strain

***in vivo* experimental models used for the comprehension of the leptospirosis pathophysiology**



Rats and mice: chronic carriers of *Leptospira*

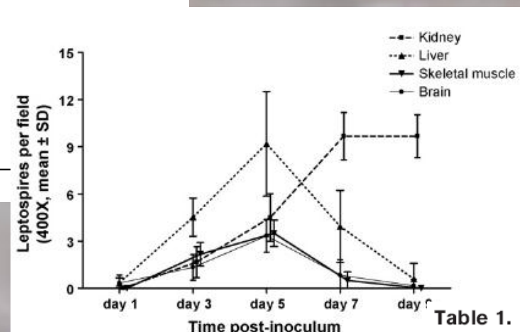
Athanazio *et al.*, Act Trop 2008

Renal colonization of *R. norvegicus* by *L. interrogans* Copenhageni Fiocruz L1-130

Time post-infection	Renal colonization/total (%) Renal/urine culture
1 Week	7/8[a] (88)
2 Weeks	8/8 (100)
3 Weeks	8/8 (100)
4 Weeks	19/21[a] (90)
2 Months	3/7[a] (43)
3 Months	5/7 (71)
4 Months	6/7 (86)

[a] One non-positive culture due to contamination.

Rattus norvegicus



Tucundiva de Faria *et al.*, J Comp Path 2007

Table 1
Histopathological changes in the kidneys of rats experimentally infected with *L. interrogans* serovar Copenhageni strain L1-130

Time post-infection	*Interstitial nephritis (n = (%))*
1 week	1/8 (12.5)
2 weeks	0/8 (0)
3 weeks	0/8 (0)
4 weeks	7/21 (33.3)
2 months	5/7 (71.4)
3 months	5/7 (71.4)
4 months	1/7 (14.3)

Santos *et al.*, J Med Microbiol 2010

Table 1. Presence of nephritis, leptospiral count and MAT titres in mice at 28 days p.i.

QD, Quartile deviation; ID, infective dose.

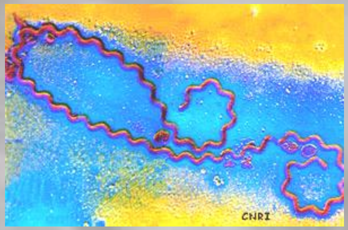
Mouse strain	% Nephritis (no./total)*			Leptospiral count (mean ± SD)	
	Control	Low ID	High ID	Low ID	High ID
A	0 (0/20)	66.7 (10/15)†	40.0 (8/20)†	20 ± 16	26 ± 21
CBA	0 (0/20)	78.9 (15/19)†	60.0 (12/20)†	10 ± 11	10 ± 10
BALB/c	0 (0/19)	0 (0/20)	0 (0/20)	5 ± 9	5 ± 7
C57BL/6	0 (0/20)	90.0 (27/30)†	92.0 (23/25)†	19 ± 27	15 ± 16

Detection and lesions in kidneys

Related to interstitial nephritis

Variability depending on mouse strain

Resistant and asymptomatic animals: not useful to study the severe symptoms of leptospirosis…



Susceptible models of leptospirosis

Mesocricetus auratus (†)

Haake, Curr Protoc Microbiol 2006

Golden Syrian hamster

Peritoneal injection

Restoring virulence (*Leptospira* culture)

Characterization of strain infectivity

Lethal Dose 50% (LD_{50}) and 100% (LD_{100})

Evaluation of potential vaccines

Examining pathology

Disadvantages

Genome not sequence

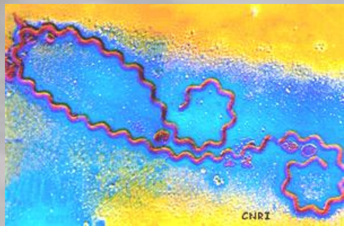
No serological kit available

Cavia porcellus (†)

