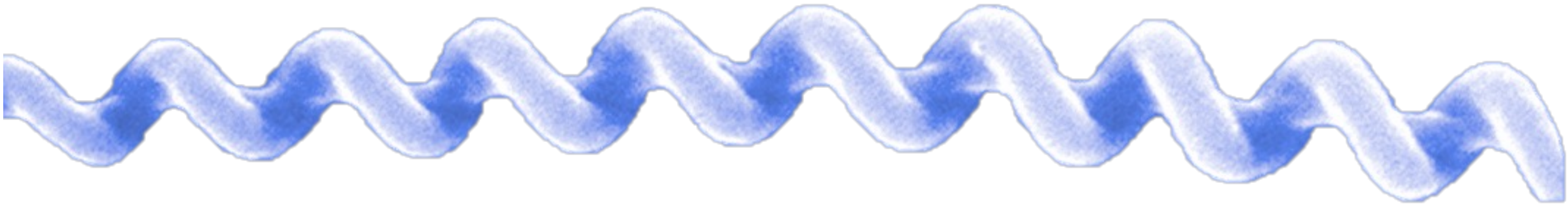
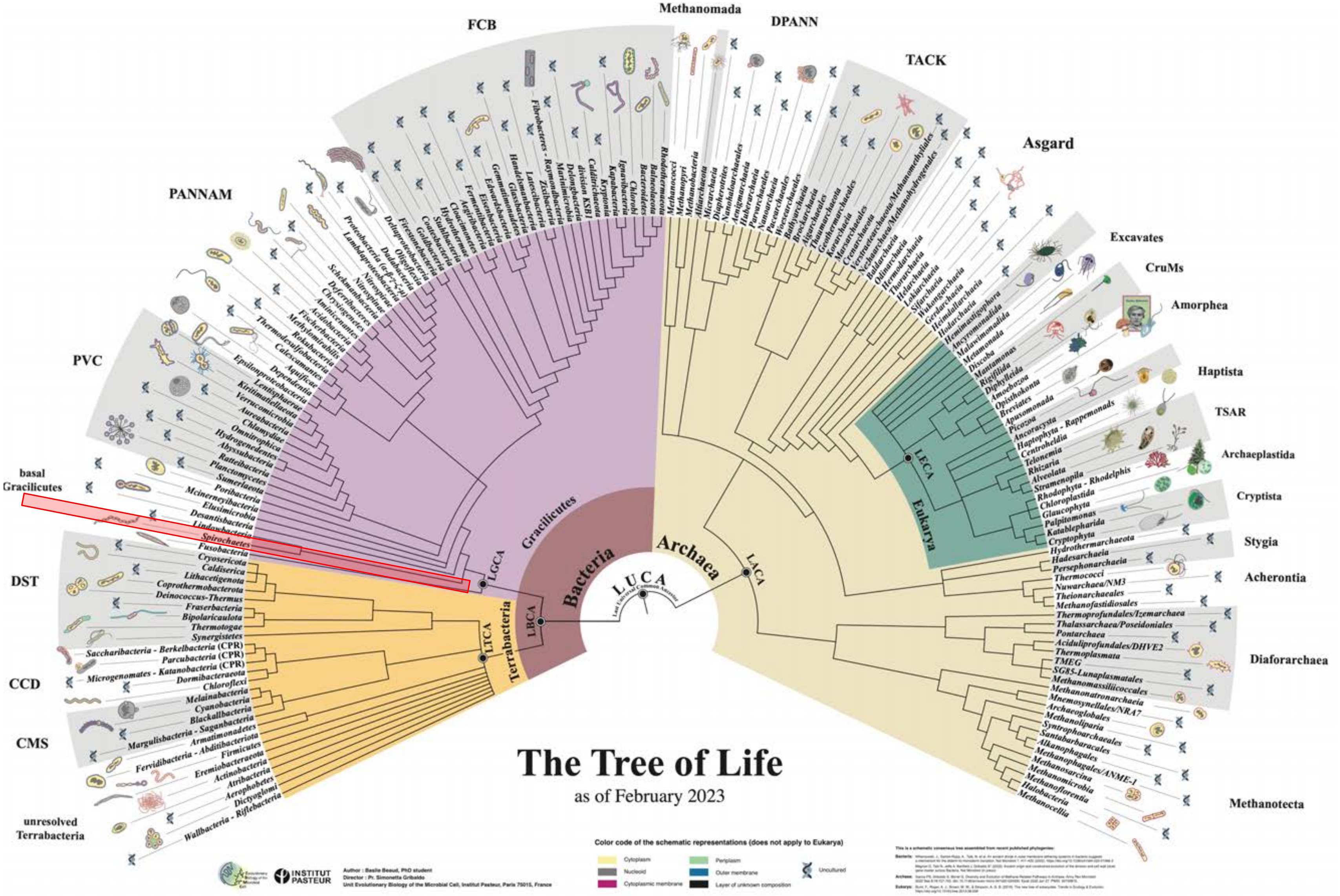
Spirochetes do it differently



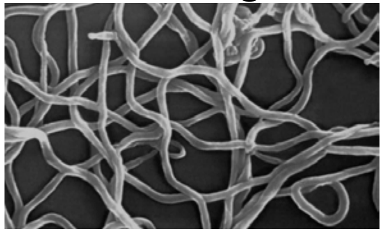
Mathieu Picardeau
Unité Biologie des Spirochètes
Centre National de Référence de la Leptospirose
Centre collaborateur OMS de référence et de recherche sur la Leptospirose
Pasteur International Unit « Leptospirosis Pasteur Network »

The phylum of *Spirochaetes*

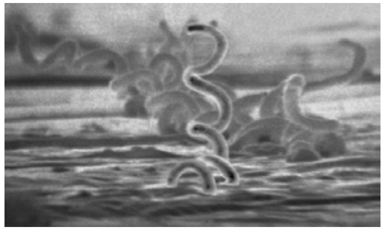


Spirochaetes, an ancestral phylum

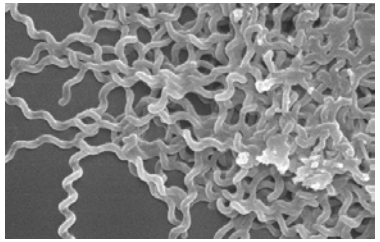
Borrelia burgdorferi

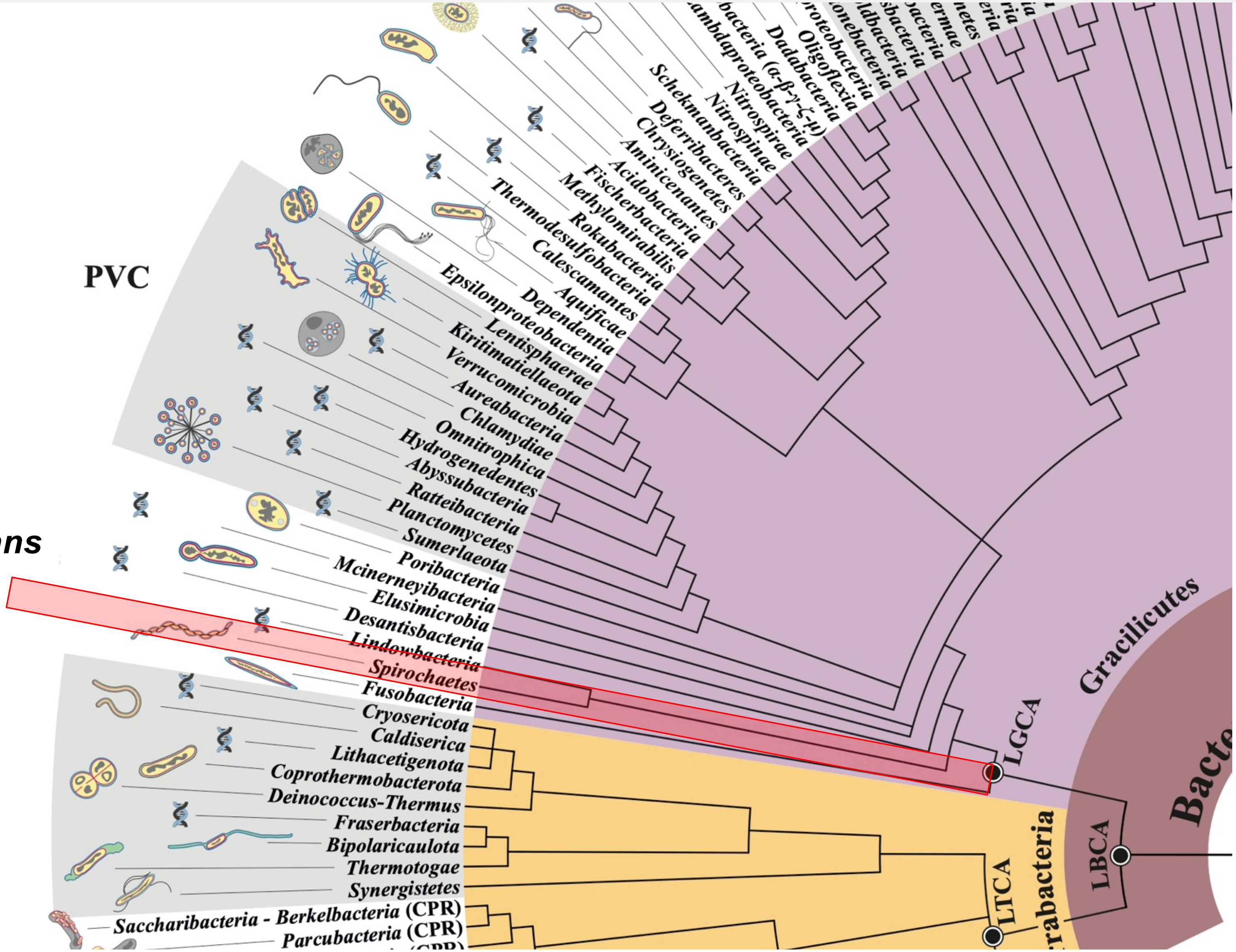


Treponema pallidum



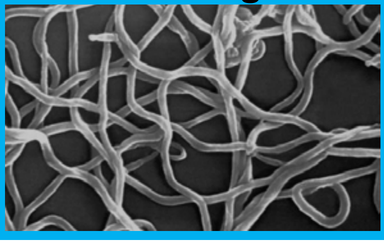
Leptospira interrogans





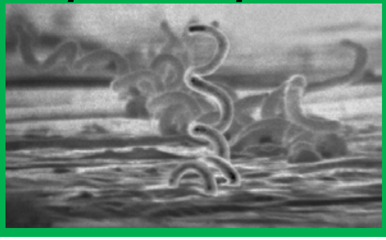
Spirochaetes are causing major animal and human diseases

Borrelia burgdorferi



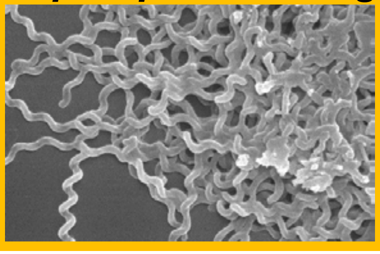
Lyme disease
300,000 cases/year in the US
90,000 cases/year in Europe
(>30,000 in France)
Fatality rate <1%

Treponema pallidum



Syphilis
6 million cases /year
(1000 cases/year in France)
Fatality rate <1%

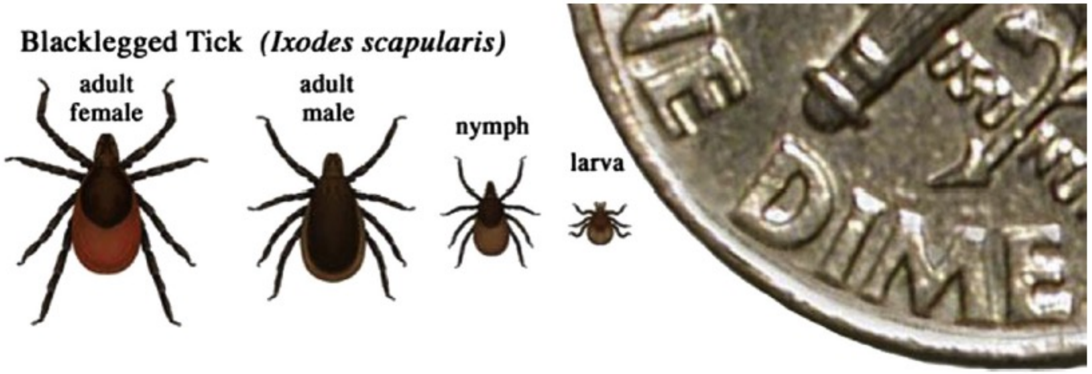
Leptospira interrogans



Leptospirosis
1 million cases/year
(700 cases in France)
Fatality rate >10%

Lyme disease

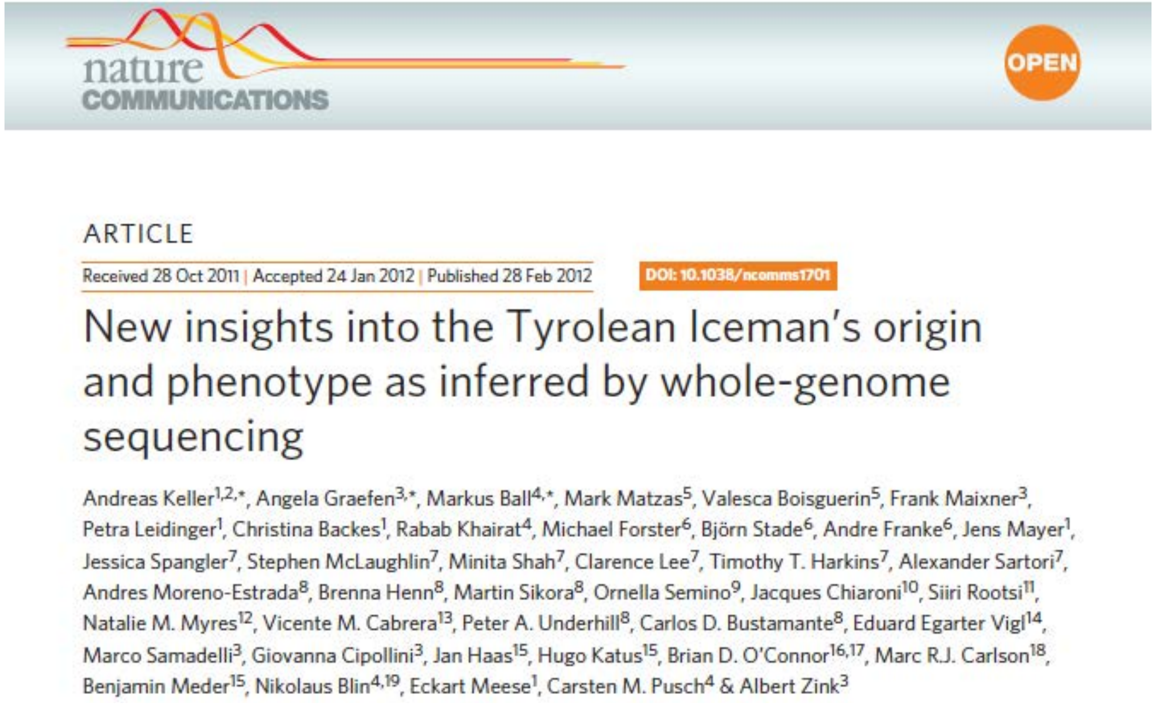
- First described in the 1970s (Old Lyme, Connecticut, USA)
- Causative agent described in 1982 (W. Burgdorfer)
- Vector-borne disease: arthropods
- Transmission by hard tick genus Ixodes
- Risk of human infections depends :
  - Prevalence of infected ticks : 3-30% in Europe
  - Tick life stage (nymph) and seasonality of tick activity
  - Duration of tick attachement (>24h)



Before/after blood meal

Reservoirs: small (mice, chipmunks, and shrews) and larger mammals (deers, squirrels, cattle, etc), birds, etc

Lyme Disease, A Prehistoric Illness?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ARTICLE

Received 28 Oct 2011 | Accepted 24 Jan 2012 | Published 28 Feb 2012

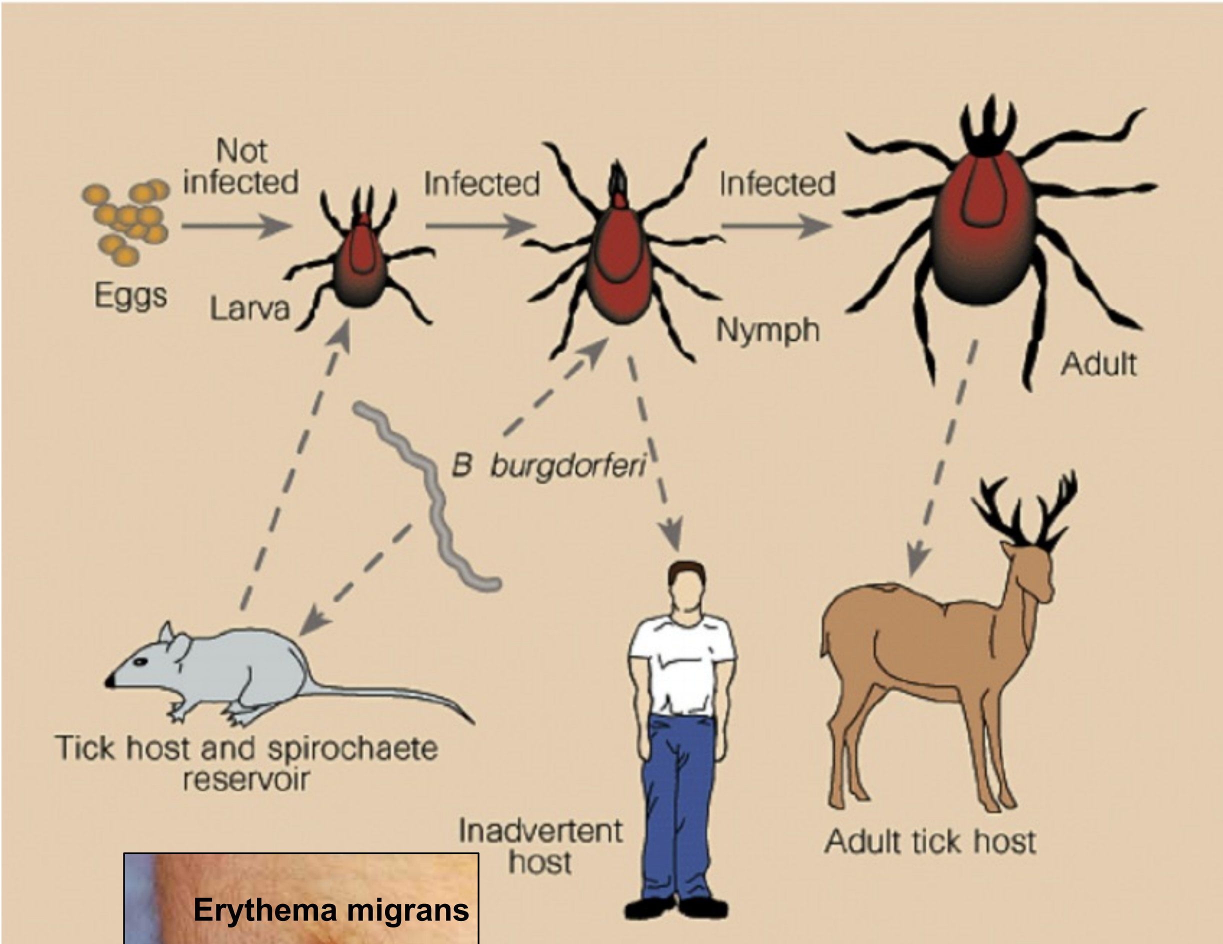
DOI: 10.1038/ncomms1701

New insights into the Tyrolean Iceman's origin and phenotype as inferred by whole-genome sequencing

Andreas Keller[1,2,*], Angela Graefen[3,*], Markus Ball[4,*], Mark Matzas[5], Valesca Boisguerin[5], Frank Maixner[3], Petra Leidinger[1], Christina Backes[1], Rabab Khairat[4], Michael Forster[6], Björn Stade[6], Andre Franke[6], Jens Mayer[1], Jessica Spangler[7], Stephen McLaughlin[7], Minita Shah[7], Clarence Lee[7], Timothy T. Harkins[7], Alexander Sartori[7], Andres Moreno-Estrada[8], Brenna Henn[8], Martin Sikora[8], Ornella Semino[9], Jacques Chiaroni[10], Siiri Rootsi[11], Natalie M. Myres[12], Vicente M. Cabrera[13], Peter A. Underhill[8], Carlos D. Bustamante[8], Eduard Egarter Vigl[14], Marco Samadelli[3], Giovanna Cipollini[3], Jan Haas[15], Hugo Katus[15], Brian D. O'Connor[16,17], Marc R.J. Carlson[18], Benjamin Meder[15], Nikolaus Blin[4,19], Eckart Meese[1], Carsten M. Pusch[4] & Albert Zink[3]