Also guinea pigs and gerbils

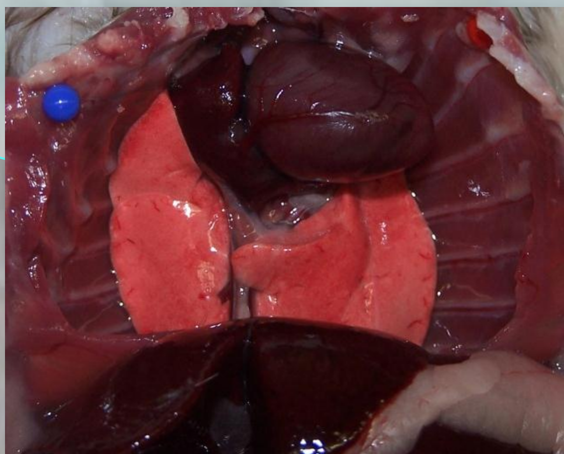
Strain virulence and vaccine efficacy

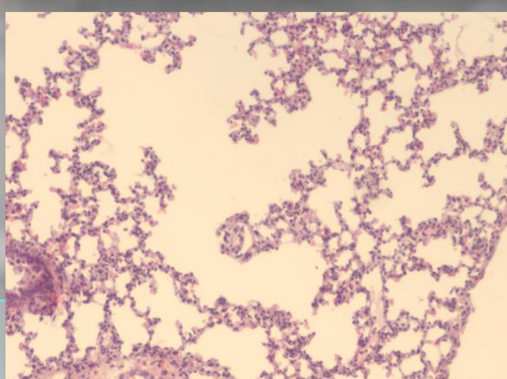
Infected hamsters used to reproduce severe lesions observed in human cases (multiple organ failures)



Hamster (1/3): pulmonary failures

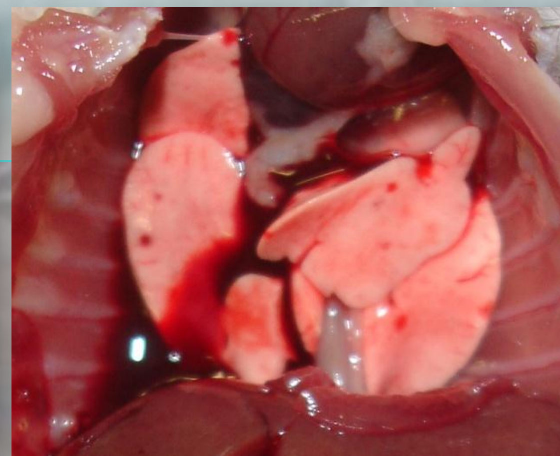
Control hamster

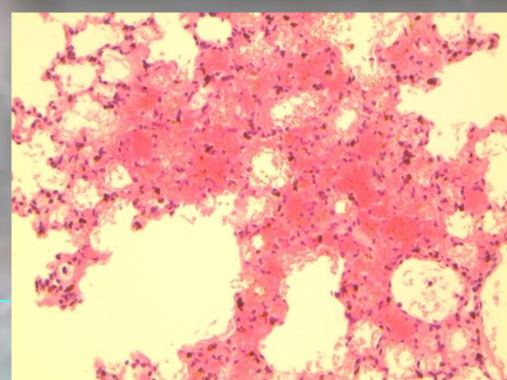




Normal alveolar architecture
(HES X200)

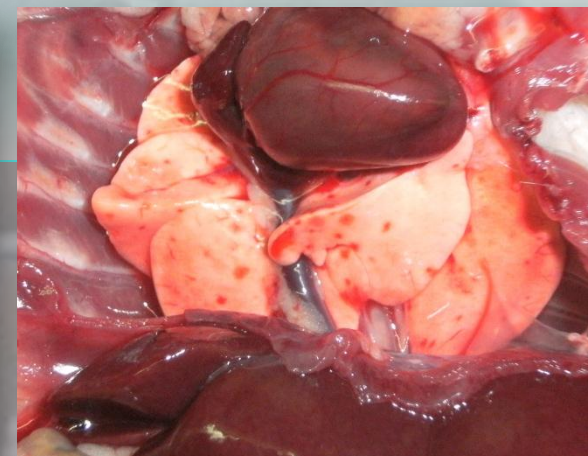
Infected hamster (day 2 pi)