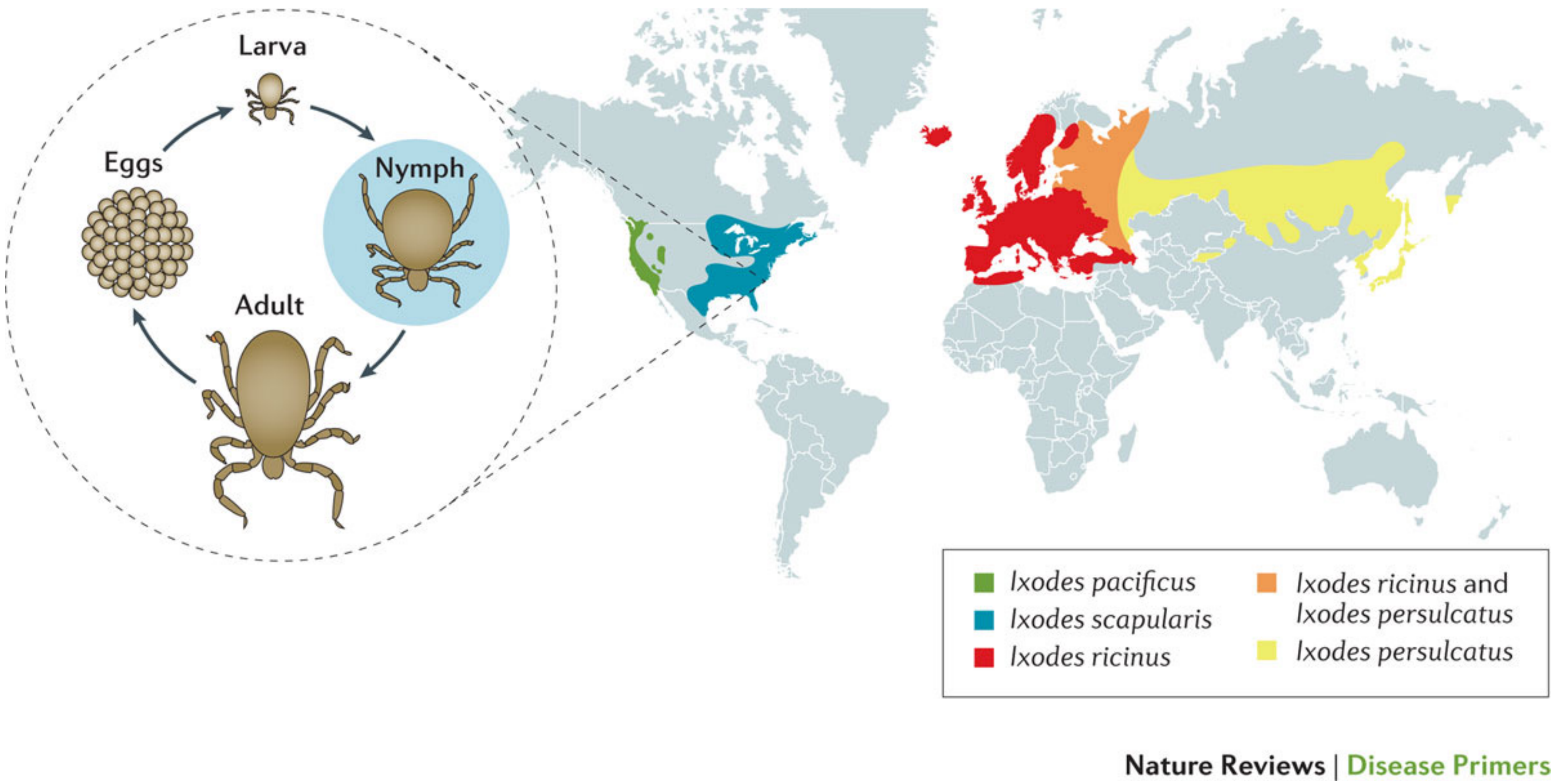
Sequences corresponding to ~60% of the genome of *Borrelia burgdorferi in a* 5,300-year-old age individual are indicative of the earliest human case of infection with the pathogen for Lyme borreliosis

Cycle of Lyme disease



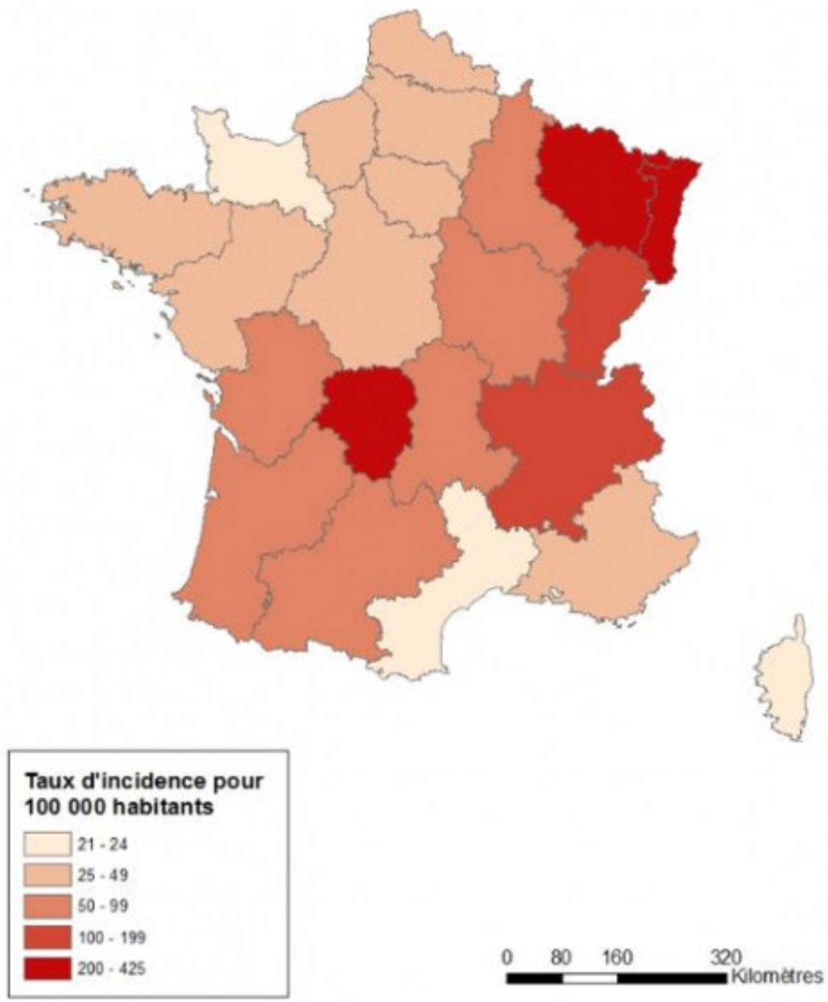
Barbour et al. Nature 1997

Distribution of ticks that transmit *Borrelia burgdorferi* s.l. to humans

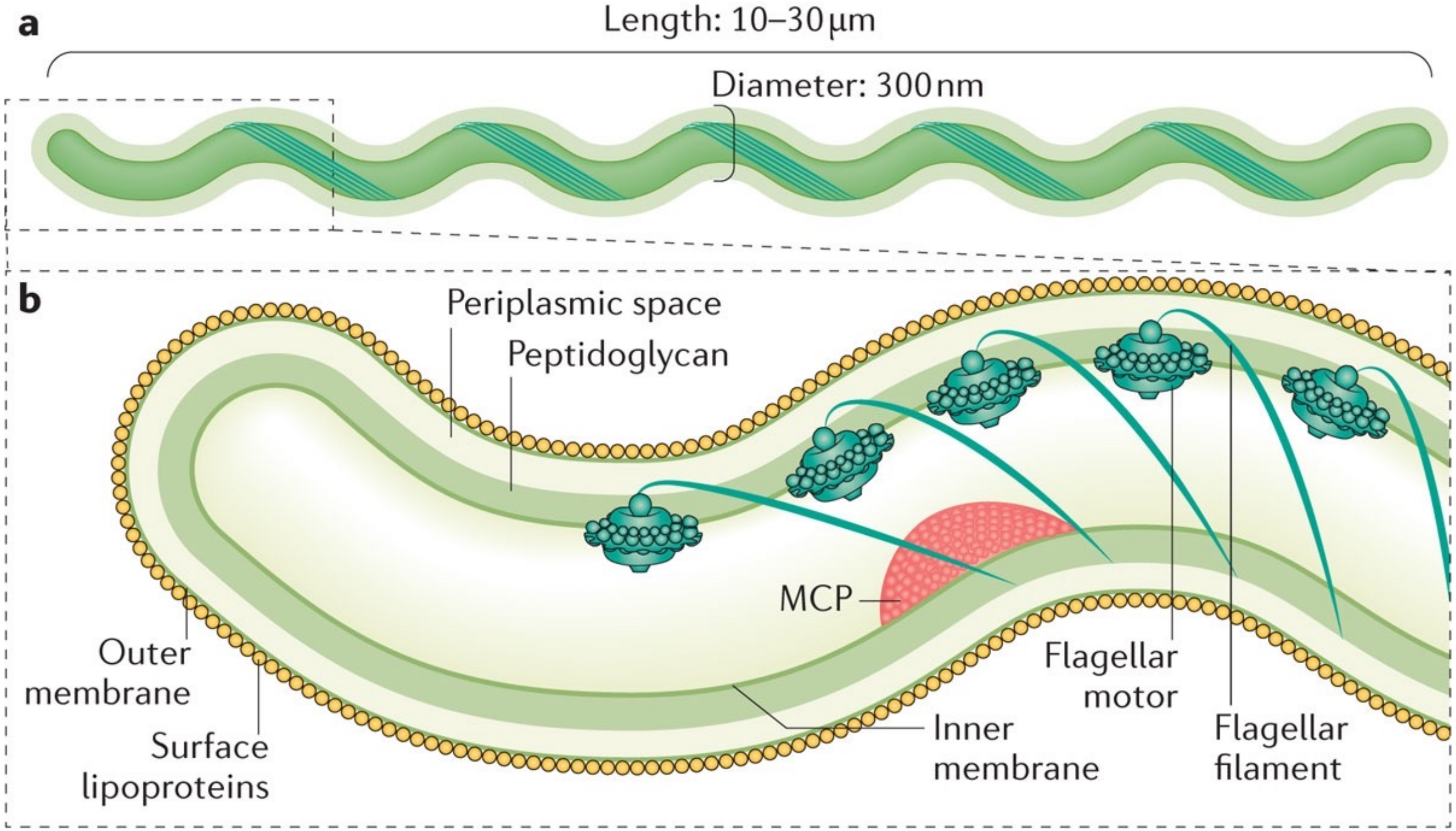


Steere, A. C. *et al.* (2016) Lyme borreliosis
Nat. Rev. Dis. Primers doi:10.1038/nrdp.2016.90

Estimation du taux d’incidence annuel moyen de la borréliose de Lyme par région, France métropolitaine, 2015 – 2019, Réseau Sentinelles



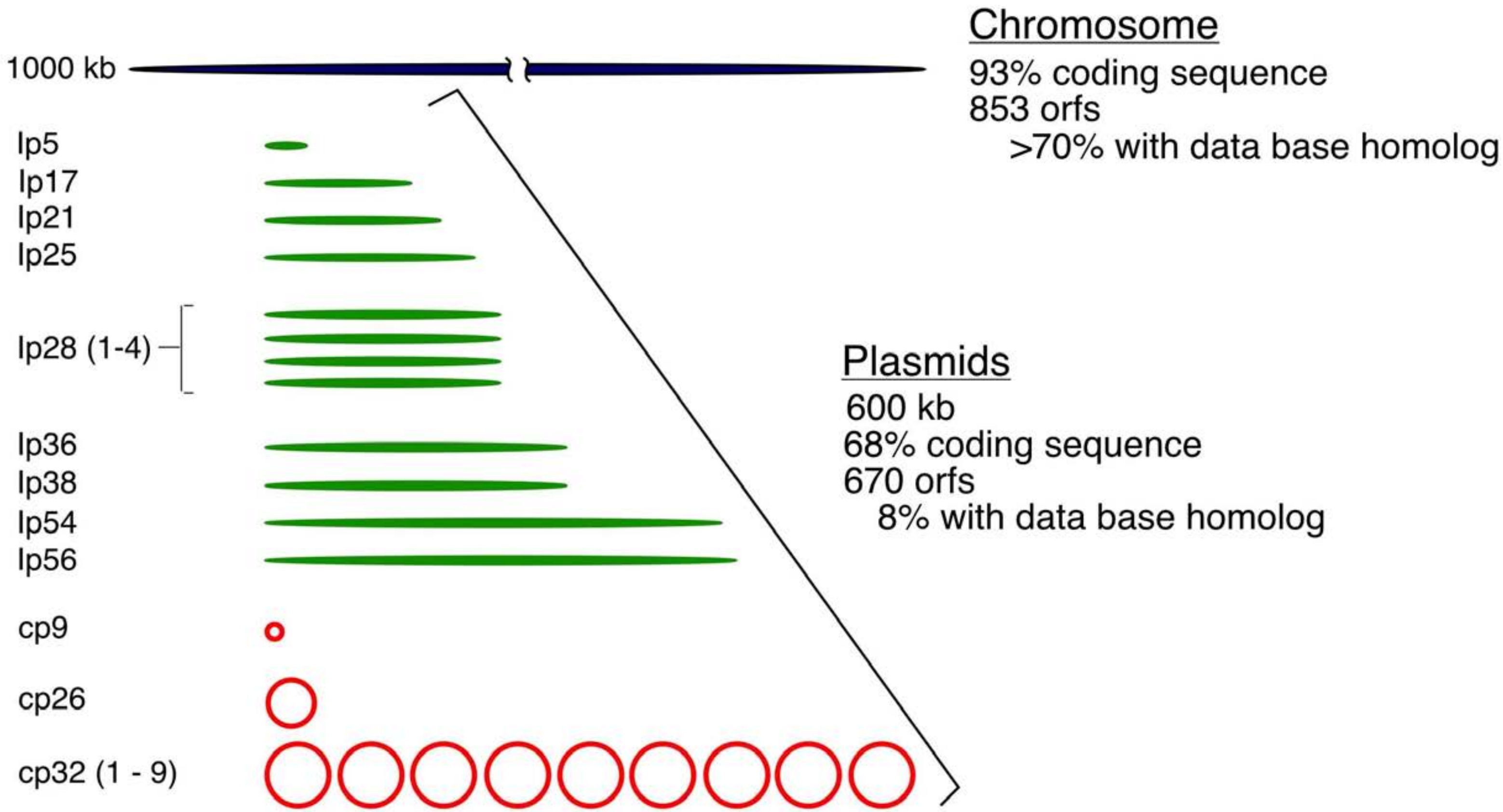
Morphology and cellular architecture of *Borrelia burgdorferi*



Steere, A. C. *et al.* (2016) Lyme borreliosis
Nat. Rev. Dis. Primers doi:10.1038/nrdp.2016.90

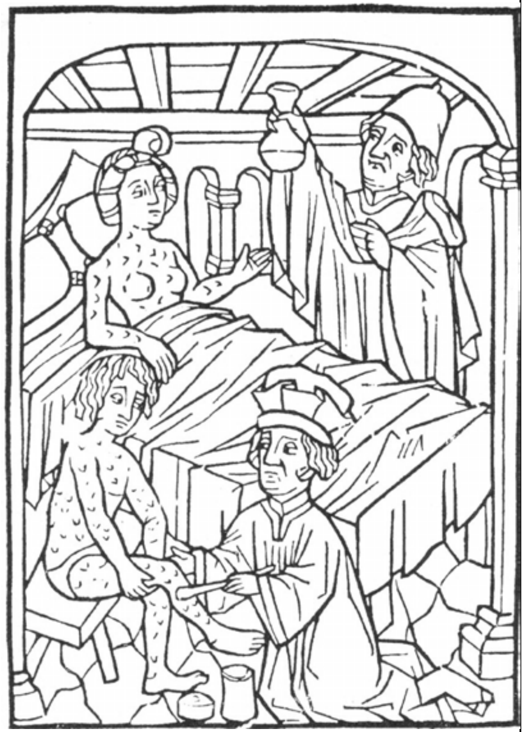
Genome of *Borrelia burgdorferi*

Multiple replicons
Linear replicons with covalently closed hairpin ends



Fraser et al, Nature, 1997 - Casjens et al, Molecular Microbiology, 2000

Treponema pallidum and syphilis



1494 - 1495

First epidemics in Europe

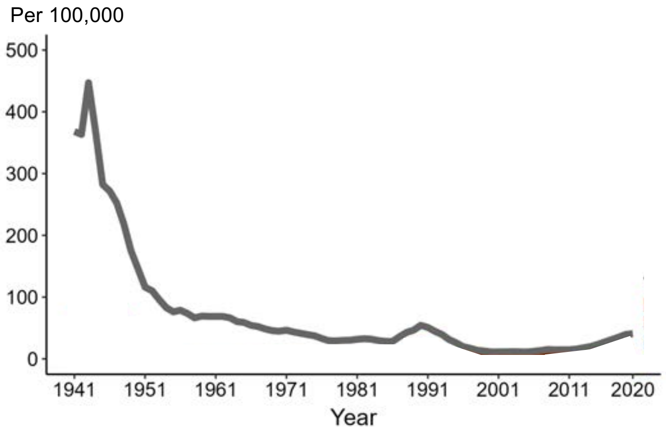
Imported from the New world (C. Columbus) ?

1905

Identification of the causative agent

(Schaudinn & Hoffman)

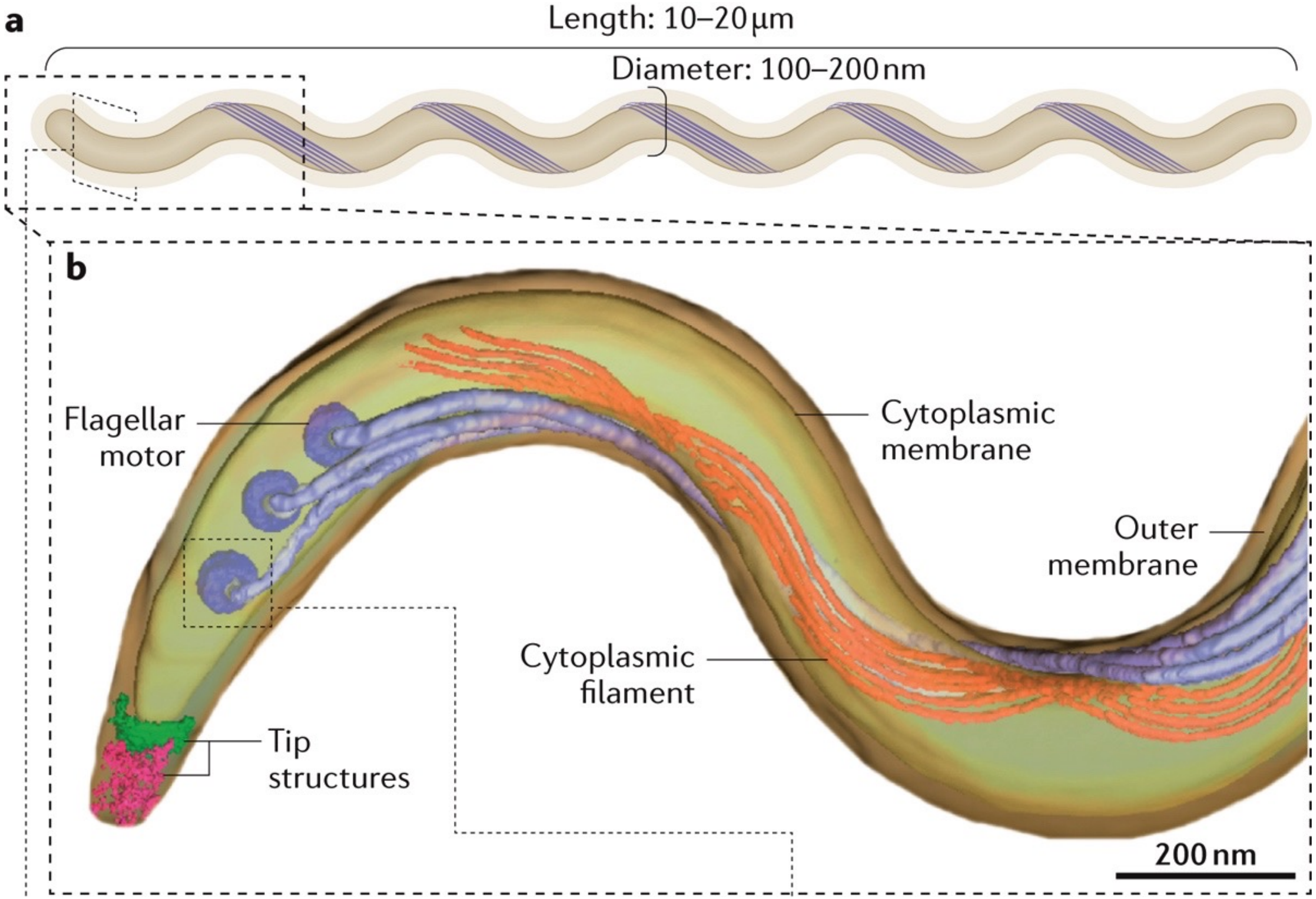
United States
(CDC surveillance report)



Today:

- 6-10 millions of cases /year
- Re-emergence of syphilis in MSM in developed countries

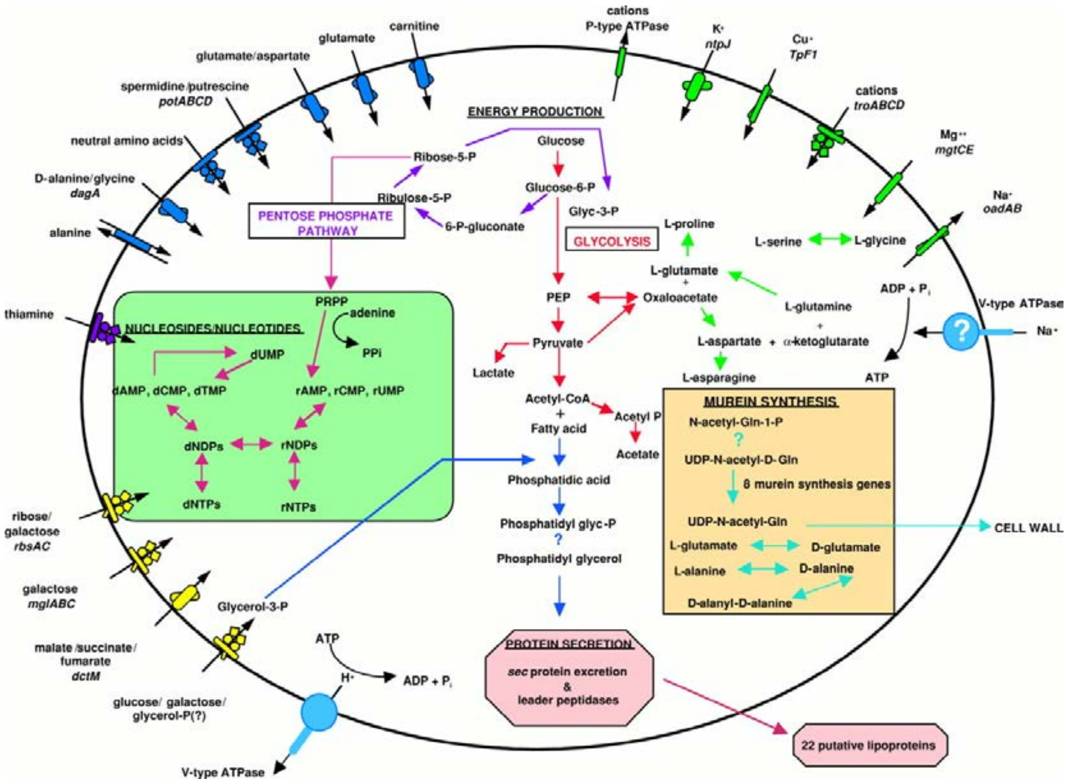
Treponema pallidum



Peeling, R. W. *et al.* (2017) Syphilis. *Nat. Rev. Dis. Primers* doi:10.1038/nrdp.2017.73

Treponema pallidum

- Not cultivable in vitro until recently
- Degenerated biosynthetic pathways
- Multiple transporters (Acquisition of nutrients)
- 1 circular chromosome of 1138 kb (Fraser *et al.*, 1998)



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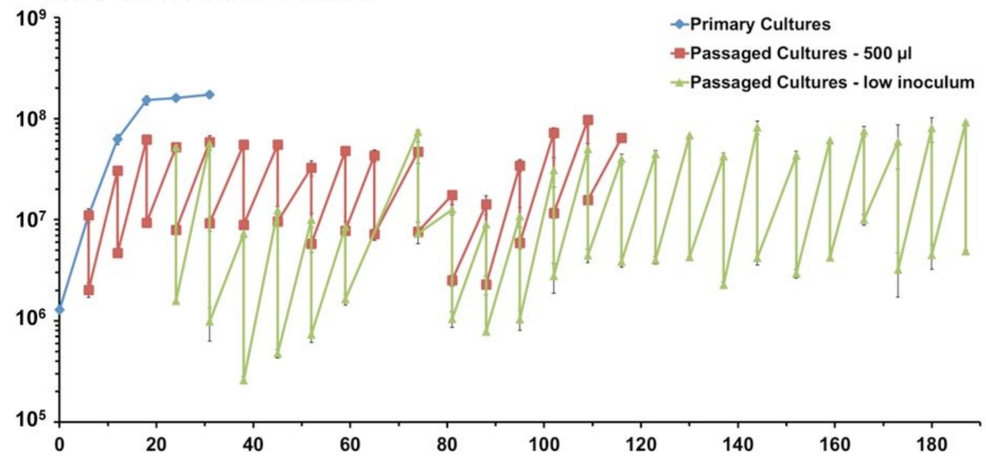
MBio. 2018 Jun 26;9(3). pii: e01153-18. doi: 10.1128/mBio.01153-18.

Long-Term *In Vitro* Culture of the Syphilis Spirochete *Treponema pallidum* subsp. *pallidum*

Diane G. Edmondson,[a] Bo Hu,[b] Steven J. Norris[a,b]

[a]Department of Pathology and Laboratory Medicine, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, Texas, USA
[b]Department of Microbiology and Molecular Genetics, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, Texas, USA

A



Long-term logarithmic multiplication of *T. pallidum* was attained through subculture every 6 to 7 days and periodic feeding using a modified medium with a previously described microaerobic, rabbit epithelial cell coincubation system

PLOS PATHOGENS

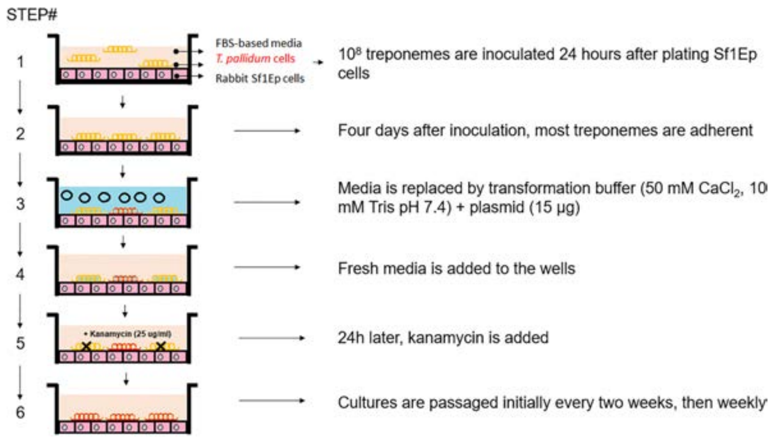
RESEARCH ARTICLE

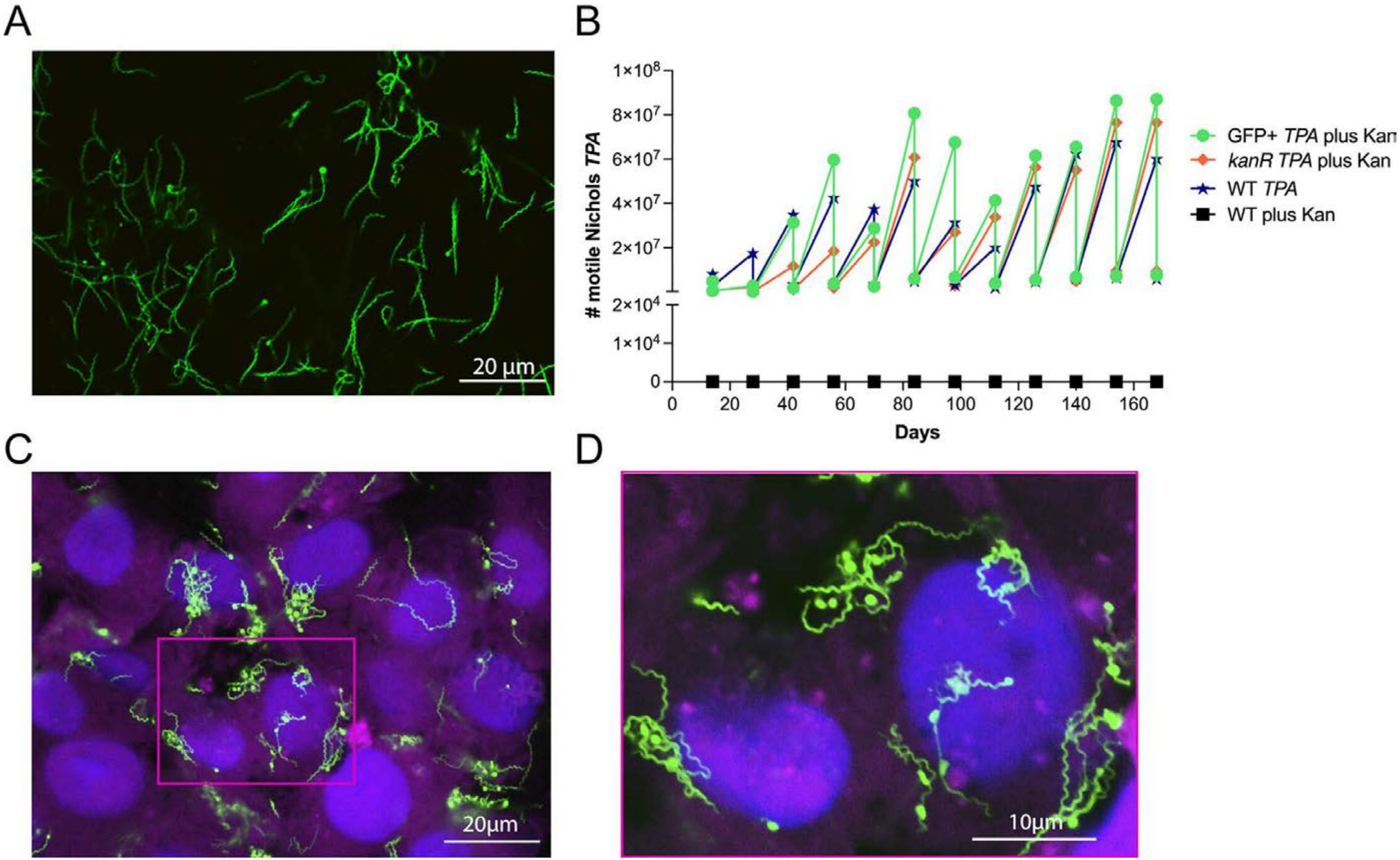
Genetic engineering of *Treponema pallidum* subsp. *pallidum*, the Syphilis Spirochete

Emily Romeis[1], Lauren Tantalo[1], Nicole Lieberman[2], Quynh Phung[2], Alex Greninger[2], Lorenzo Giacani[1,3]*

1 Department of Medicine, Division of Allergy and Infectious Diseases, University of Washington, Seattle, Washington, United States of America, **2** Department of Laboratory Medicine, University of Washington, Seattle, Washington, United States of America, **3** Department of Global Health, University of Washington, Seattle, Washington, United States of America

PLOS Pathogens | https://doi.org/10.1371/journal.ppat.1009612 July 6, 2021





Delgado et al. 2024

A brief history of leptosspirosis

THE ETIOLOGY, MODE OF INFECTION, AND SPECIFIC THERAPY OF WEIL'S DISEASE (SPIROCHÆTOSIS ICTEROHÆMORRHAGICA).

By RYOKICHI INADA, M.D., YUTAKA IDO, M.D., ROKURO HOKI, M.D., RENJIRO KANEKO, M.D., AND HIROSHI ITO, M.D.

(From the First Medical Clinic of the Imperial University in Kyushu, Fukuoka.)

PLATES 56 TO 62.

(Received for publication, December 1, 1915.)

THE RAT AS A CARRIER OF SPIROCHÆTA ICTEROHÆMORRHAGIÆ, THE CAUSATIVE AGENT OF WEIL'S DISEASE (SPIROCHÆTOSIS ICTEROHÆMORRHAGICA).*

By YUTAKA IDO, M.D., ROKURO HOKI, M.D., HIROSHI ITO, M.D., AND HIDETSUNE WANI, M.D.

(From the First Medical Clinic of the Imperial University in Kyushu, Fukuoka, Japan.)

(Received for publication, February 20, 1917.)

THE PROPHYLAXIS OF WEIL'S DISEASE (SPIROCHÆTOSIS ICTEROHÆMORRHAGICA).

By YUTAKA IDO, M.D., ROKURO HOKI, M.D., HIROSHI ITO, M.D., AND H. WANI, M.D.

(From the First Medical Clinic of the Imperial University in Kyushu, Fukuoka.)

(Received for publication, June 27, 1916.)

THE JOURNAL OF EXPERIMENTAL MEDICINE VOL. XXIII. PLATE 62.

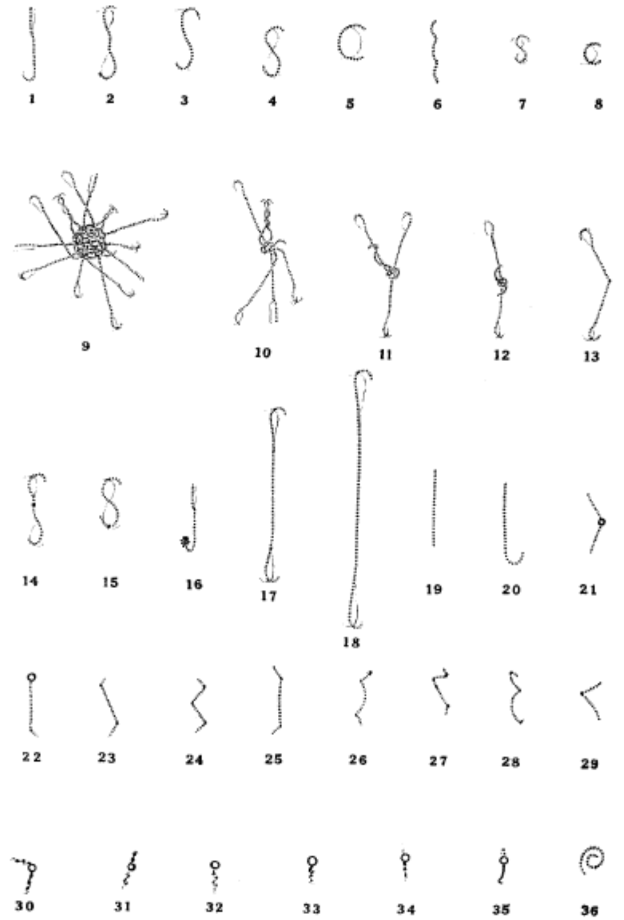


FIG. 26.

(Inada, Ido, Hoki, Kaneko and Ito: Weil's Disease.)

MONOGRAPHIES DE L'INSTITUT PASTEUR

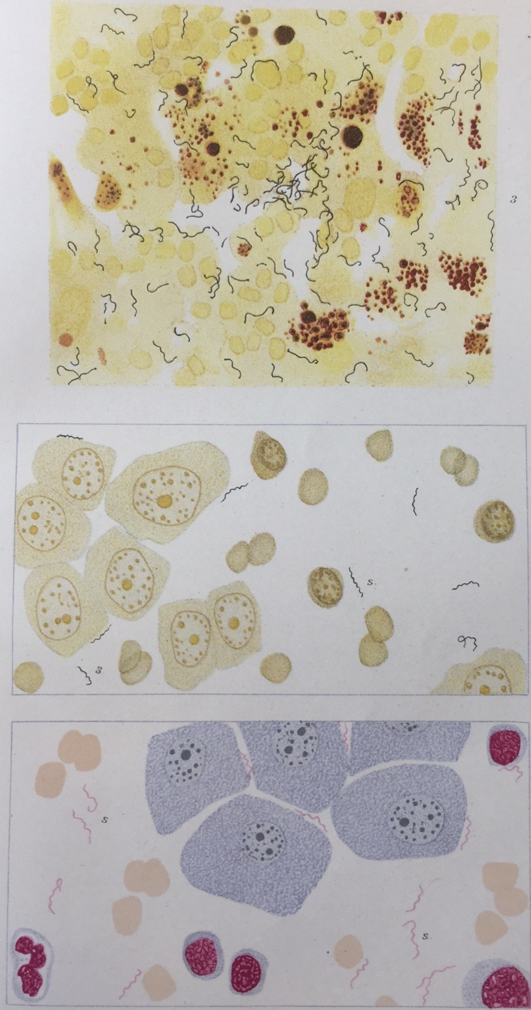
SPIROCHÉTOSE
ICTÉROHÉMORRAGIQUE

PAR

Louis MARTIN Auguste PETTIT

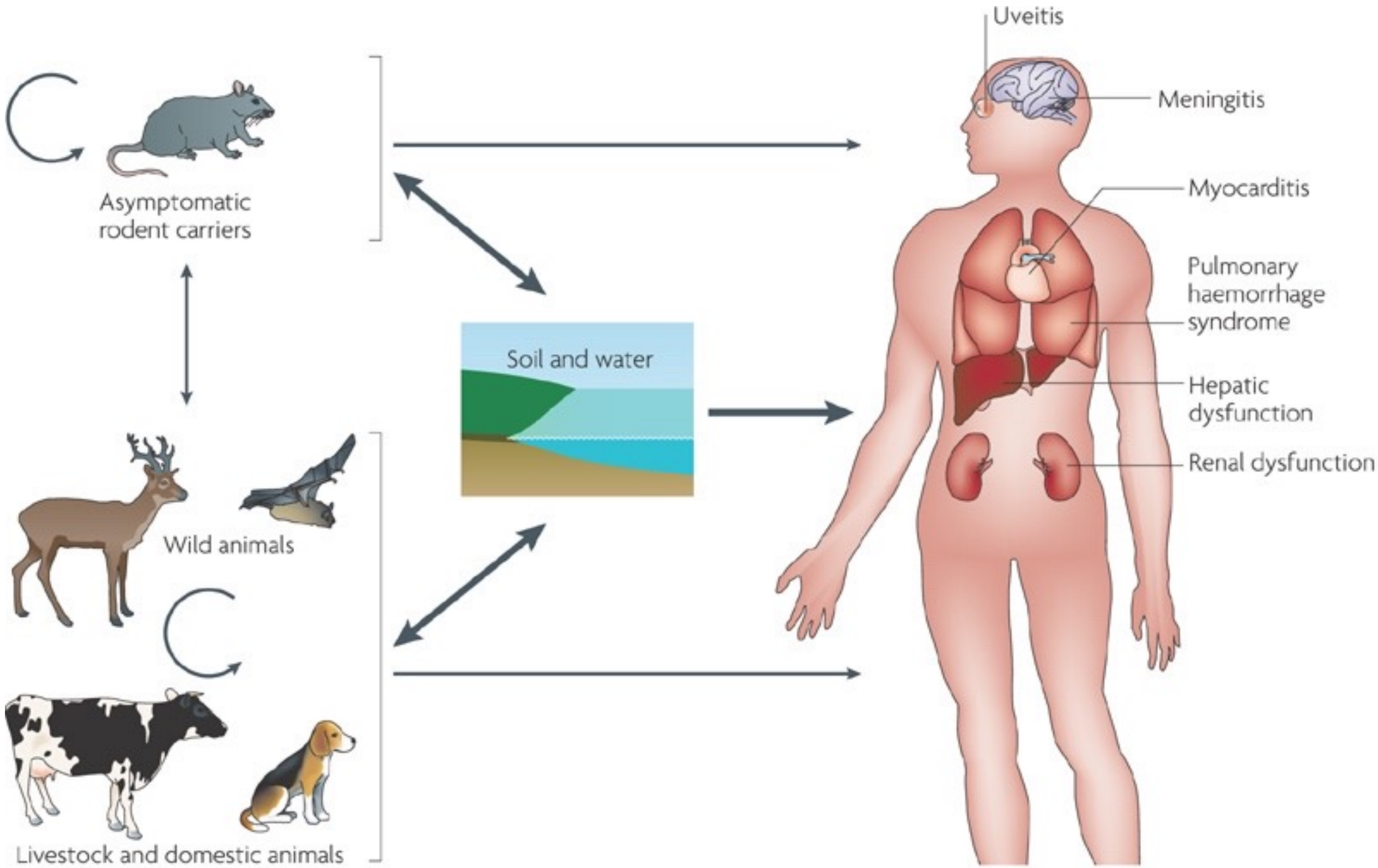
AVEC 13 PLANCHES HORS TEXTE
EN NOIR ET EN COULEURS

MASSON ET C[ie], ÉDITEURS
LIBRAIRES DE L'ACADÉMIE DE MÉDECINE
120, BOULEVARD SAINT-GERMAIN, 120, PARIS (VI[e])
1919



C. CONSTANTIN del. lith. Imp. LAFONTAINE.

The cycle of leptospirosis



Ko *et al.* 2009

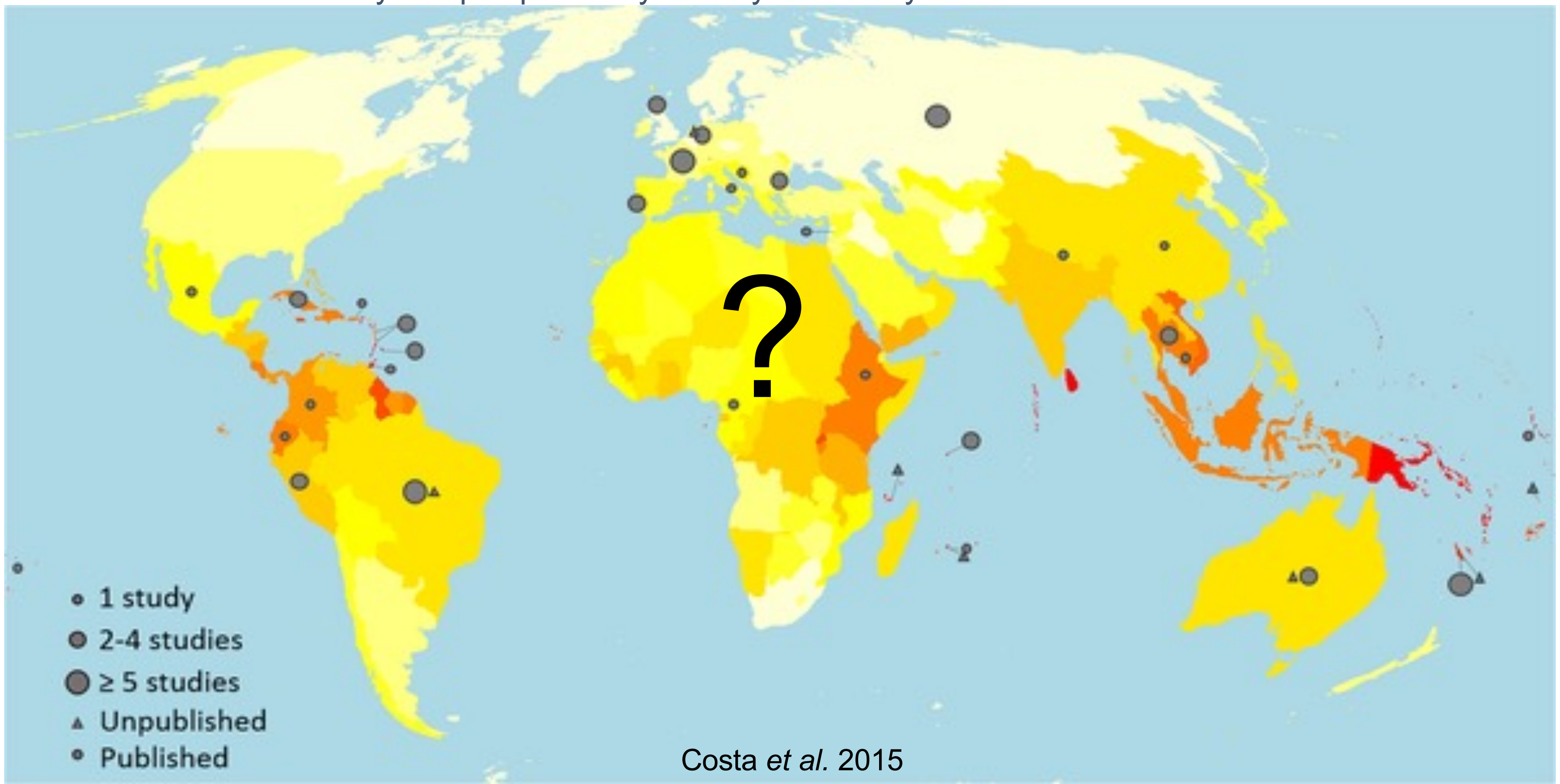
Differential diagnosis

-Influenza
-Hepatitis
-Meningitis
-Yellow fever
-Viral haemorrhagic fever
-Dengue fever
-Malaria
-Hanta virus
-Lyme disease
-Rift valley fever
-Legionellosis
-Nipah and Hendra virus
-Plague
-Typhoid fever
-Brucellosis
-Q fever
-Toxic shock syndrome
-COVID 19

Global burden of leptospirosis

>1 million severe cases and 60,000 deaths due to leptospirosis / year
An under-recognized cause of acute fever

Estimated annual morbidity of leptospirosis by country or territory

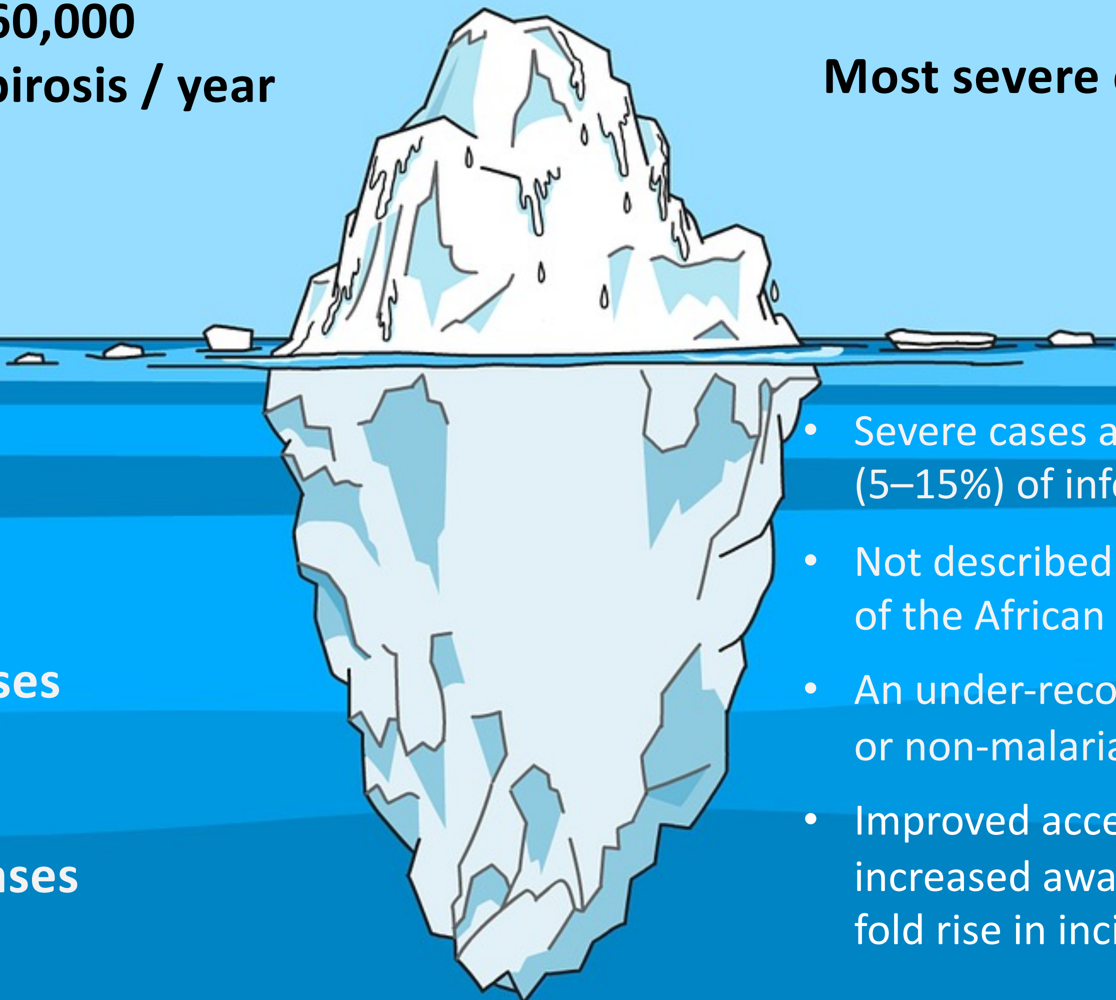


Annual disease incidence is represented as an exponential colour gradient from white (0–3), yellow (7–10), orange (20–25) to red (over 100), in cases per 100,000 population.