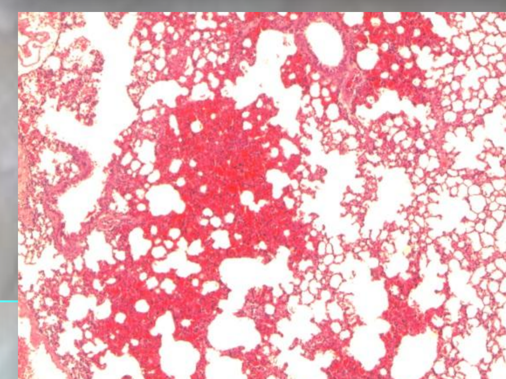




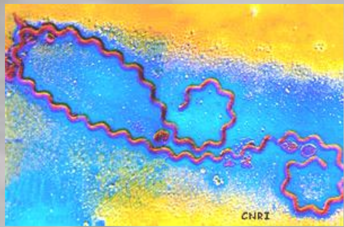
Edema and hemorrhages
(HES X200)

Infected hamster (day 4.5 pi, †)



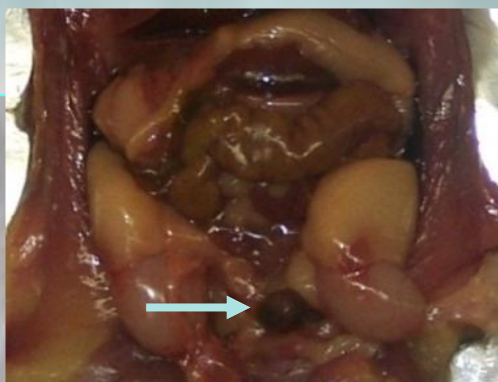


Large foci of hemorrhages
(HES X100)

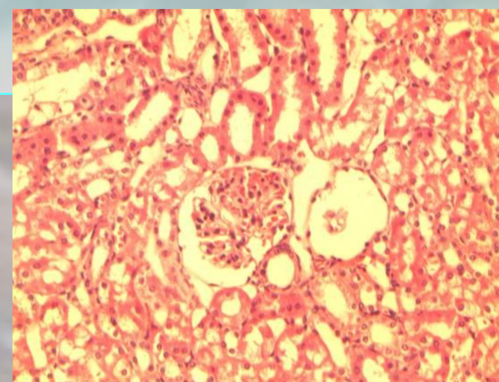


Hamster (2/3): renal and hepatic dysfunction

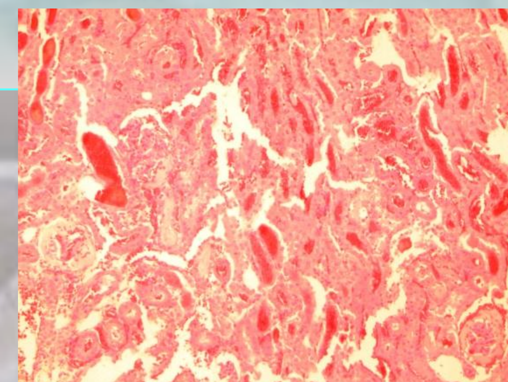
Icteric tissue and blood in the bladder (arrow)
(infected, day 4 pi)

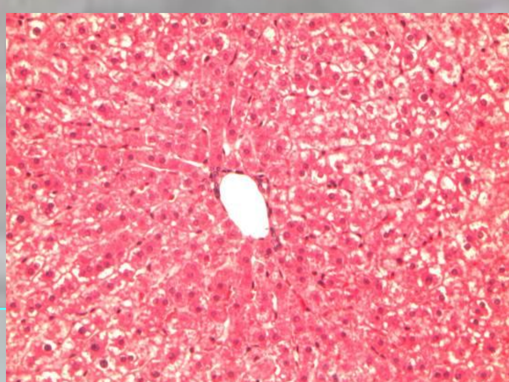


Normal glomeruli and renal tubules
(control, HES X200)

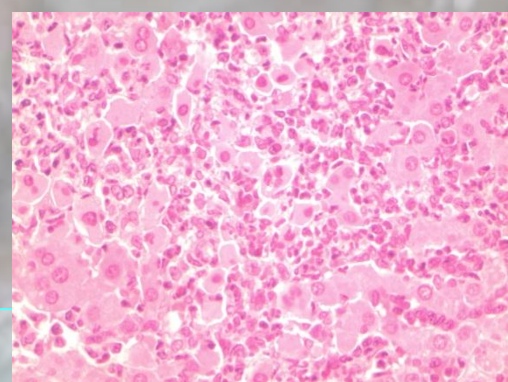


Hemorrhage in renal tubules
(day 4.5 pi, †, HES X100)