Burden of leptospirosis: only the tip of the iceberg

>1 million cases and 60,000 deaths due to leptospirosis / year
Costa et al. 2015

Most severe cases



Mild cases

Undiagnosed cases

Misdiagnosed cases

- Severe cases account for a small fraction (5–15%) of infections
- Not described in human or animals in half of the African countries (Allan et al. 2015)
- An under-recognized cause of acute fever or non-malarial fever cases
- Improved access to diagnosis and increased awareness have led to a 3- to 4-fold rise in incidence (Cassadou et al. 2016)

Leptospirosis, a neglected and emerging disease

Neglected disease

- Limited information on disease burden
- Affects the most neglected and marginalized populations
- Lack of adequate diagnostics
- No effective control measures
- Universal vaccines are not available
- Limited understanding of the epidemiology and pathogenesis

Emerging disease

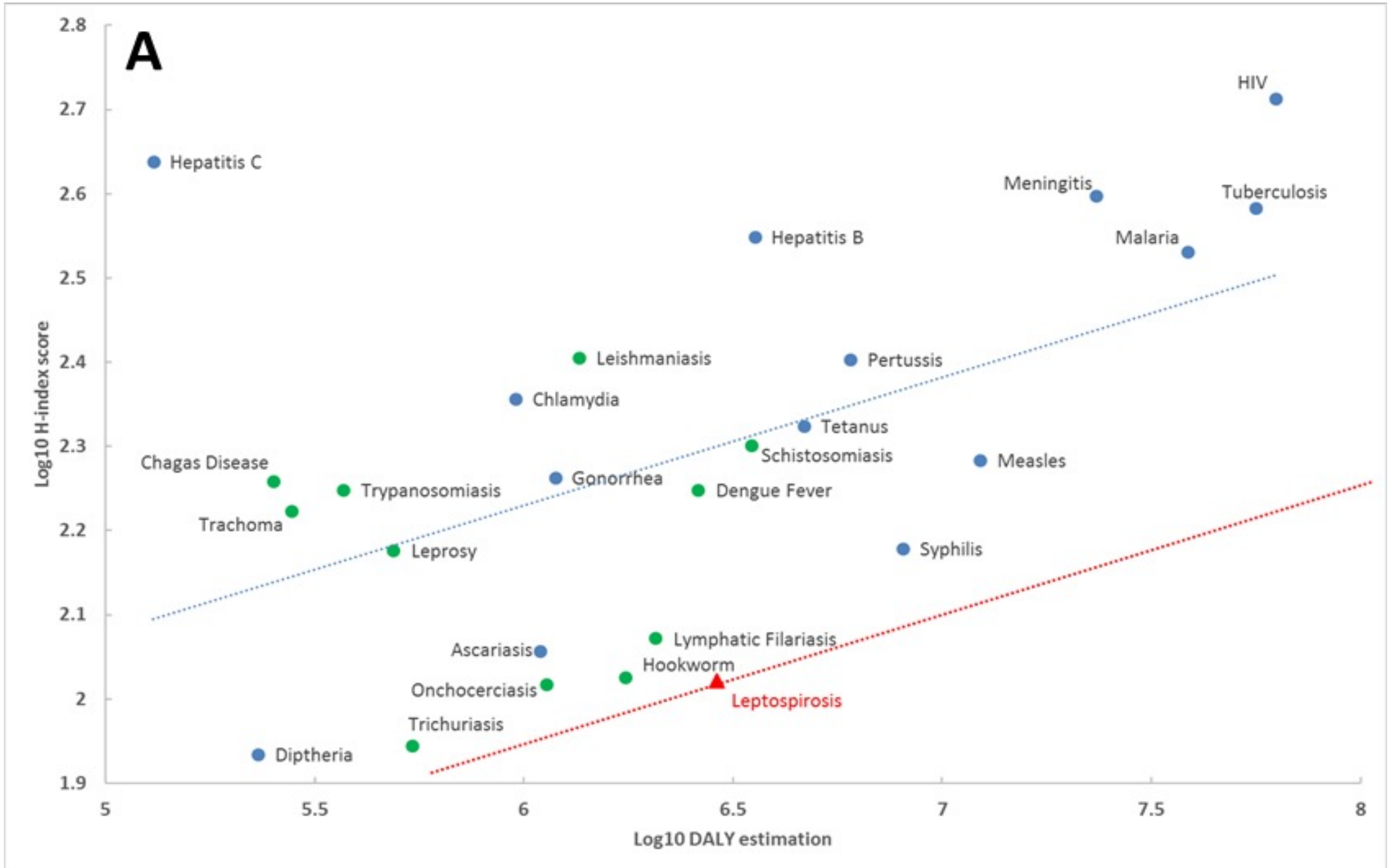
- **Demographic / ecologic changes**
  - One billion individuals live in slums (rat-borne transmission)
  - The urban slum population will double in the next 25 years
- **Climatic changes**
  - Global warming
  - Extreme climatic events (heavy rainfalls, etc)

Neglected Tropical diseases

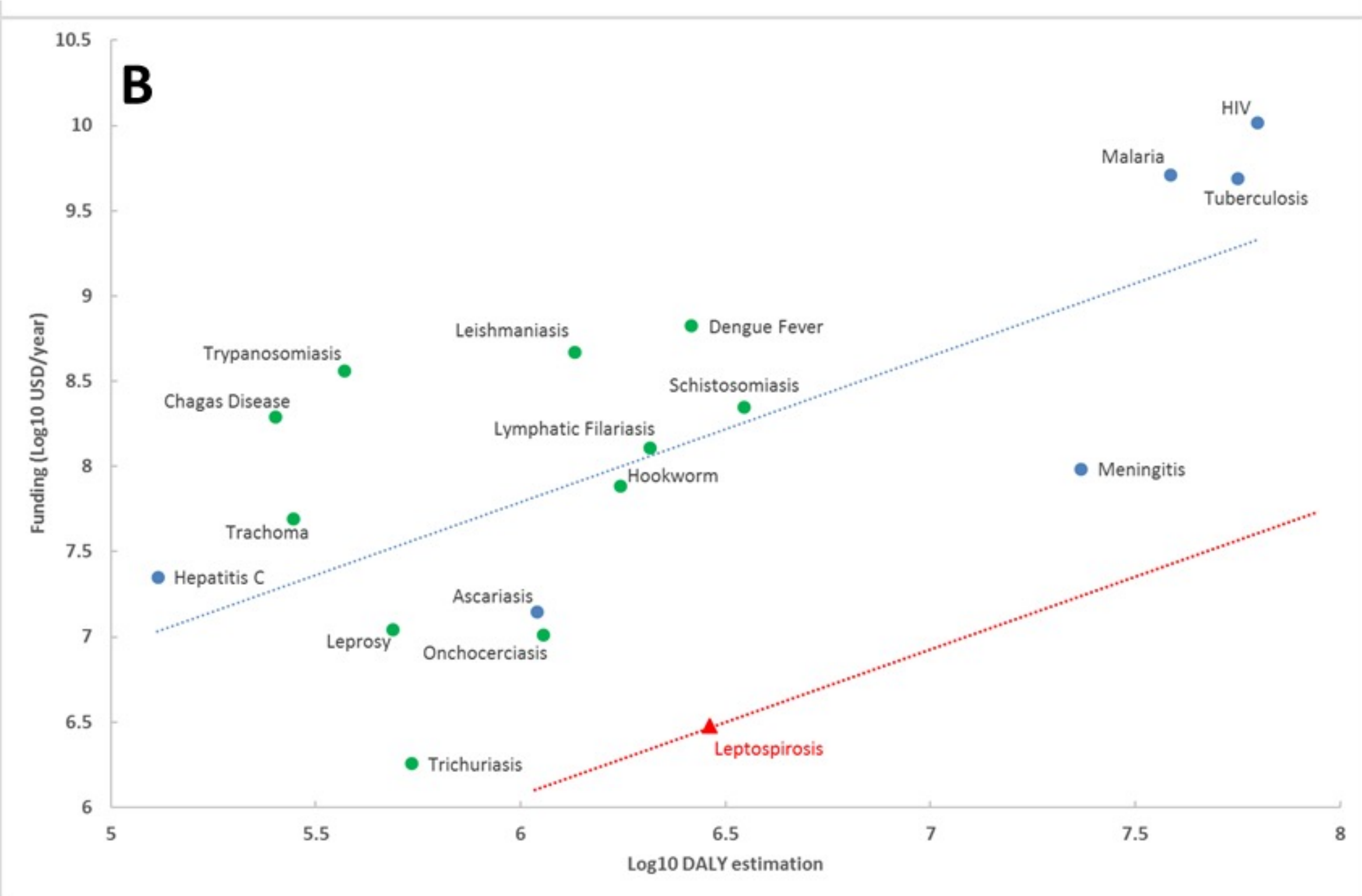
- >1 billion people around the world are affected
- Common in tropical countries
- Lack Public health attention at global and national level
- Mainly affect people living in poverty with inadequate access to clean water, basic sanitation and heath care

Leptospirosis: flying under the bibliometrics radar increases neglect

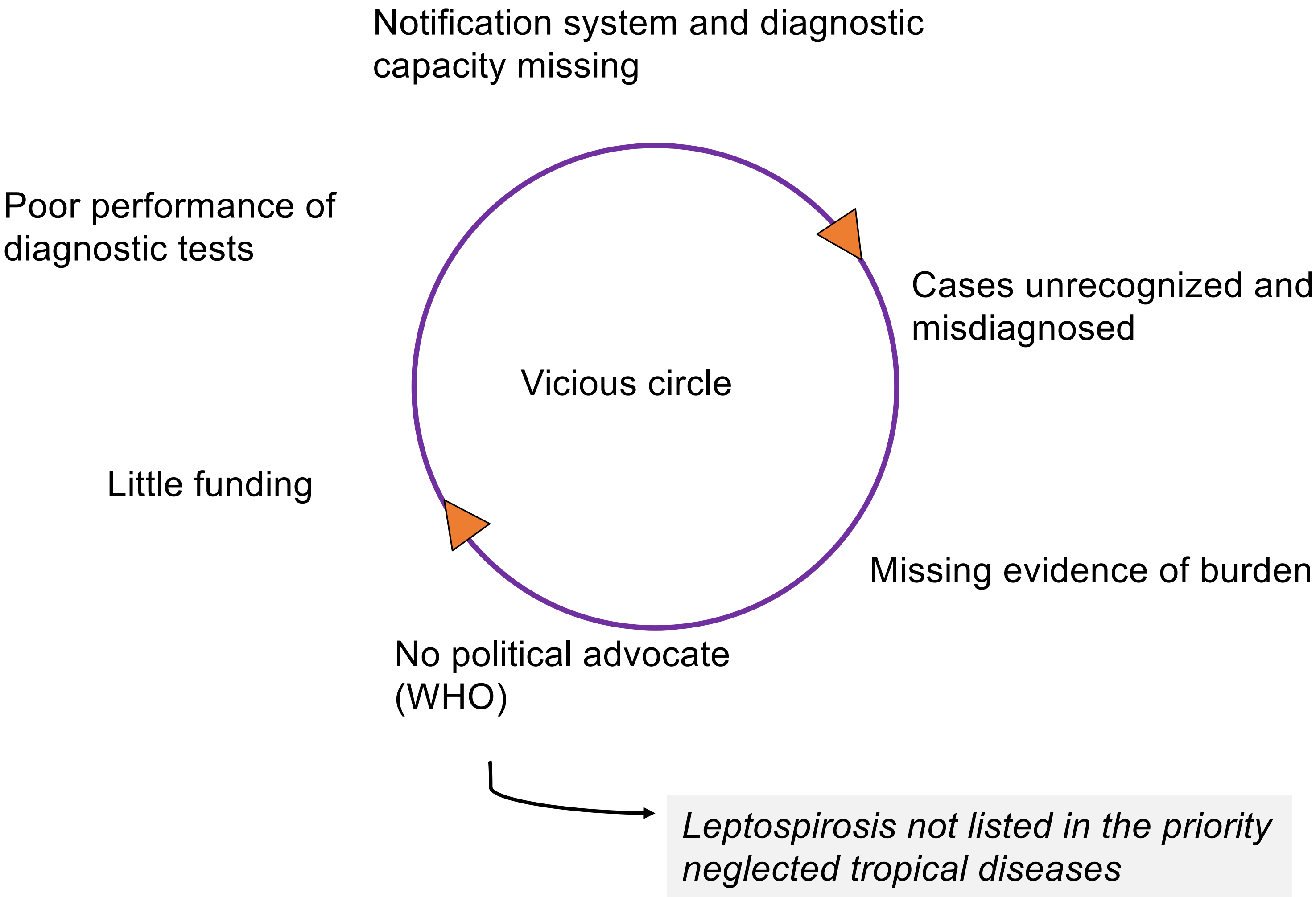
C. Goarant, M. Picardeau, S. Morand, K.M. McIntyre



Leptospirosis: flying under the bibliometrics radar increases neglect
C. Goarant, M. Picardeau, S. Morand, K.M. McIntyre



The vicious circle of a neglected disease



Adapted from Anou Dreyfus

List of priority neglected tropical diseases (WHO)

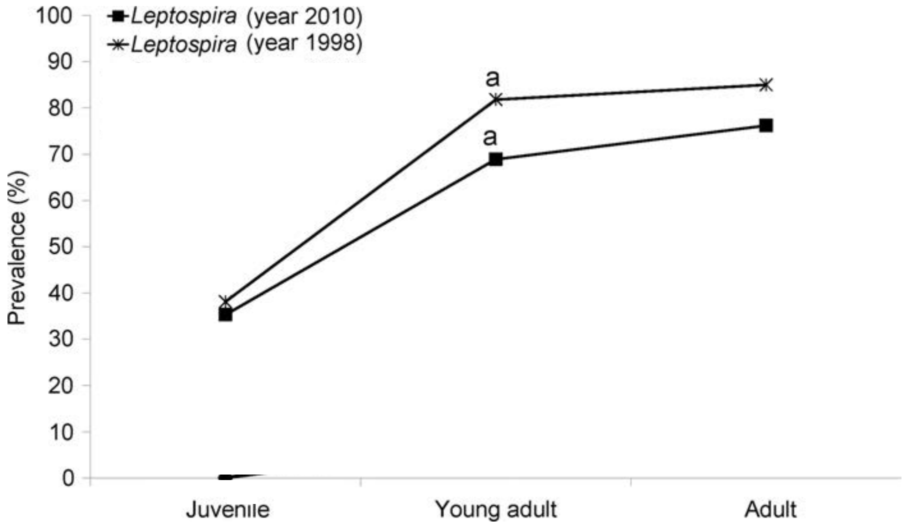
Category	Disease
Protozoan infections	1. Chagas disease 2. Human African trypanosomiasis 3. Leishmaniasis
Helminth infections	4. *Taenia solium* (neuro) cysticercosis/ Taeniosis 5. Dracunculiasis 6. Echinococcus 7. Foodborne trematodiases 8. Lymphatic filariasis 9. Onchocerciasis 10. Schistosomiasis 11. Soil-transmitted helminthiases (ascariasis, Hookworm diseases, trichuriasis, strongyloidiasis)
Bacterial infections	12. Buruli ulcer 13. Leprosy 14. Trachoma 15. Yaws
Viral infections	16. Dengue and chikungunya fevers 17. Rabies
Fungal Infections	18. Mycetoma, chromoblastomycosis, deep mycosis
Ectoparasitic infections	19. Scabies, Myiasis
Venom	20. Snakebite envenoming

Why not Leptospirosis ?

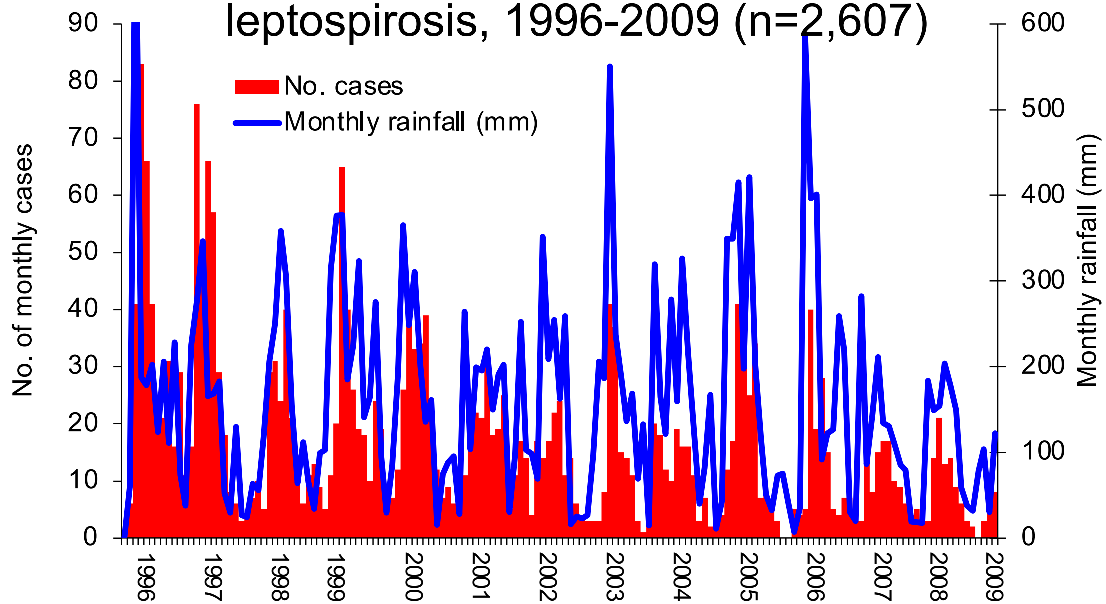
The burden of malaria and neglected tropical diseases expressed in disability-adjusted life years (DALYs)

Diseases	DALYs per 100,000	Reference
Malaria	897.6 (728.1-1094.8)	Murray et al. 2015
Melioidosis	84 (57-120)	Birnie et al. 2019
Cholera	65 (49–84)	Murray et al. 1990
Leishmaniasis	58.6 (48.2-69.7)	Murray et al. 2015
Schistosomiasis	42.1 (23.3-77.8)	Murray et al. 2015
Leptospirosis	42 (18.1-66)	Torgerson et al. 201
Lymphatic filariasis	28.9 (15.7-47.1)	Murray et al. 2015 [65]
Rabies	17.3 (12.7-21.2)	Murray et al. 2015 [65]
Dengue	15.8 (10.1-27.4)	Murray et al. 2015 [65]

Prevalence for three age groups of rats
(Costa *et al.* 2014)



Annual rainfall-associated epidemics of severe leptospirosis, 1996-2009 (n=2,607)



Pau da Lima community, Salvador, Brazil (*Albert Ko, Yale School of Public Health*)

Leptospirosis and the environment

- Leptospirosis is a waterborne disease
- Pathogenic *Leptospira* can survive (but not multiply) in water for months

Preservation of *Leptospira interrogans* virulence *in vivo*

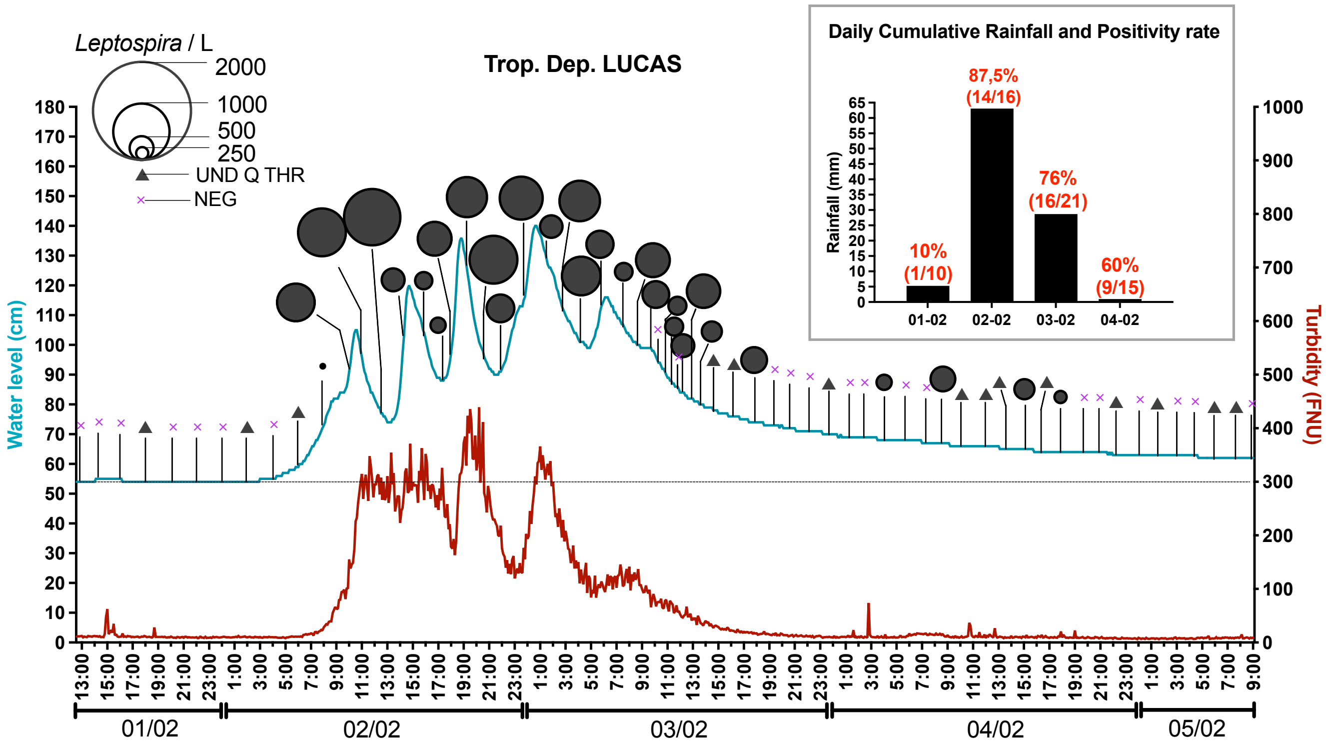
	Waters	2 dpi	15 dpi	2 mpi	4 mpi	9 mpi	12 mpi	15 mpi	24 mpi
Leptospira interrogans Manilae L495	Mont-Dore®		+		+				–
	Evian®						+		
	Contrex®	+							
	Volvic®			+		+		–	
Leptospira interrogans 2016-001	Mont-Dore®		+		+	+	+	–	–
	Evian®	+							
	Volvic®			+					

Virulence was assessed by direct intraperitoneal injection into hamster. Plus signs designate positive culture from cardiac puncture. Note that not all conditions were tested at each timepoint

Bierque et al. 2020

- Pathogenic *Leptospira* are in a resting state in the soil and are able to proliferate with increased water content (Yanagihara et al. 2022)

Does heavy rainfalls cause a concentration or dilution of *Leptospira* in freshwater?



- •Pathogens were absent in the river before the flood, but high concentrations were detected during the rainfall event
- •Several samples remained positive for pathogens even after water levels decreased

Thibeaux et al. 2024

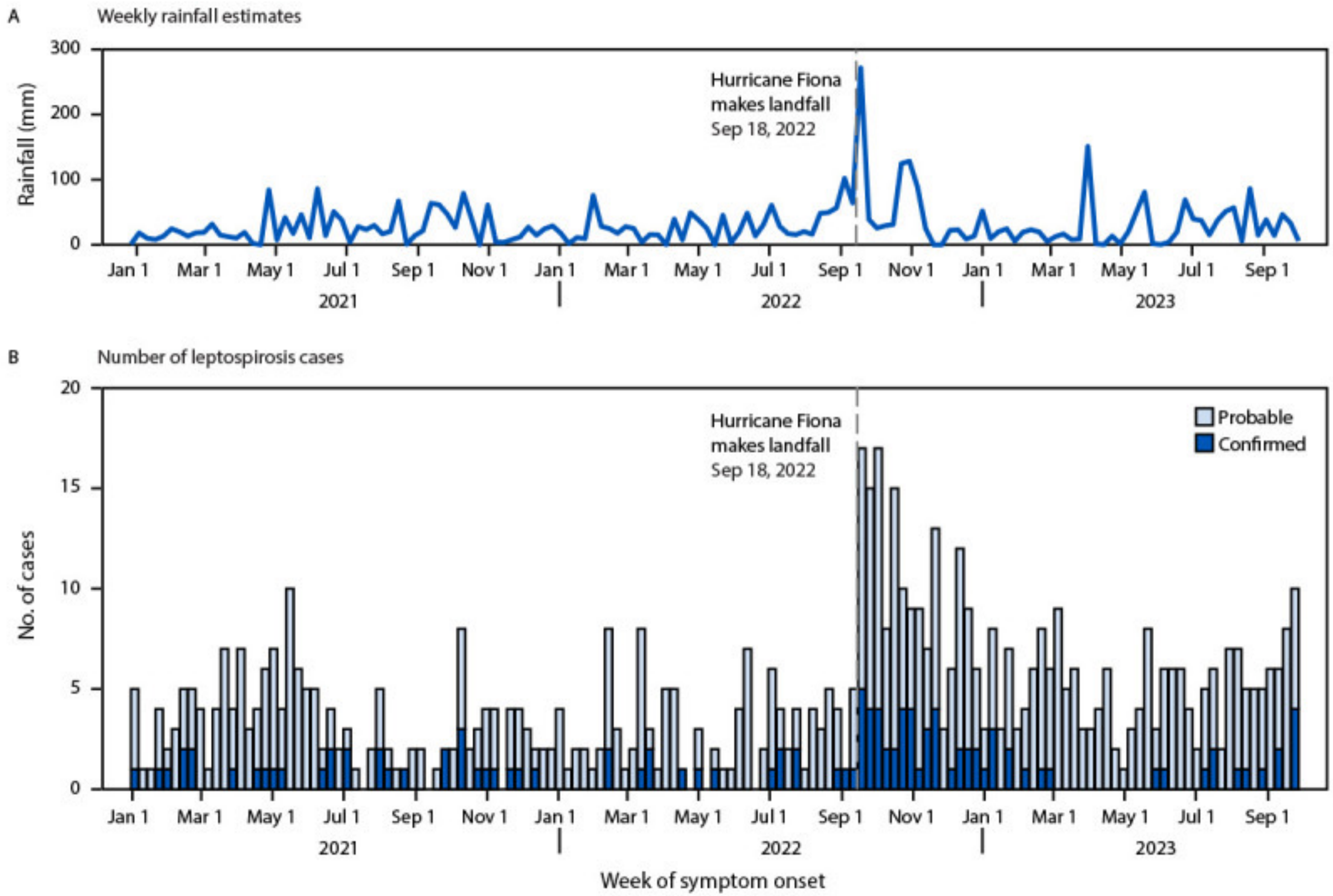
Indirect contamination from the environment

Excretion of pathogenic *Leptospira* from the urine of animal reservoirs

During heavy rainfall :
- Resuspension of soil particles
- Contamination of rivers, etc

Survival of pathogenic *Leptospira*
Exposure to contaminated water

Emilie Bierque, Institut Pasteur de Nouvelle-Calédonie

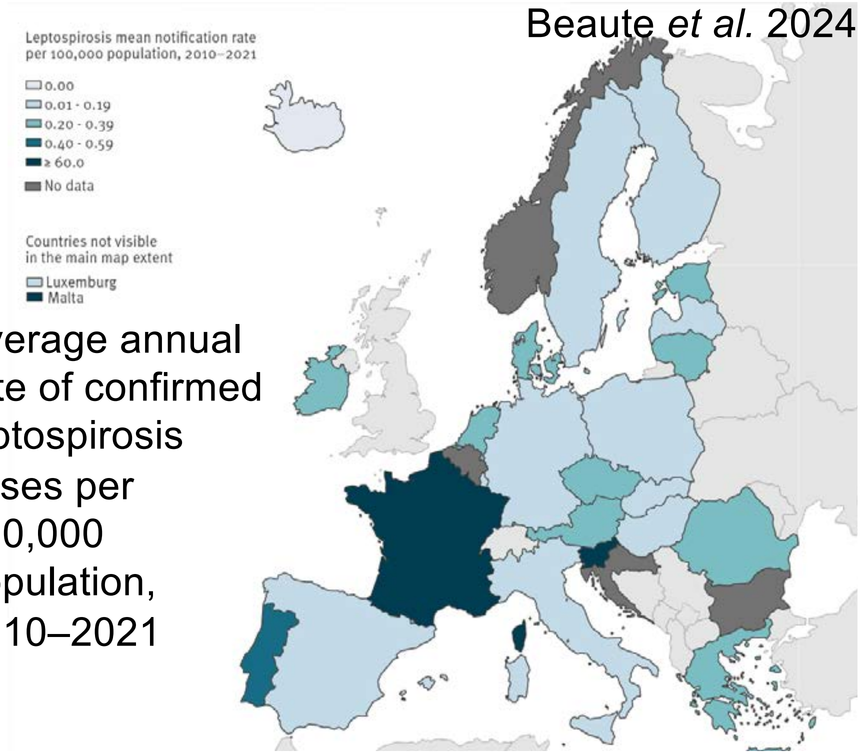


Jones et al. 2024

Weekly rainfall estimates* (A) and number of probable and confirmed leptospirosis cases (B) before and after Hurricane Fiona landfall — Puerto Rico, January 3, 2021–September 30, 2023

Leptospirosis is not exclusively a tropical disease

- 1886: Adolf Weil (Austria)
- 1915: R. Inada and Y. Ido (Japan)
- 1915: H. Ubener and H. Reiter & P. Uhlenhuth and W. Fromme (Germany)
- 1916: L. Martin and A. Pettit (France)

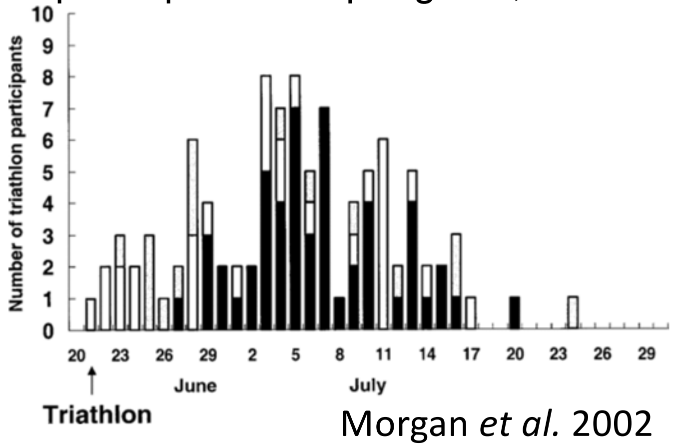


Beaute *et al.* 2024

Average annual rate of confirmed leptospirosis cases per 100,000 population, 2010–2021

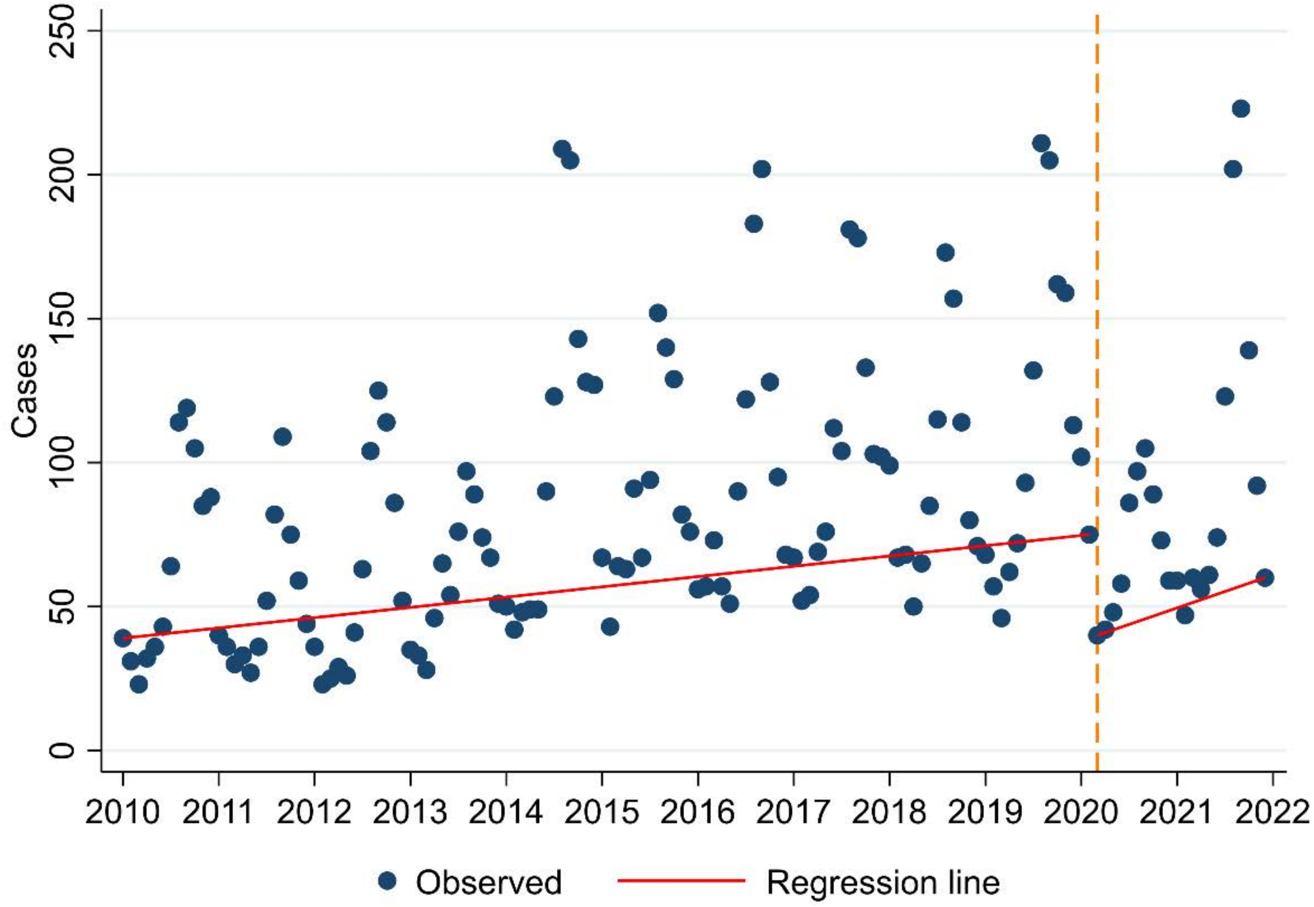
In France, approx. 600-700 cases / year
Occupational (people who work outdoors or with animals) and recreational exposures

Leptospirosis among triathlon participants in Springfield, IL



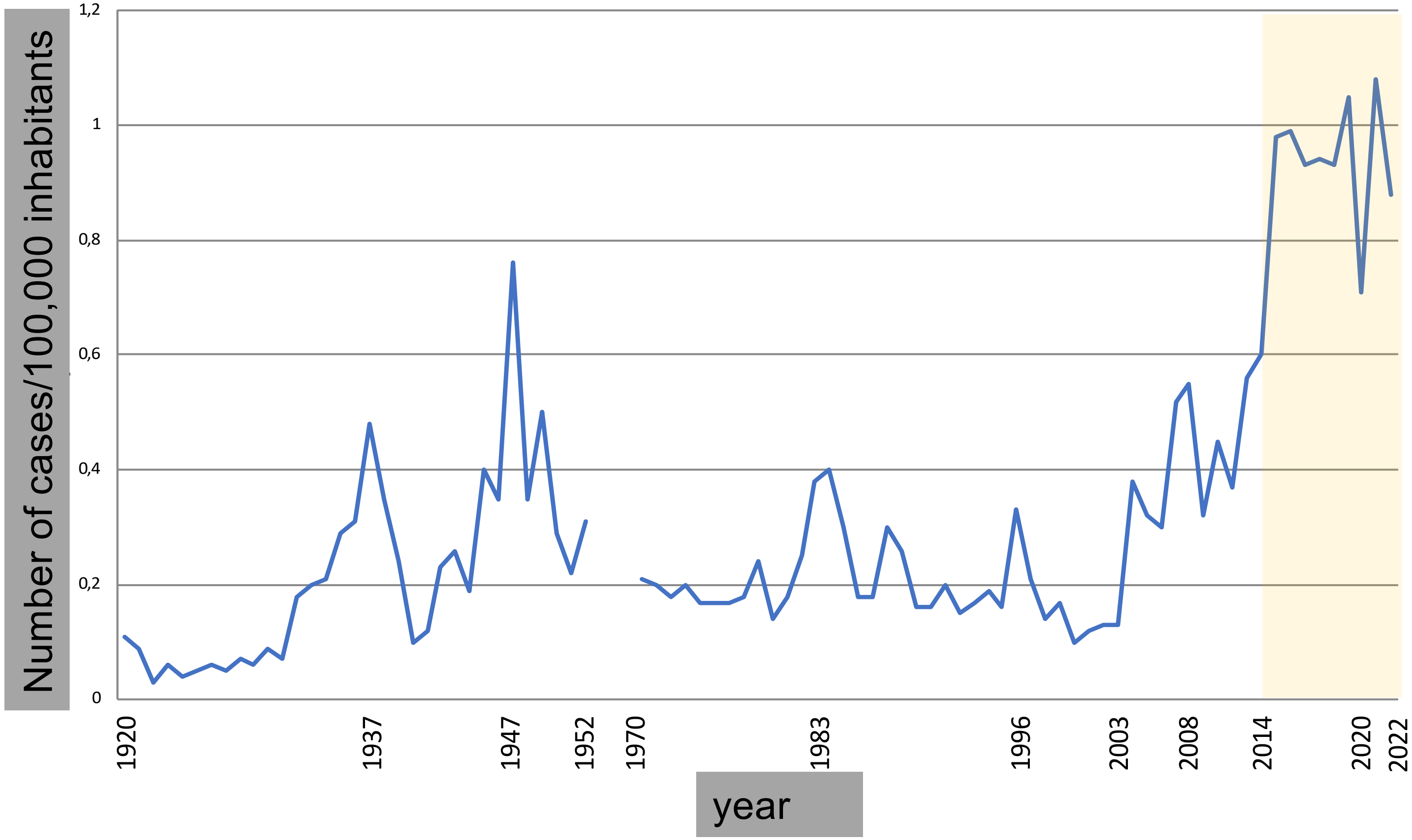
Morgan *et al.* 2002

Monthly number of confirmed leptospirosis cases by month used for statistics with interrupted time series trend line, European Union/European Economic Area, 2010–2021 (12 180). **23 countries**



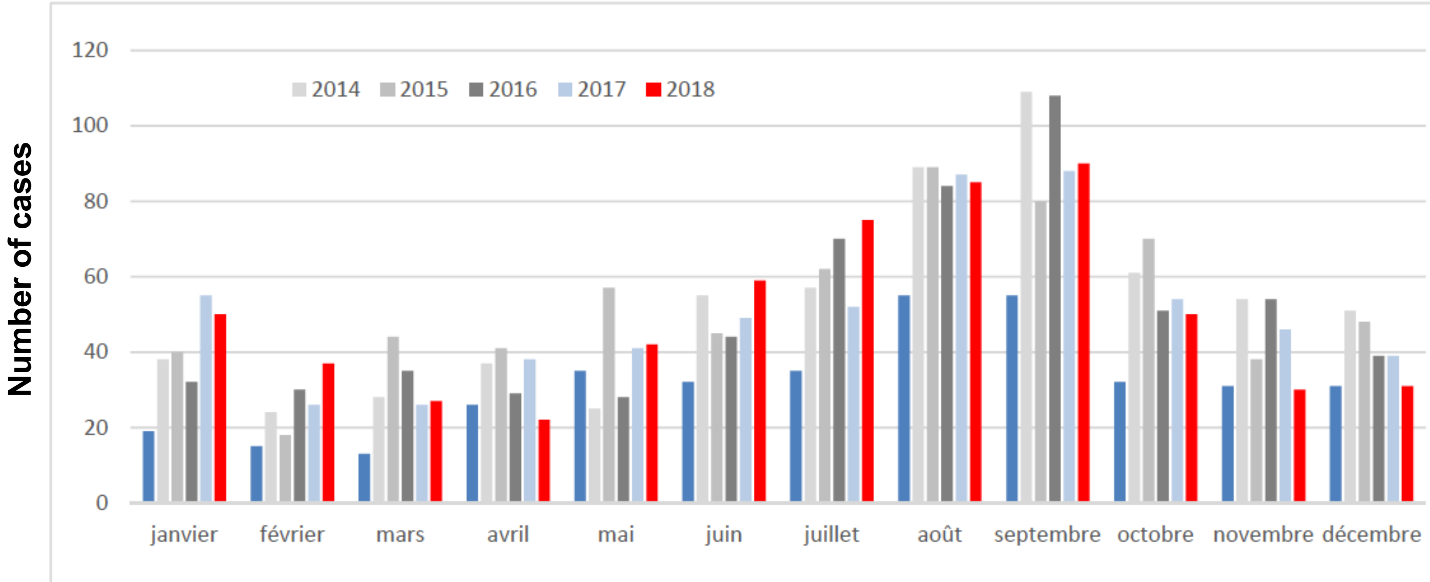
Julien Beaute *et al.* Eurosurveillance. 2024

Leptospirosis incidence in Mainland France, 1920-2022
(data from the NRC for Leptospirosis, Institut Pasteur)



Leptospirosis in mainland France

Disribution of cases by month of the year



The incidence is much higher in french overseas territories in comparison to mainland France

(2021 data from the NRC for Leptospirosis)