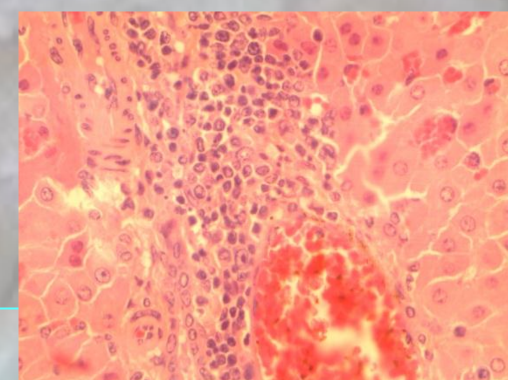




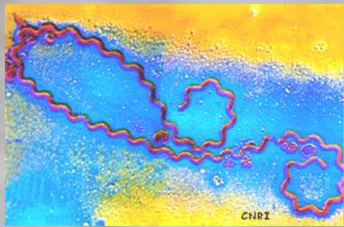
Control hamster
Normal hepatic strutures
(HES X200)



Infected hamster (day 2 pi)
Necrotic hepatocytes
(HES X400)



Infected hamster (day 4.5 pi,†)
Inflammation of portal vein
(HES X400)



Hamster (3/3): charaterization of virulence

Several Brazilian isolates

(I) To test isolate virulence

(II) To standardize LD_{50}

Variability of virulence (lethality for 5/7 isolates)

Highly virulent strains (< 200 *Leptospira*)

LD_{50} of virulent *Leptospira* strai

Species	Strain	Mean LD_{50} (±S.D.) F	Mean LD_{50} (±S.D.) M
L. interrogans	L1-130	105 (44.8)	36.7 (16.6)
L. interrogans	Kito	2.8 (0.54)	2.5 (1.29)
L. noguchii	Cascata	33.9 (15.9)	57.2 (32.4)
L. noguchii	Hook	115.4 (56.5)	18.4 (8.71)
L. noguchii	Bonito	2.7 (0.8)	3.3 (2.0)

S.D. = Standard deviation; F = Fe

Silva *et al.*, Vaccine 2008

Zuerner *et al.*, Vet Path 2012

Table 1. Microscopic Analysis of Steiner-Steiner-Stained Sections: Tissue Distribution of Serovar Hardjo Strains

Section	Strain 203[a]	Strain JB197[b]
Bladder	– (Urine only)	+
Brain	+ (Inconsistent)	+
Heart	–	+
Intestine	No data	+
Liver	+ (Rare)	+
Lung	–	+
Kidney	+	+
Pancreas	+ (Inconsistent)	+
Spleen	+	+
Uterus	–	+ (Inconsistent)

Detection in hamster organs

Two different Hardjo strains

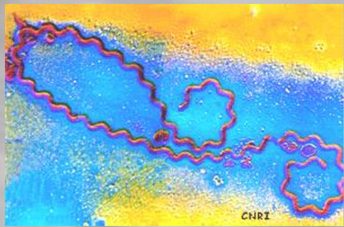
Chronic carriage for one strain

≠ strains used in laboratory

Verdun (LD_{50} = 1.10^8) *vs.* Fiocruz (LD_{50} <200)

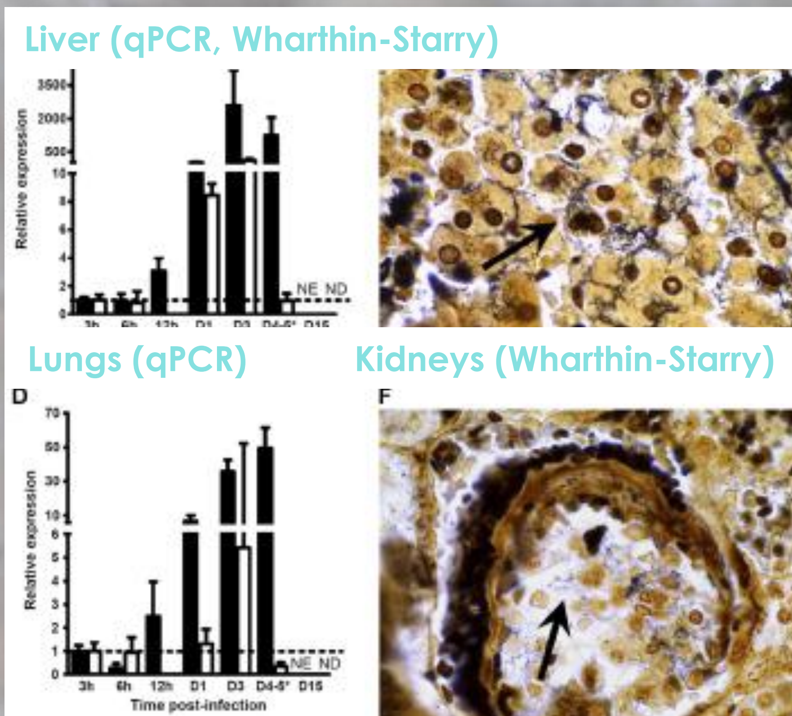
Characterization is primordial

Disparity of virulence (LD_{50}) but also variability in tissue lesions and colonization depending on strains



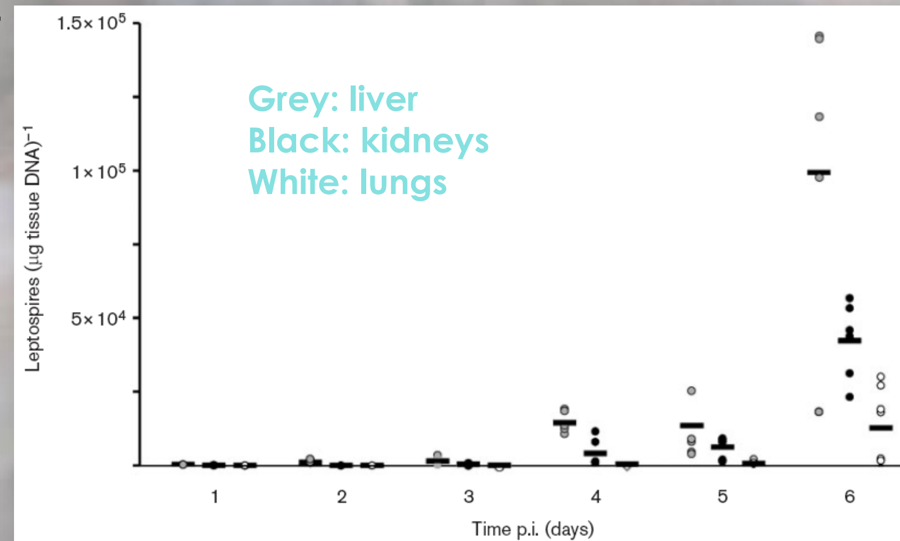
Pathogen dissemination and bacterial load in susceptible animal models

Detection in hamster (†) organs (full bars)



Matsui *et al.*, Infect Immun 2011

Lourdault *et al.*, J Med Microbiol 2009



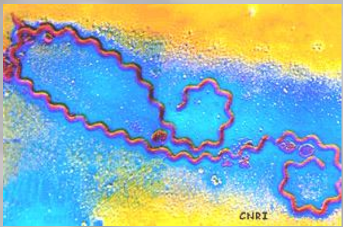
Bacterial load in guinea pig (†) organs

Kinetic (qPCR) – Fiocruz strain

Low level of bacteria load in lungs

Difference in bacterial load depending on organs

Pathogenesis in organs failures?