1 case /100,000 inhabitants

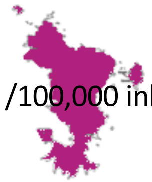
Martinique

31 cases /100,000 inhabitants

Guadeloupe

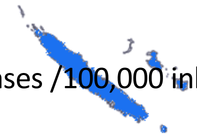
37 cases /100,000 inhabitants

Mayotte



70 cases /100,000 inhabitants

New Caledonia

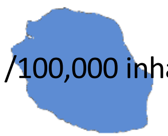


85 cases /100,000 inhabitants

French Guiana

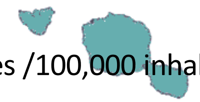
43 cases /100,000 inhabitants

La Reunion



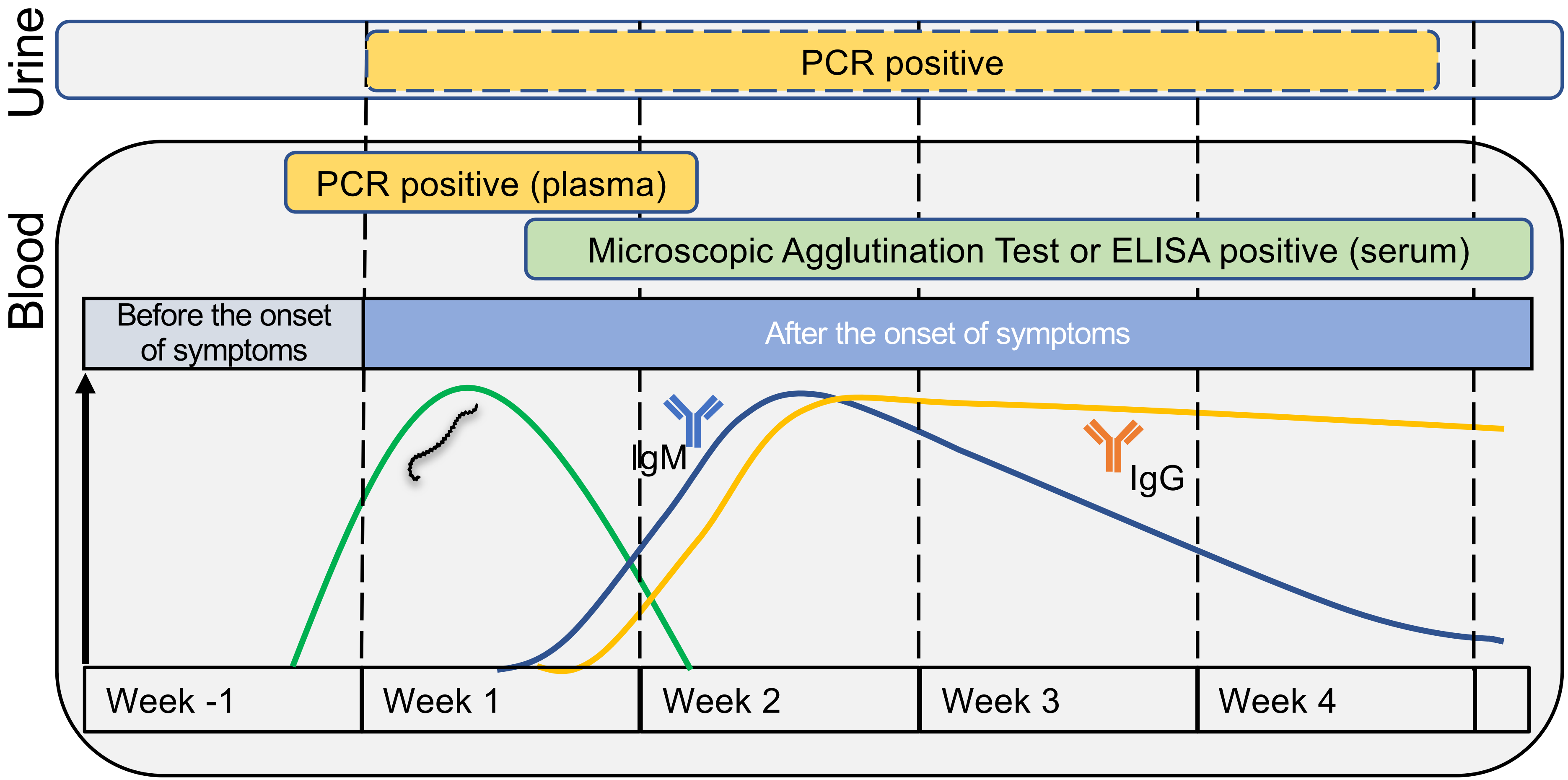
16 cases /100,000 inhabitants

French Polynesia



59 cases /100,000 inhabitants

Diagnosis of leptospirosis



Consigny et al. *2022*

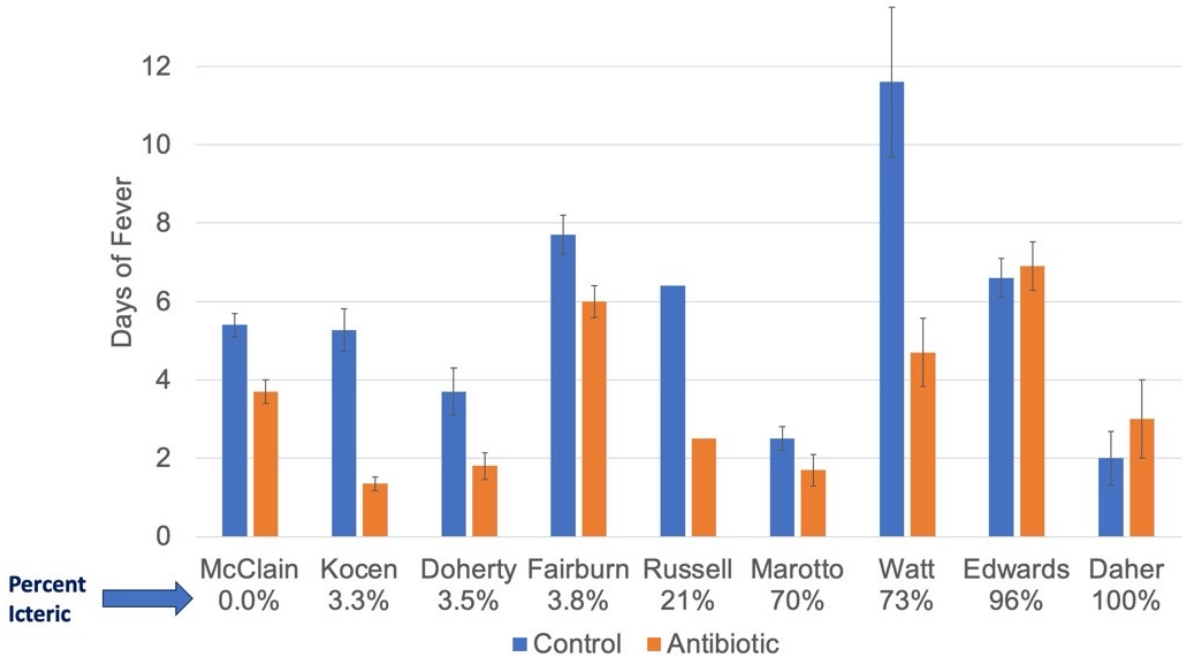
Which assay and which sample to use according to the kinetics of infection ?

	≤10 days post onset symptoms	≥10 days post onset symptoms
PCR plasma	yes	
PCR urine	yes	yes
IgM ELISA serum	yes	yes
MAT serum		yes

- Leptospirosis has varied clinical presentations
- Misdiagnosis common (malaria, dengue, etc)
- Culture isolation : no diagnostic value (slow-growing bacteria) but epidemiological value (identification of circulating species / serovars)
- **Early diagnosis can be challenging**

Early antibiotherapy is effective

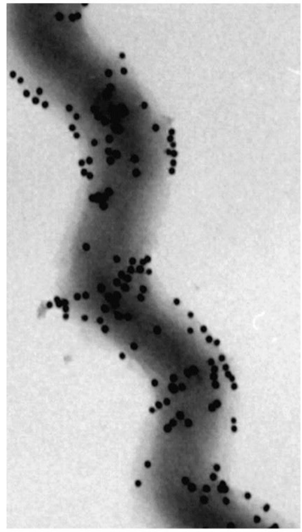
- Cochrane database of systematic reviews (Win et al. 2024) : « *Antibiotics may have no effect on mortality (death) and side effects associated with leptospirosis infection. However, due to the limited evidence, these findings may change if more trials of high quality are conducted* »
- No reported cases of antibiotic-resistance strains
- Early antibiotherapy is effective (doxycycline, ampicillin, amoxicillin, azithromycin, penicillin, ceftriaxone)



Controlled studies comparing fever duration +/- antibiotic therapy for leptospirosis.
David Haake et al. 2025

Vaccines against leptospirosis

- Protection using killed spirochetes / immune sera (Inada et al. 1916)
- Natural infection does not confer immunity to a subsequent infection with a homologous serovar (Felzemburgh et al. 2014)
- Protective immunity is mediated by antibodies directed against LPS
- Bacterins confer little or no cross-protection between serovars
- 12/31 (39%) of *Leptospira*-positive cultures in cattle with recent vaccination (<12 months) (Zarantonelli et al. 2018)
- 30/60 (50%) of *Leptospira*-positive PCRs in vaccinated dogs (Garcia-Lopez et al. 2023)
- A live motility-deficient mutant can induce cross-protective immunity (Wunder et al. 2021)



L. interrogans coated with gold-labeled anti-LPS monoclonal antibodies (Haake et al. 2015)

Spirolept, a formalin-inactivated Icterohaemorrhagiae vaccine

1. Primary vaccination
2 injections of 1 mL at 15-day intervals
2. First booster
4 to 6 months after primary vaccination
3. Subsequent boosters
Every 2 years

- High-risk occupations:

People with close contacts with contaminated environment and/or animals (sewer workers, fish farmers, veterinarians, etc)

- Countries:

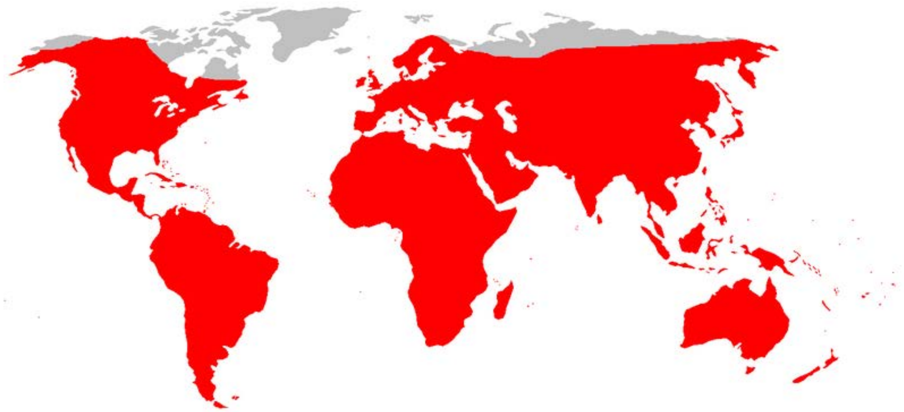
France (mainland and oversea-territories), Belgium, Luxembourg, Switzerland

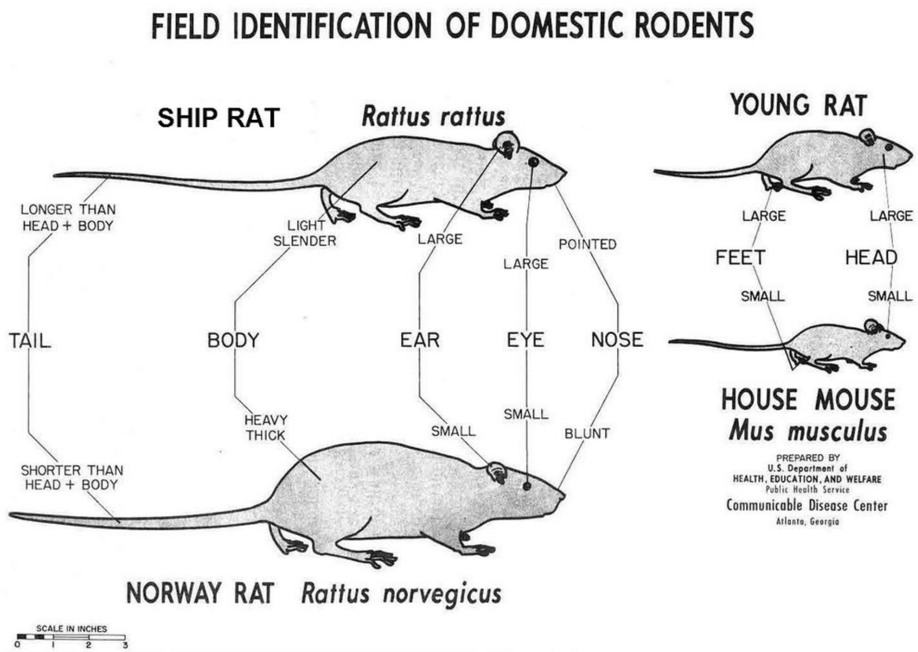
Rats are the main reservoirs

- Worldwide distribution
- Carriers of the most virulent strains (*L. interrogans* sg Icterohaemorrhagiae)

Global distribution of black rats (R.rattus)

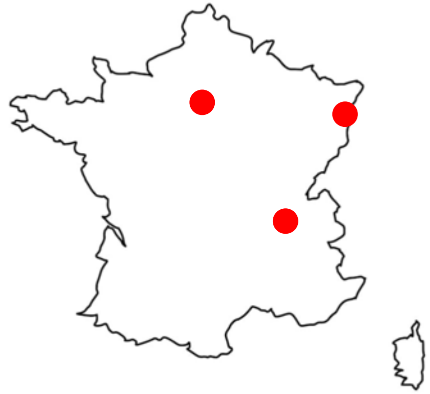
Global distribution of brown rats (R.norvegicus)





Leptospirosis and reservoirs

Rattus norvegicus

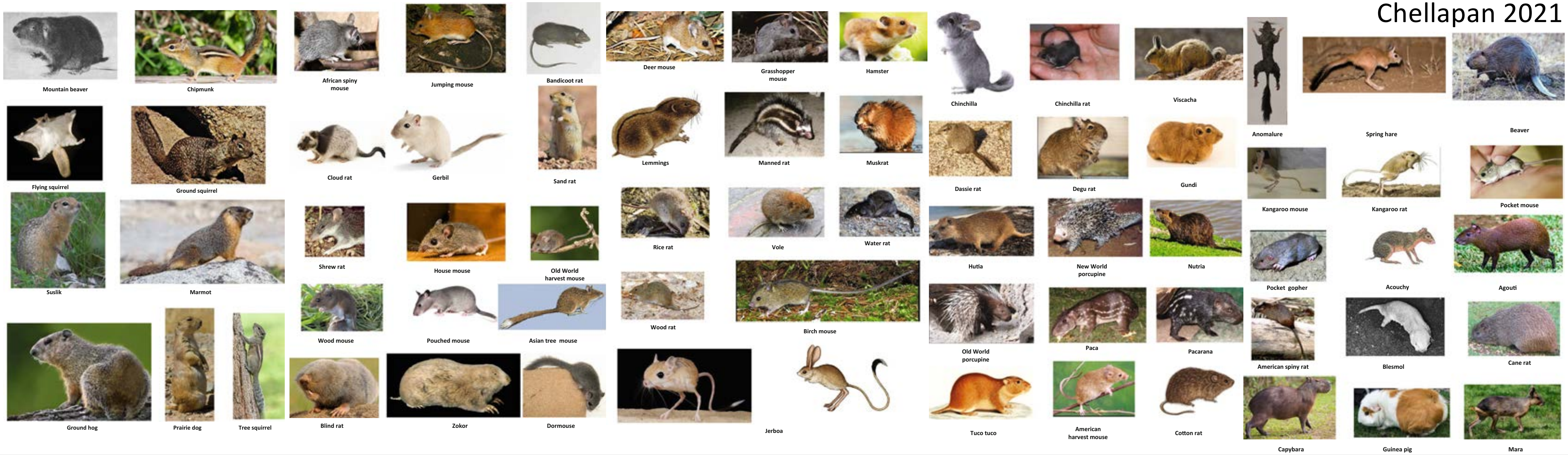


- Prevalence in rats > 20% in Lyon (2011-2013), Paris (2021-2023) and Strasbourg (2023)
- 100% of *L. interrogans* serogroup Icterohaemorrhagiae

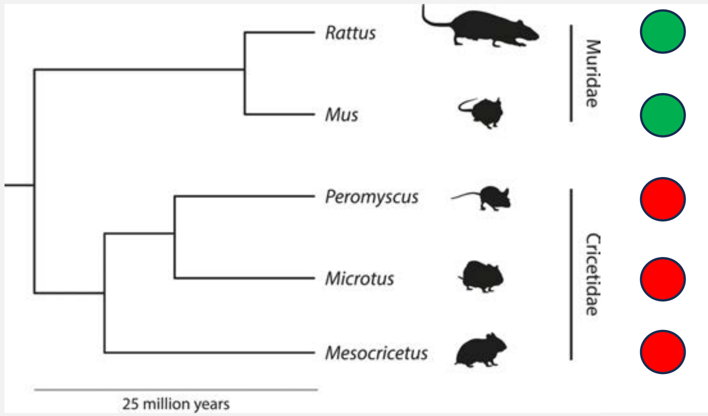
Ayral *et al.* 2015. PLoS ONE.
Bourhy *et al.* unpublished

Leptospirosis and rodents

Chellapan 2021



gerbils, guinea-pigs, hamsters
(highly susceptibles)
vs
rats, mice
(resistants)

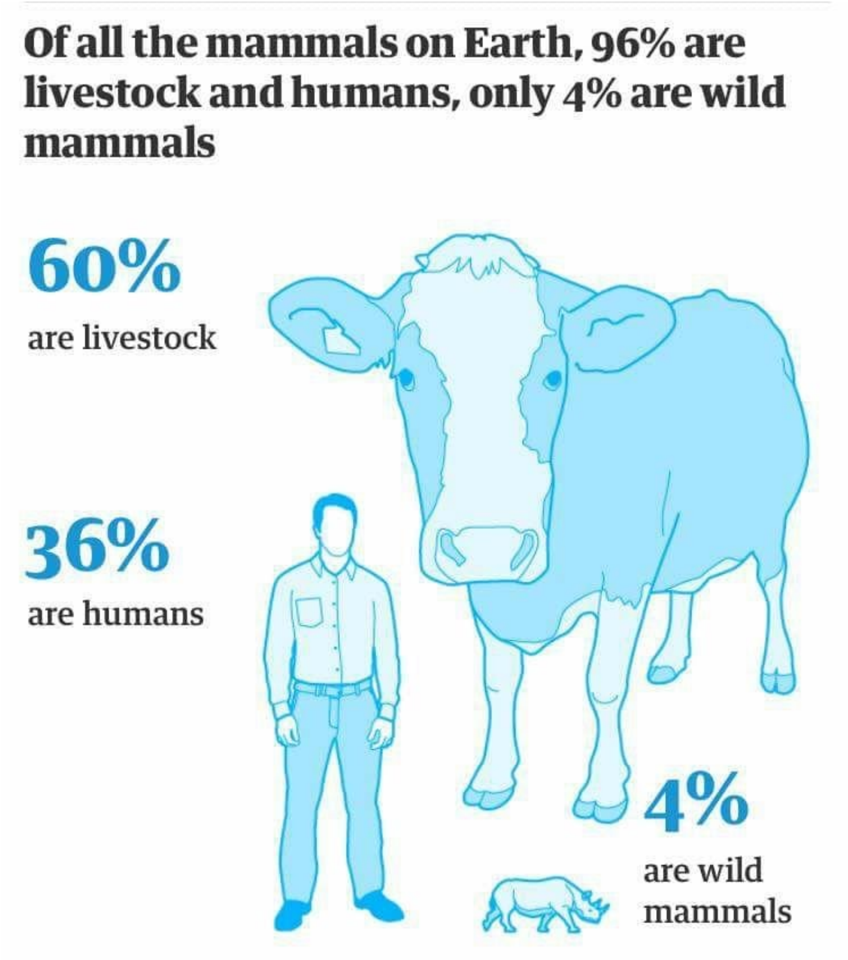


needs to be confirmed
needs to be confirmed

Bedford et al. 2015

Leptospira infect a wide array of hosts

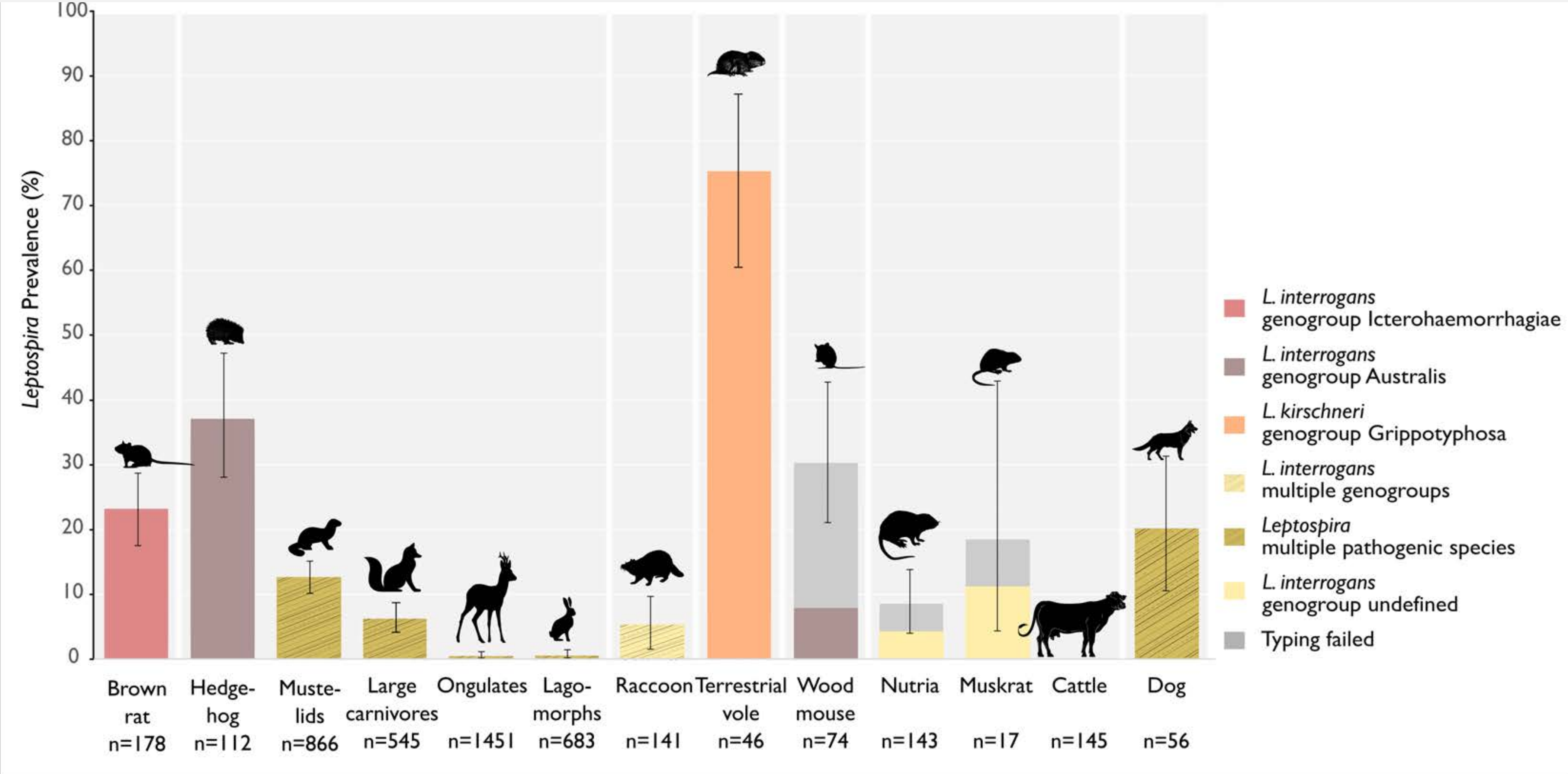
What is the economic impact of leptospirosis in livestock ?