Pathogenesis during acute infection in guinea pigs

Guinea pig (†) lungs

Ig and C3 complement deposit

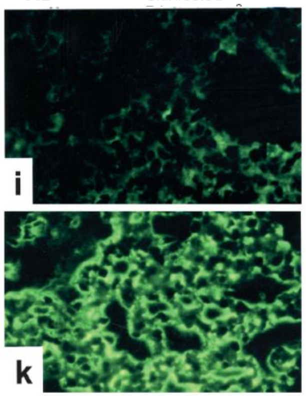


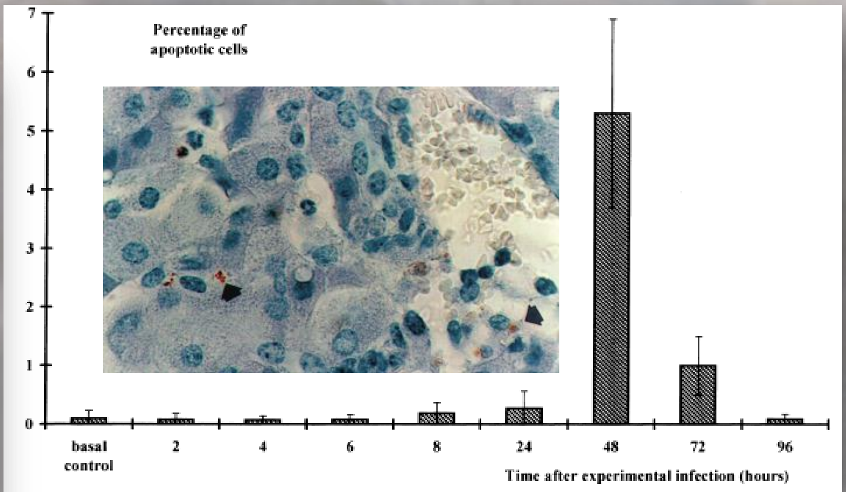
Table 2. Distribution of Immunofluorescent Patterns in Lung Tissues of Guinea Pigs Infected with 10^3, 10^5, or 10^7 of RJ15958 o RJ16441

Guinea pig no.	Strain, dose	Day of sacrifice	IF IgM	IF IgG	IF IgA	IF C3
380	Uninfected	7	–	–	–	–
381	Uninfected	10	–	–	–	–
358	Uninfected	11	–	–	–	–
362	Uninfected	11	–	–	–	–
		10	AS + L	AS	AS + L	–
		10	AS	–	–	A
		10	AS + L	AS + L	–	–
		7	AS + L	AS + L	AS + L	AS + L
		7	AS	AS	AS	AS
		7	AS	AS	AS	–
		6	AS	AS	AS	AS
		7	AS	AS	AS	AS
		7	AS	AS	AS	–
		7	AS	AS	AS	AS
		7	–	–	–	–
		6	AS + L	AS	–	AS + L
		7	–	–	–	–
		6	A	A	A	A
		7	AS	AS	AS	AS
		7	AS	–	–	–
		6	AS	AS	AS	AS
		5	–	AS	AS	–
		7	AS	AS	AS	AS
		6	AS + L	–	AS + L	–
		4	AS + L	AS + L	–	AS
		4	–	–	–	–

Three staining patterns were observed for IgM, IgG, IgA, and C3 as shown in Figure 5 including: staining along the alveolar septum (AS) addition to linear staining along the alveolar septum, there was more amorphous, faint intra-alveolar staining adjacent to the alveolar surface, indica of periseptal leak (AS + L); and intra-alveolar amorphous material filling alveolar spaces (A).

Nally *et al.*, Am J Pathol 2004

Merien *et al.*, FEMS Microbiol Lett 1998

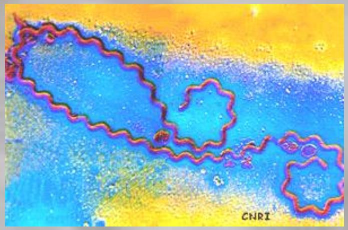


Guinea pig (†) liver

Hepatocyte apoptosis

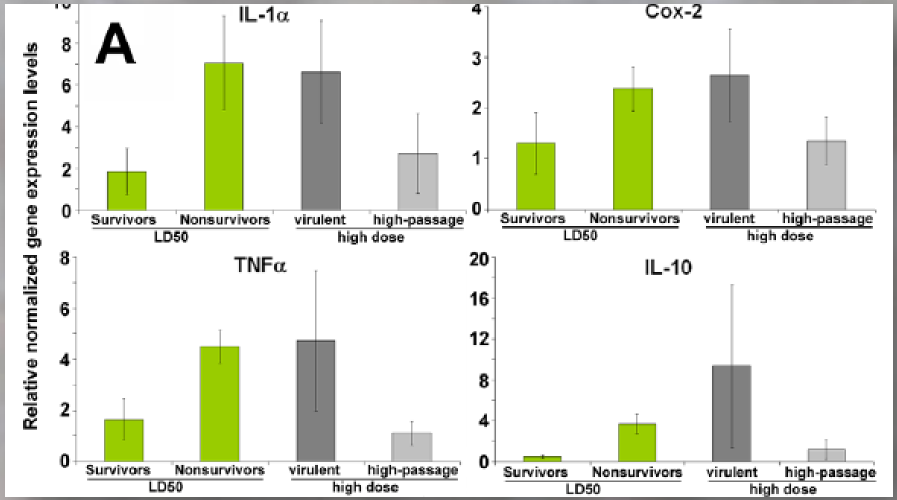
Pic of apoptosis at 48h pi

Various mechanisms involved in the pathogenesis in susceptible hosts: role of immune system?



Comparative studies between models: cytokine regulation

Vernel-Pauillac & Goarant, Plos NTD 2010



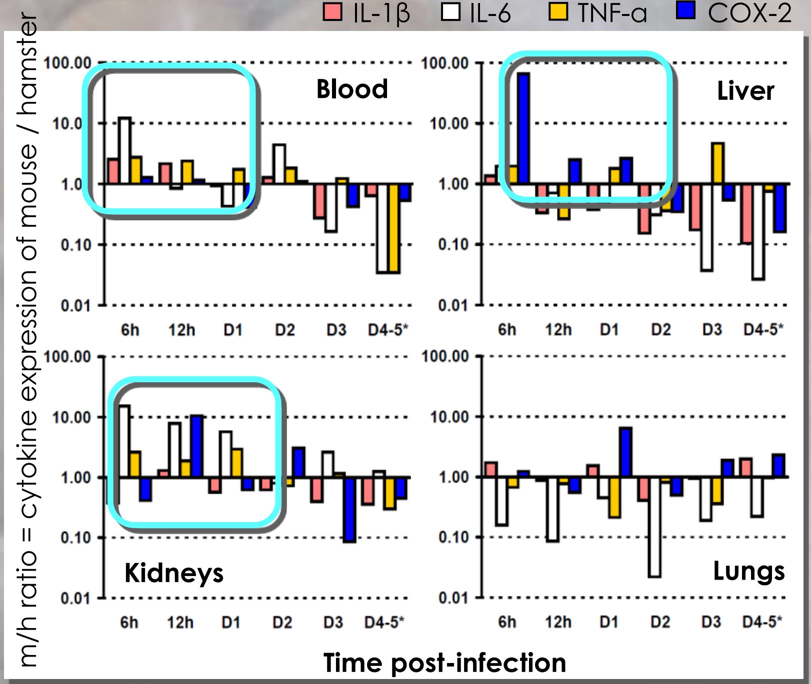
Cytokine expression in hamster blood (Day 3 pi)

Survival vs. lethality

Lower expression level for survival

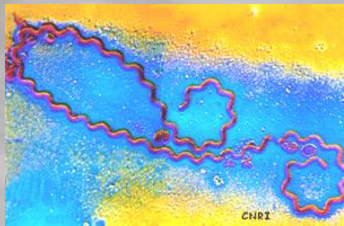
Hamster (†) vs. mouse

Early regulation of pro-inflammatory response in mice



Matsui *et al.*, Infect Immun 2011

Trouble in the cytokine balance: maintained in hamster (†) while rapidly restored in mouse or survival



Use of genetically modified mouse models

Athanazio *et al.*, Act Tropica 2008

Table 2
Inflammatory lesions in infected animals at 28-day after infection in combined analysis of lower and higher inocula

Mouse strain	Nephritis ++	Nephritis +	None
C57BL/6 TNFR-KO	7/9	1/9	1/9
C57BL/6 IFN-KO	1/8	2/8	5/8
C57BL/6 wild-type	2/15	8/15	5/15
BALB/c IL4-KO			4/4
BALB/c wild-type			4/4