- Leptospirosis in cattle: abortion, fetal death, loss in milk production, etc
- Beef and dairy exports rank among the most important national income sources in South American countries
- In Uruguay : 4 times more cattle than inhabitants
- Approximately 20% of cattle were found to be shedding pathogenic *Leptospira* in their urine (Zarantonelli et al. 2018)

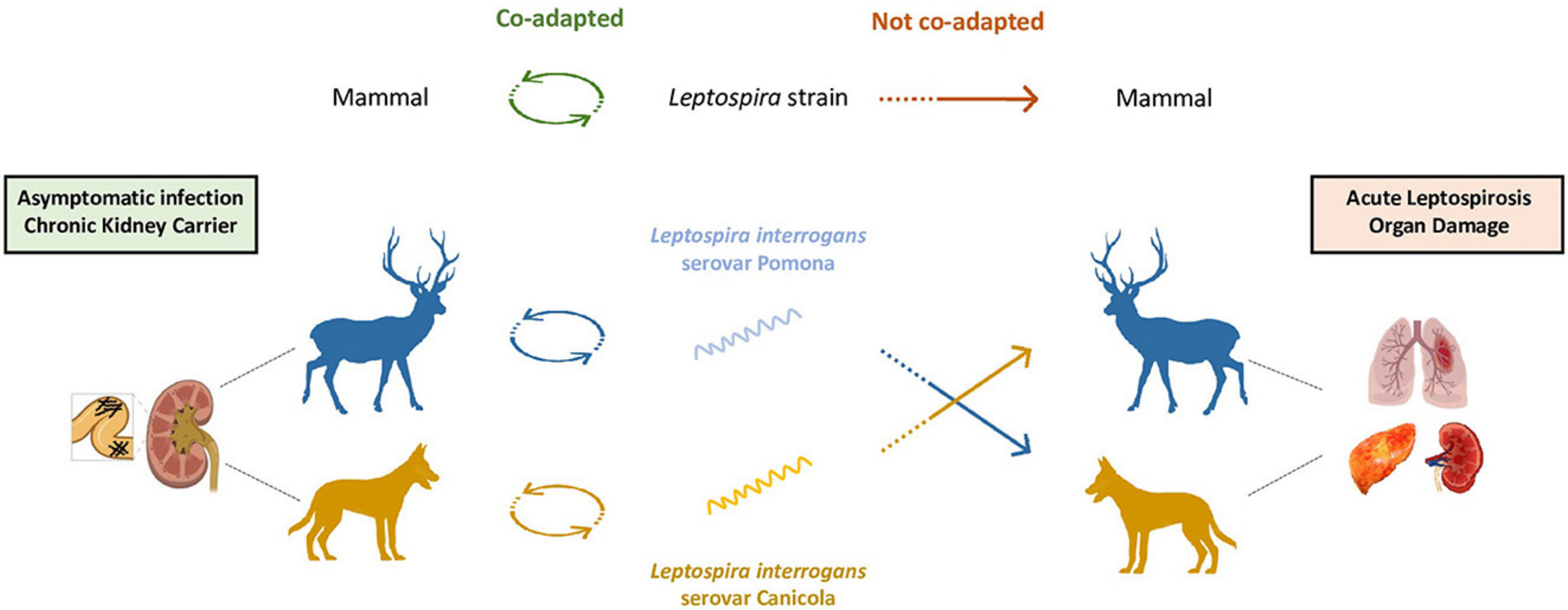
The biomass distribution on Earth. Bar-On YM, Phillips R, Milo R. Proc Natl Acad Sci U S A. 2018 Jun 19;115(25):6506-6511.

Leptospirosis and reservoirs in France



Data from Florence Ayral, VetAgro Sup

Leptospirosis susceptibility in incidental hosts



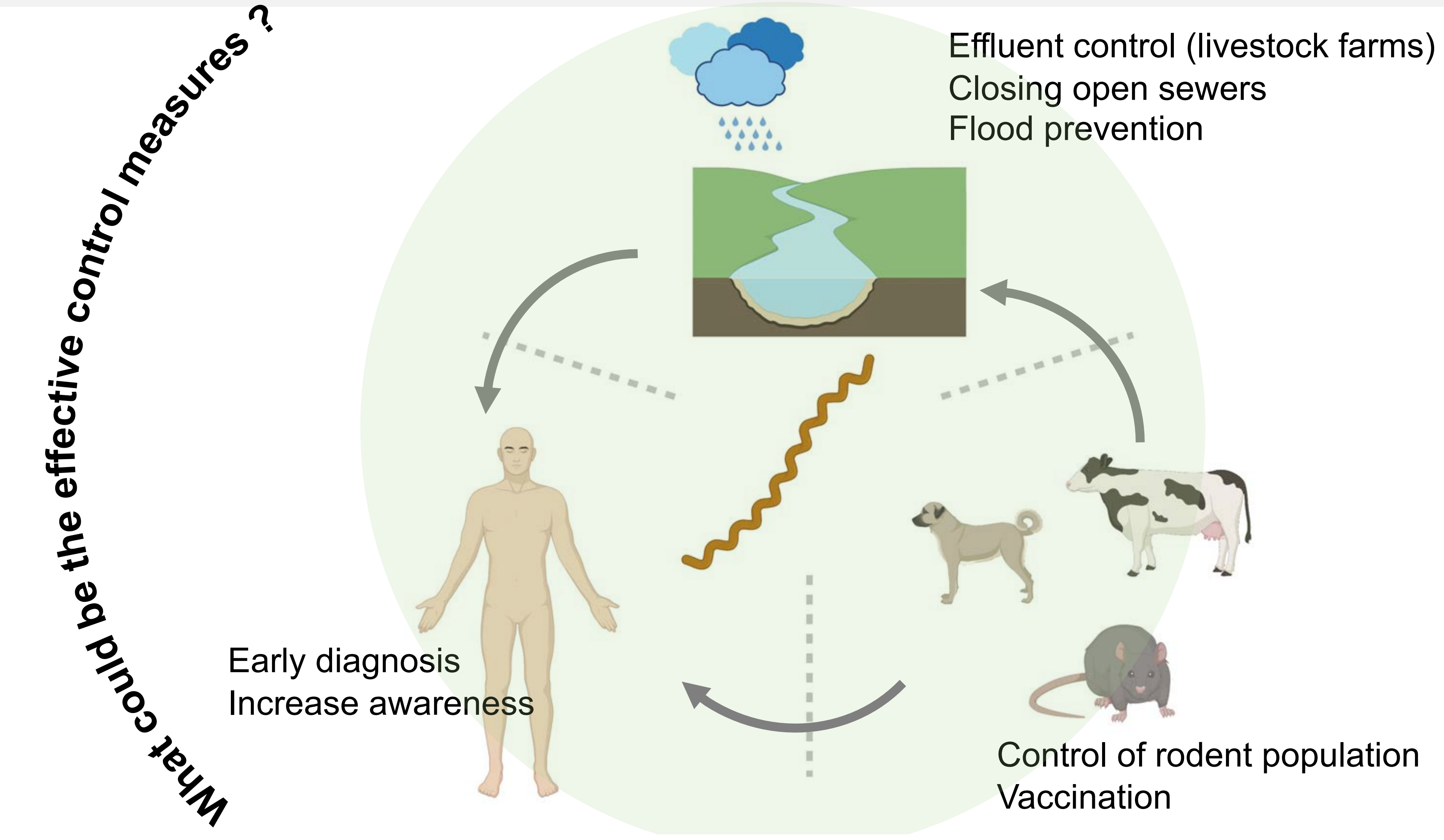
Davignon *et al.* 2023

Typical reservoir hosts of common serovars

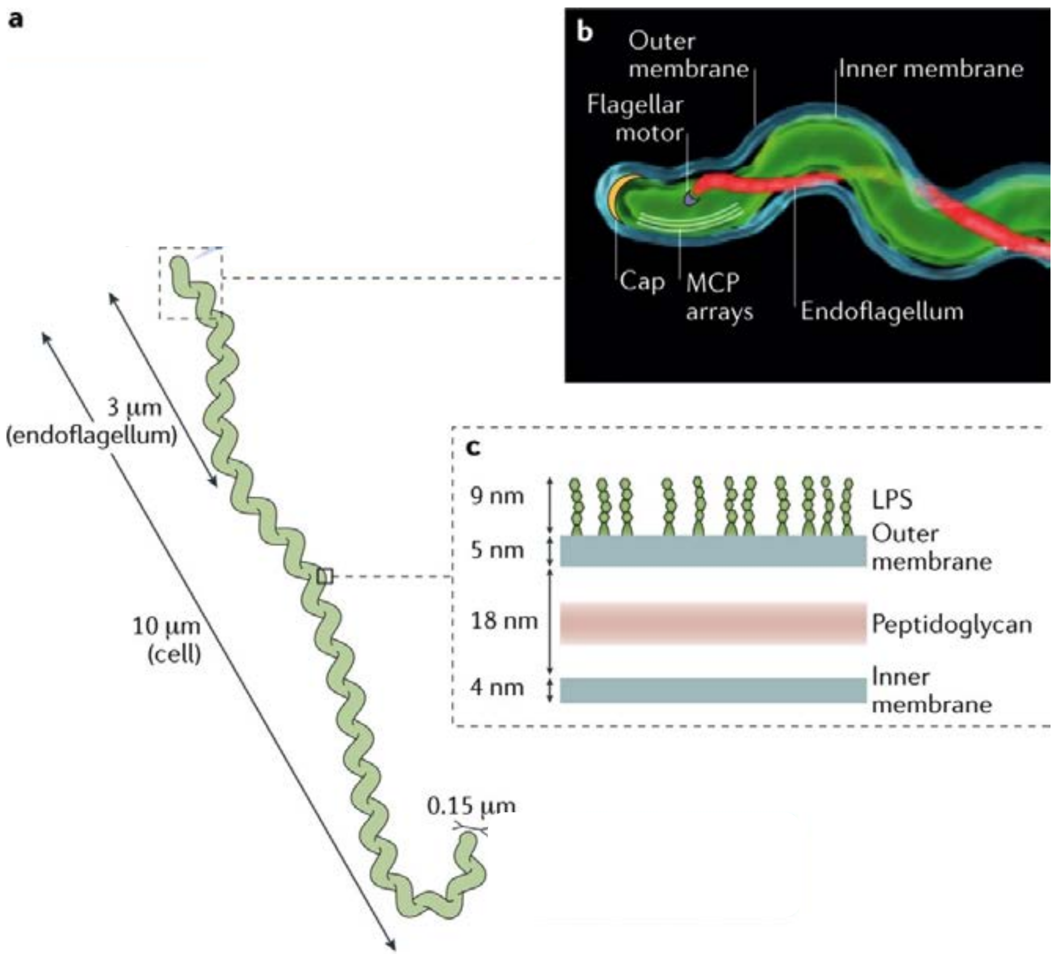
- Animal reservoirs represent the natural source of infection and of environmental contamination

Reservoir host	Host-adapted serovars
Pig	Pomona, Tarassovi
Cattle	Hardjo, Pomona
Horse	Bratislava
Dog	Canicola
Rat	Icterohaemorrhagiae, Copenhageni
Mouse	Ballum, Arborea, Bim
Bat	Cynopteri, Wolffi

Leptospirosis as a One Health model

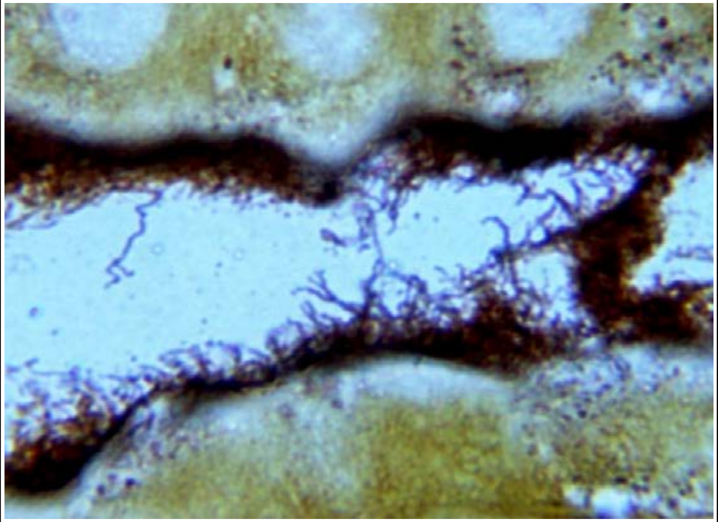


What does the agent of leptospirosis look like ?

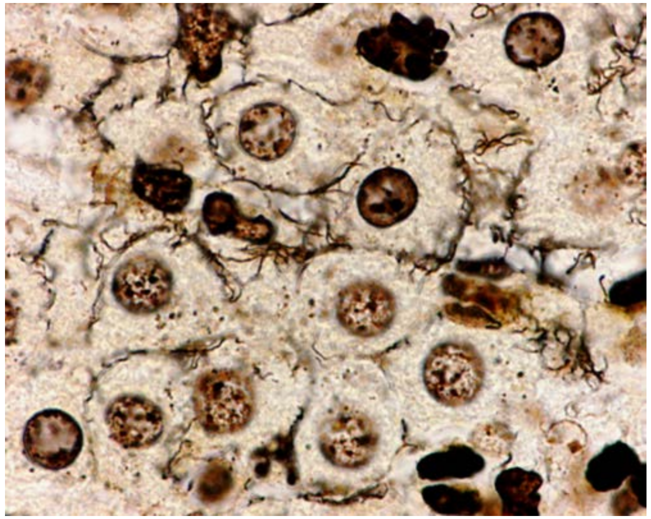


Picardeau 2017

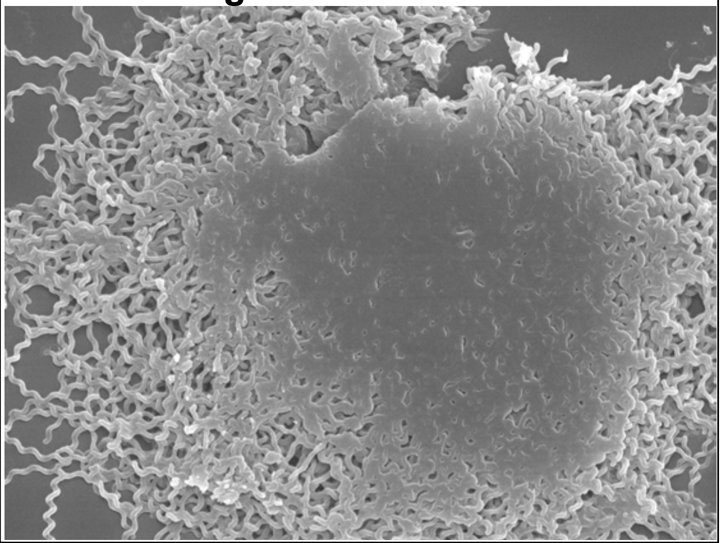
Kidney from *Rattus norvegicus*



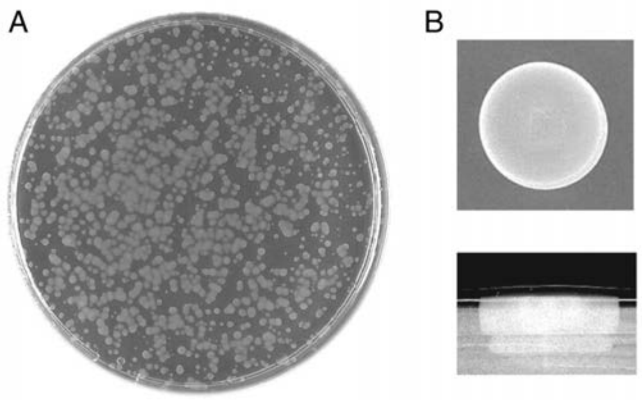
Liver from an infected guinea pig



Biofilm on a glass surface

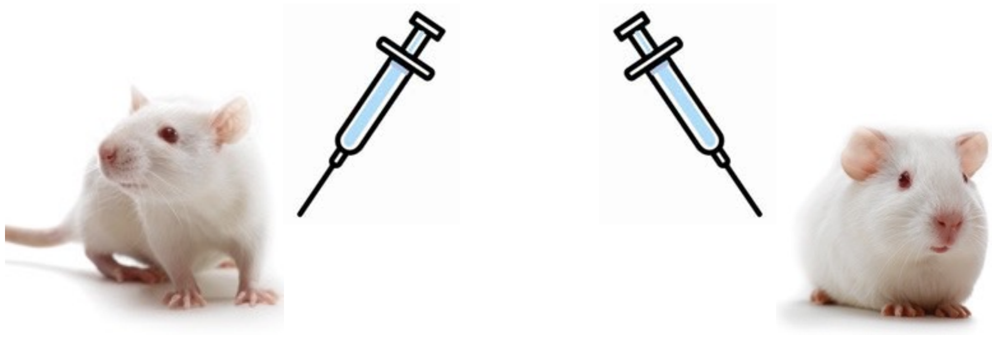


Sub-surface colonies



Animal models of leptospirosis

Leptospira interrogans



reservoirs

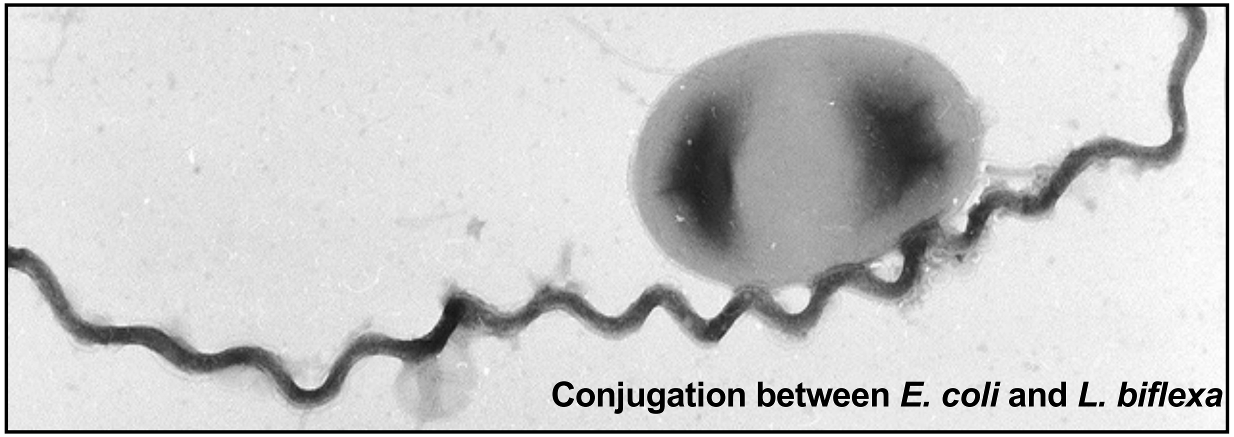
- Rats and mice : Chronic asymptomatic disease, with persistent carriage in the renal tubules

susceptible hosts

- Guinea-pigs, hamsters, or gerbils : Systemic infection with severe manifestations (hepatic and renal insufficiencies and hemorrhages)

Genetic toolbox for *Leptospira* spp.