<u>Mouse deficient in cytokine (IL-4, IFNγ) or receptor (TNF-a)</u>

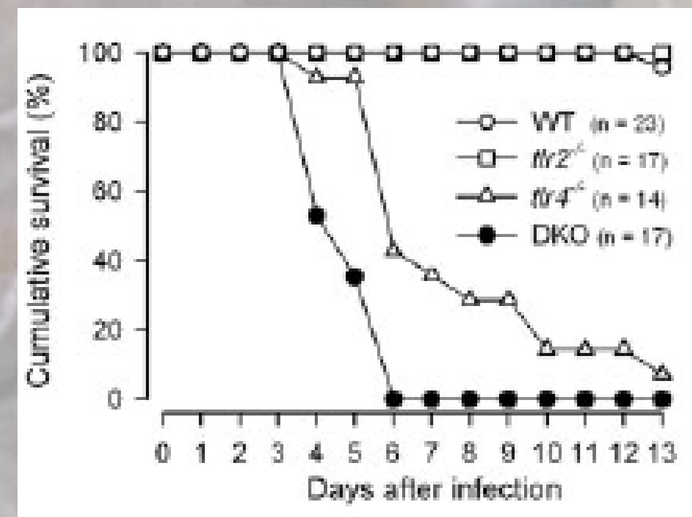
Survival but ↑ lesions for TNFR-KO mouse

Importance of TNF-a receptor in the early control of infection

<u>Mouse deficient in Toll-like receptor (TLR)</u>

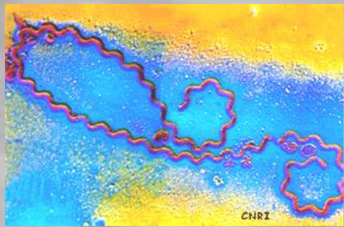
Lethality in TLR4 KO mouse and DKO

<u>TLR2 and TLR4 important in the survival</u>



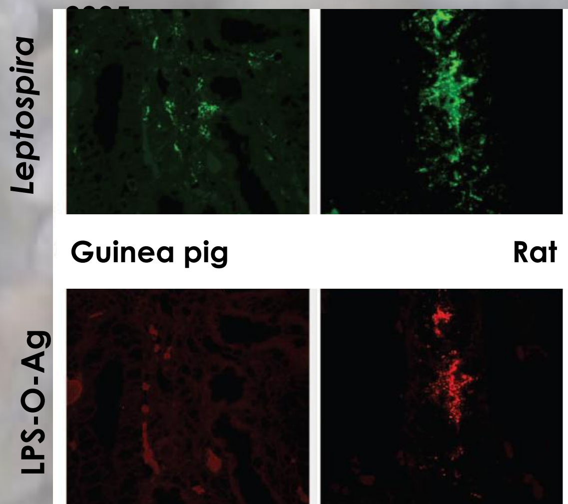
Chassin *et al.*, J Immunol 2009

Contribution of these models in the discover of atypical *Leptospira* recognition receptors (mouse TLR2/TLR4, human TLR2)



Pathogen adaptation *in vivo* depending on infected host

Nally *et al.*, Plos Infect Immun



Shift in the LPS-O-Ag production in kidneys

Guinea pig (†) *vs.* rat

Shift related to virulence in host

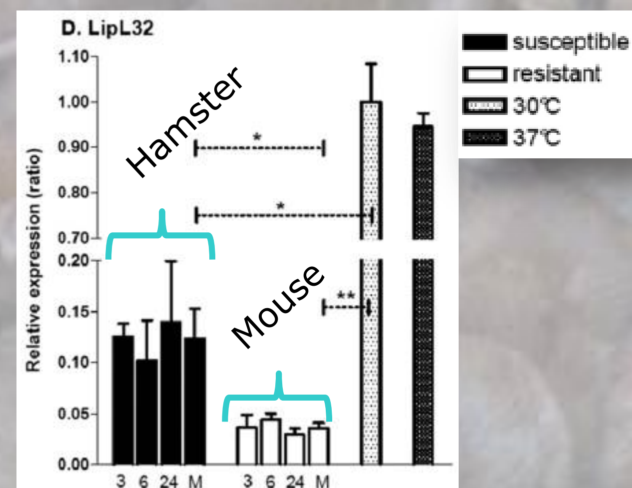
***Leptospira* gene expression in blood**

Hamster (†) *vs.* mouse

LipL32, major protein, **binding TLR2**

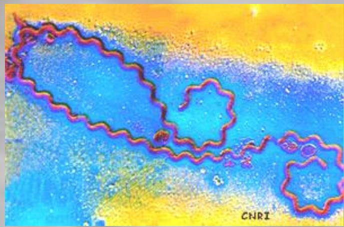
Lower expression level for mice

→ Evasion strategy from host immunity?



Matsui *et al.*, Appl Environ Microbiol 2012

Utility of *in vivo* models used in comparative study for a better comprehension of leptospirosis pathophysiology



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Thank you for your attention!