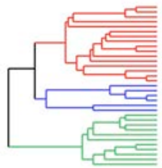
	Saprophytes (*L. biflexa*)	**Pathogens (*L. interrogans*)**	
Doubling time (liquid medium)	5 hours	20 hours	
Colonies (solid medium)	1 week	4 weeks	
Targeted mutagenesis	++	-/+	Allelic exchange, Cas9+NHEJ, Cas9-PE
Gene silencing	+	+	TALE, dCas9
Random Tn mutagenesis	+++	+	*Himar*1
Replicative vector(s)	yes	yes	pGKLep, pMaORI



Conjugation between *E. coli* and *L. biflexa*

Whole-genome sequencing of *Leptospira*

- Gene content and genome organization
- Comparative genomics:
  - Phylogenetic tree (diversity)



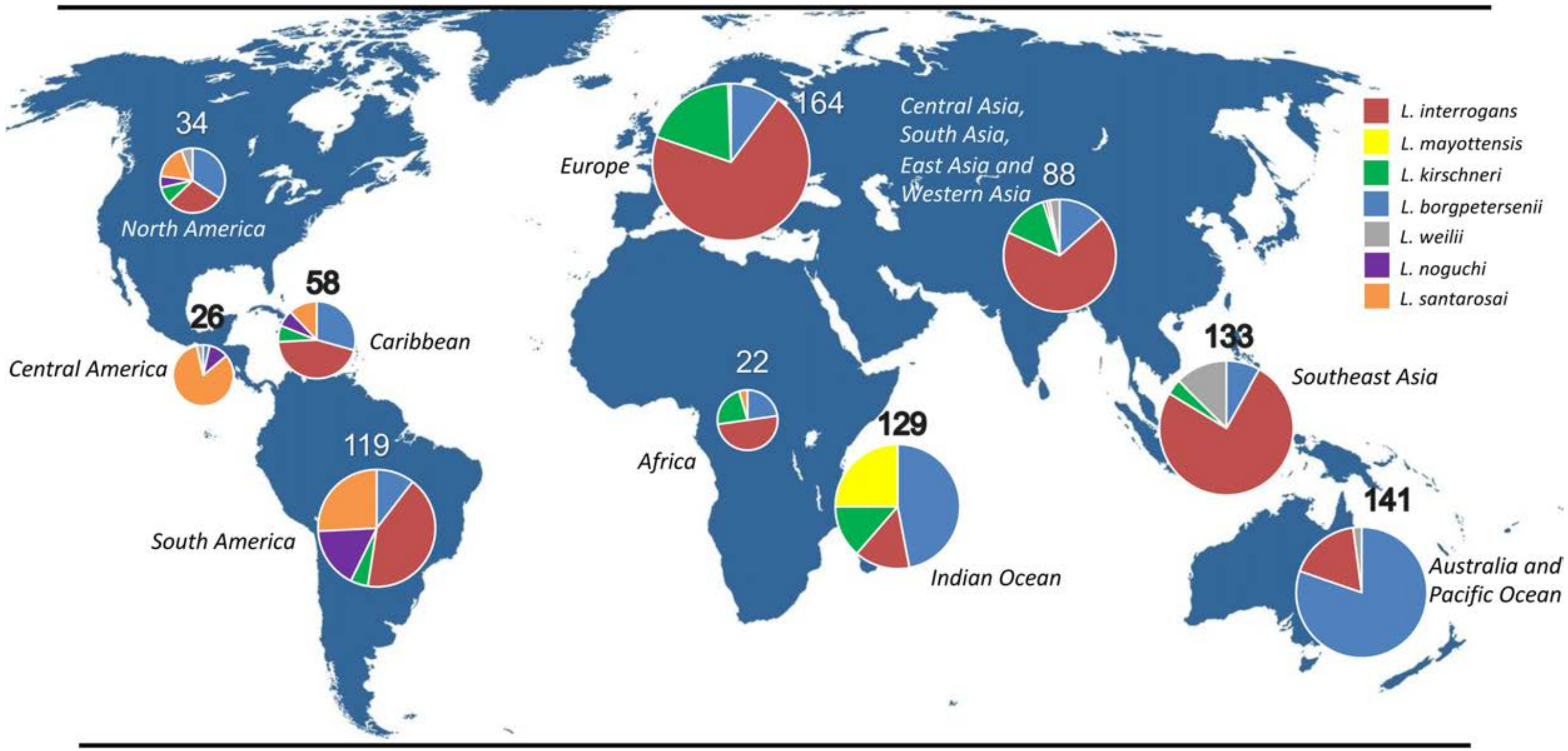
  - Pangenome analysis

  - Global mapping of circulating strains
  - Virulence evolution

What are the agent of leptospirosis circulating worldwide?

- A great biodiversity in the genus, probably still under-estimated
  - 69 species, including 8 pathogenic species (*L. interrogans*, etc)
  - >300 serovars (Icterohaemorrhagiae, etc)
- Distribution of species and serovars varies at the region / country level
- Refine current molecular /serological diagnostic tools according to the local epidemiology
  - Use of local strains for MAT
  - For ELISA tests, evaluation of the recommended cutoff
  - Use of PCR assays that allows the detection of locally circulating strains

Geographic origins of the most frequent pathogenic *Leptospira* species in our genome database (n = 914)



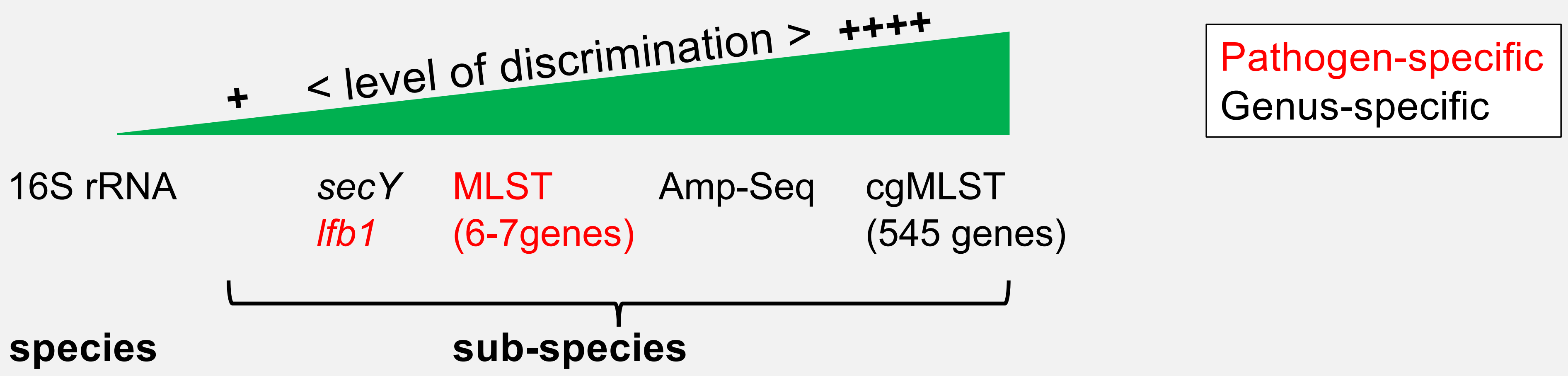
Chinchilla *et al.* PLoS Negl Trop Dis. 2023

Genetic diversity in *Leptospira*

- Accurate description of microbial diversity is the first step towards:

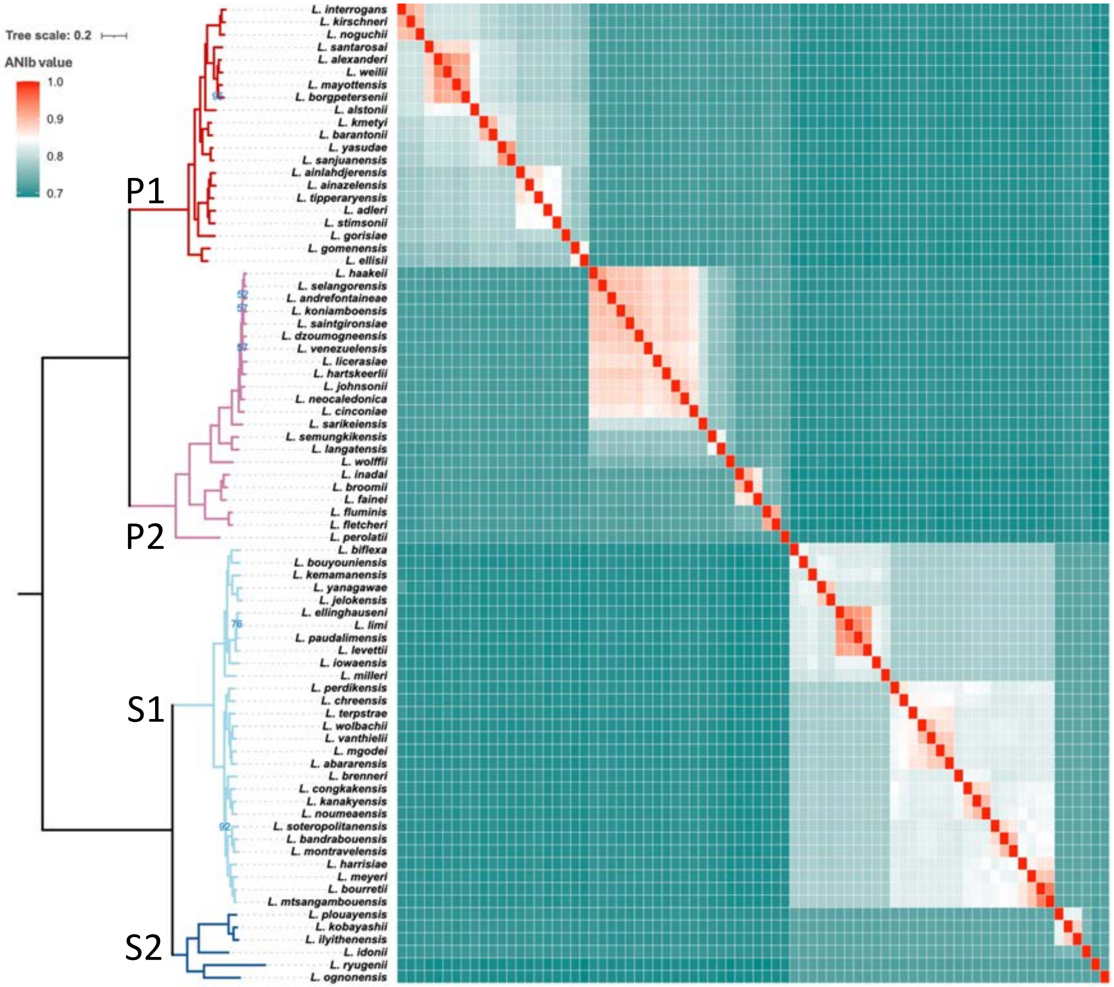
i) improving diagnostic tools
ii) identifying animal reservoirs
iii) tracking emergence of clones
iv) generating control strategies (eradication of reservoirs, vaccines, etc)

- Culture isolation is challenging
- *Leptospira* are slow-growing bacteria

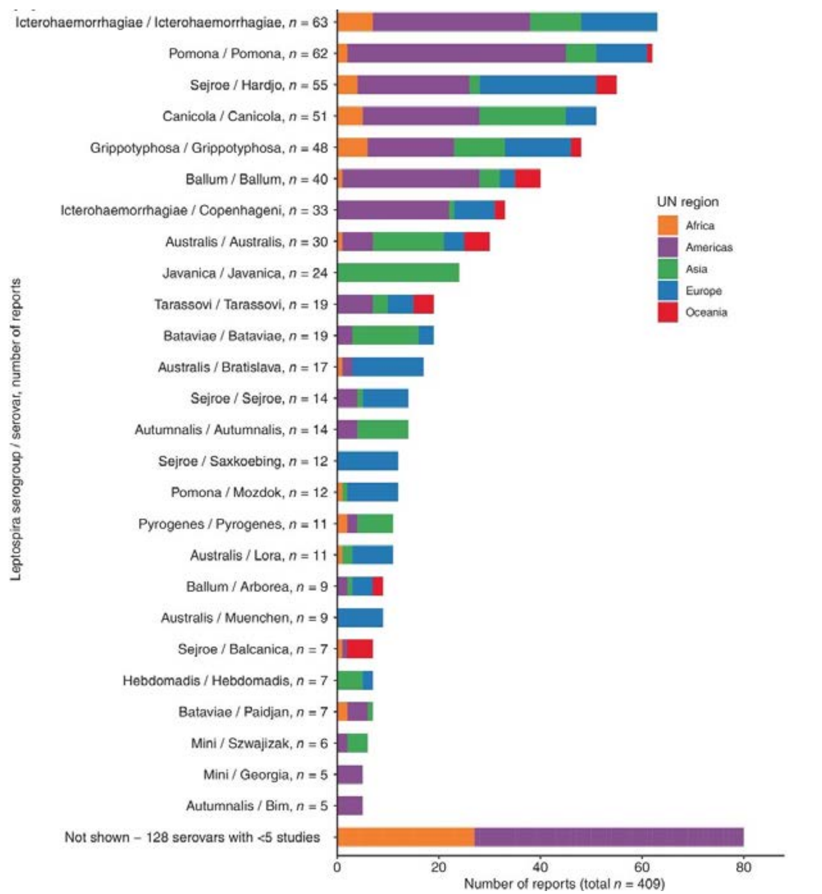


Leptospira: a highly heterogeneous genus

- 77 species and >250 serovars (150 with data on animal hosts)

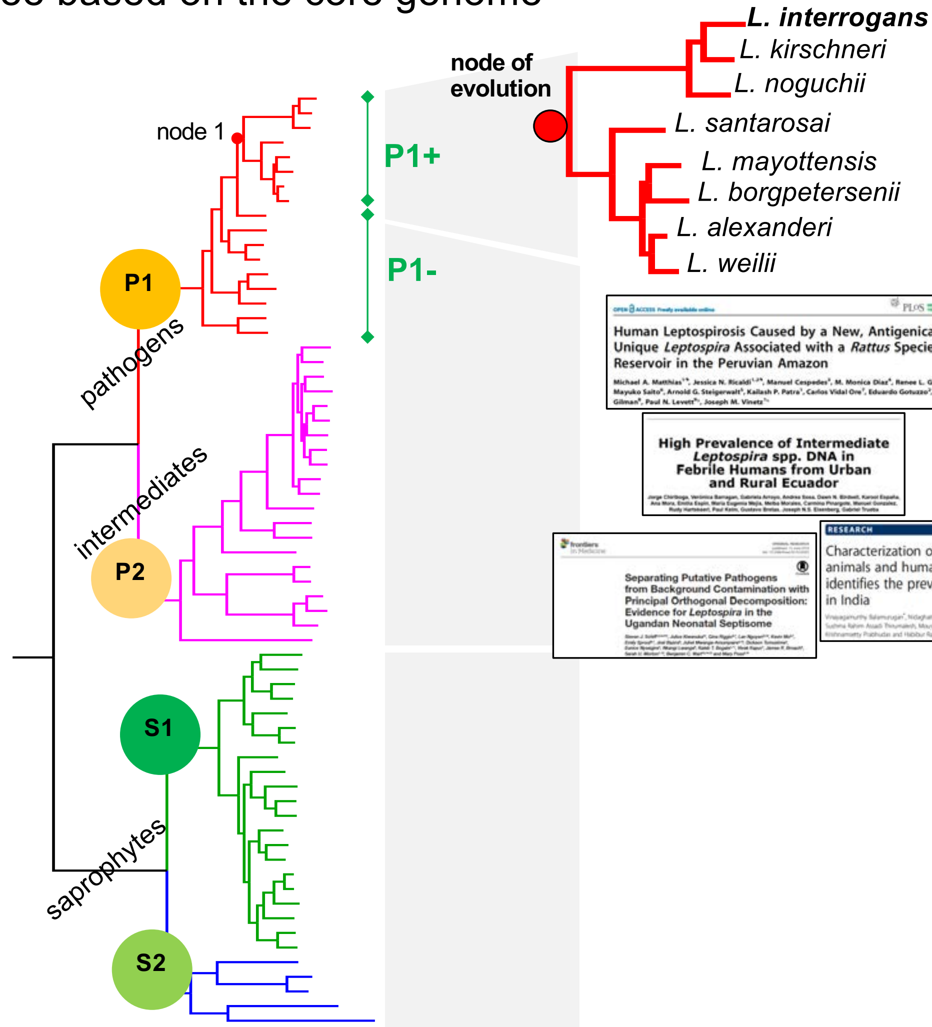


Hagedoorn et al. 2024



Only P1+ species are responsible for severe infections in both human and animals

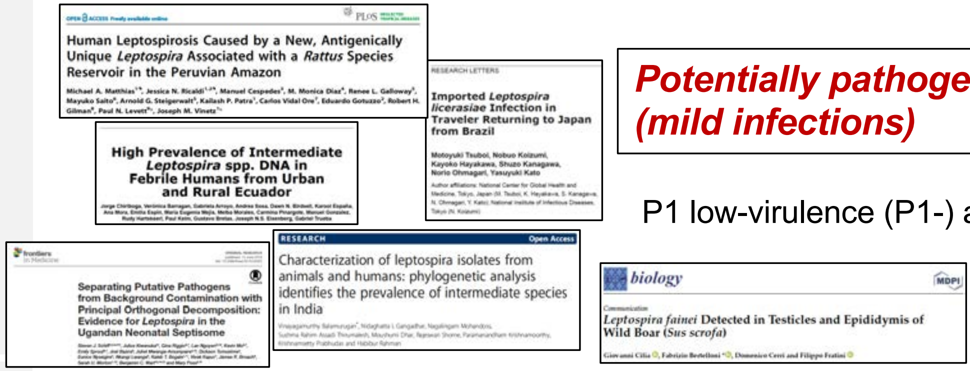
Phylogenetic tree based on the core genome



← Responsible for the most severe infections

Pathogens (mild to severe infections)

P1 high-virulence (P1+)

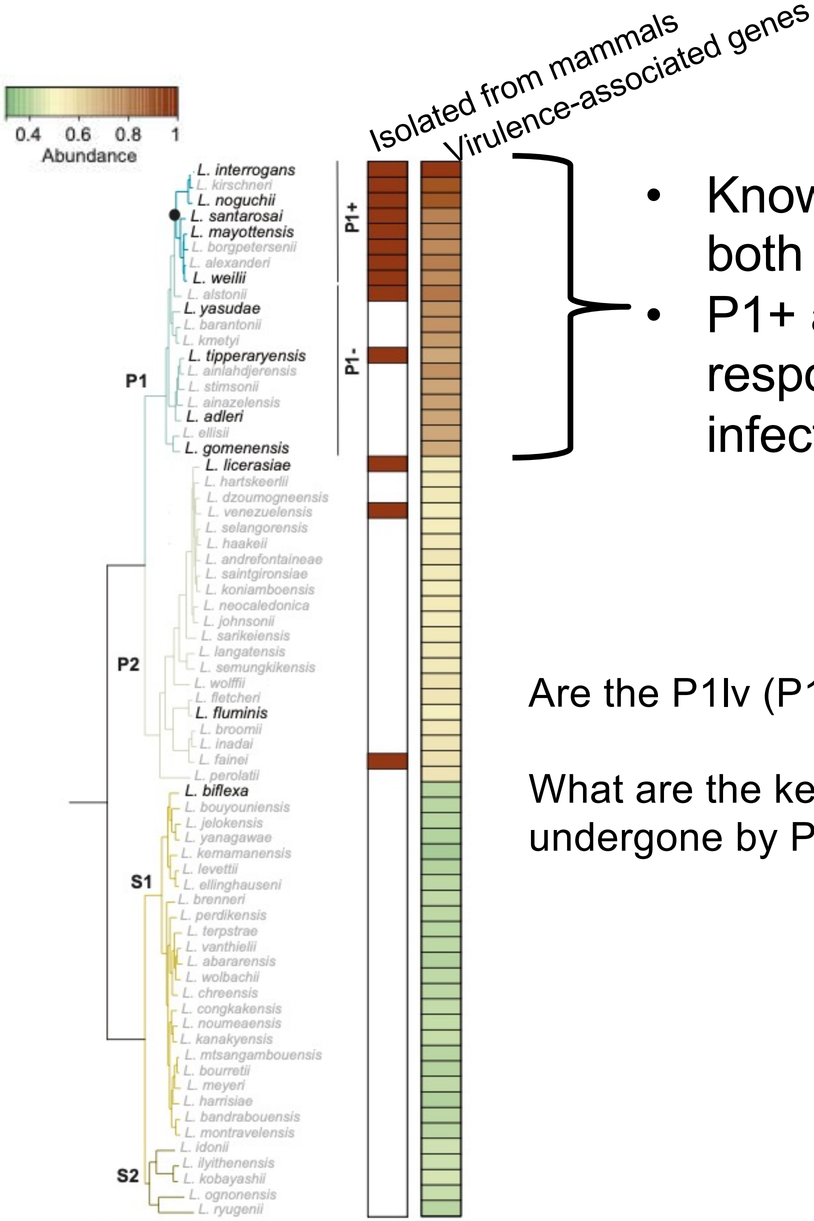


Potentially pathogens (mild infections)

P1 low-virulence (P1-) and P2

Non pathogens

S1 and S2



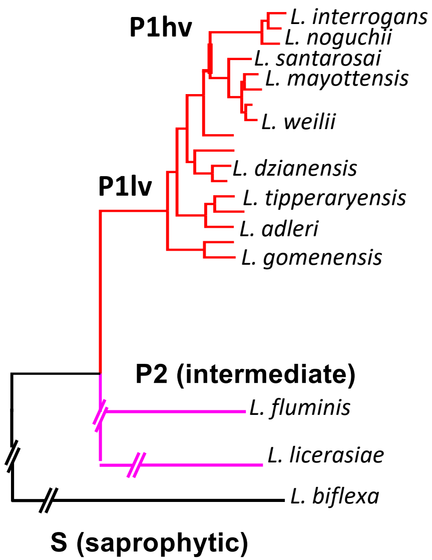
- Known virulence factors are present in both P1+ and P1
- P1+ are isolated from mammals and responsible for the most severe infections

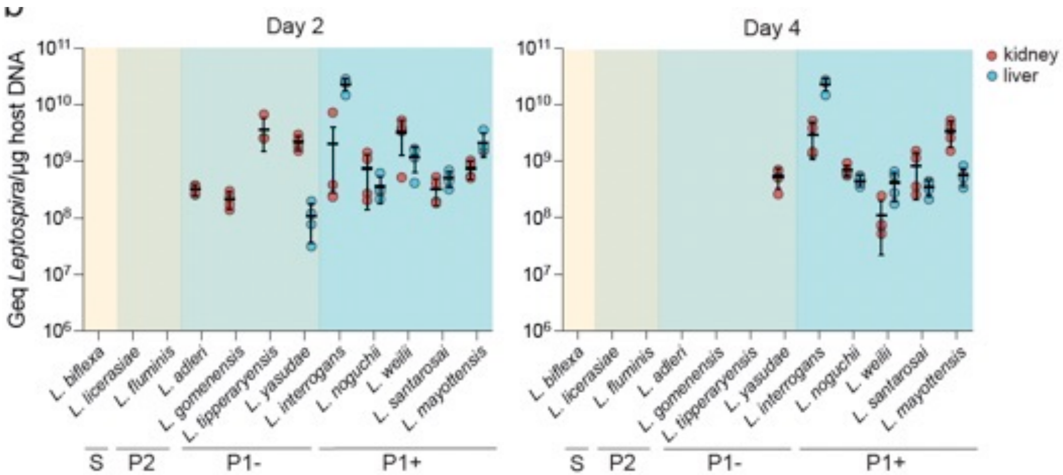
Are the P1lv (P1-) potentially pathogens ?

What are the key genetic and phenotypic changes undergone by P1hv (P1+) ?

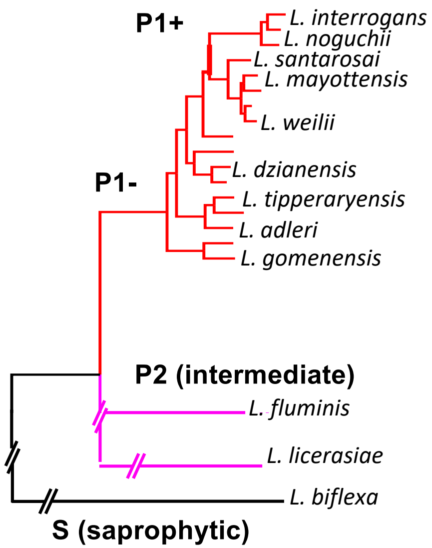
P1- are not virulent in the hamster model

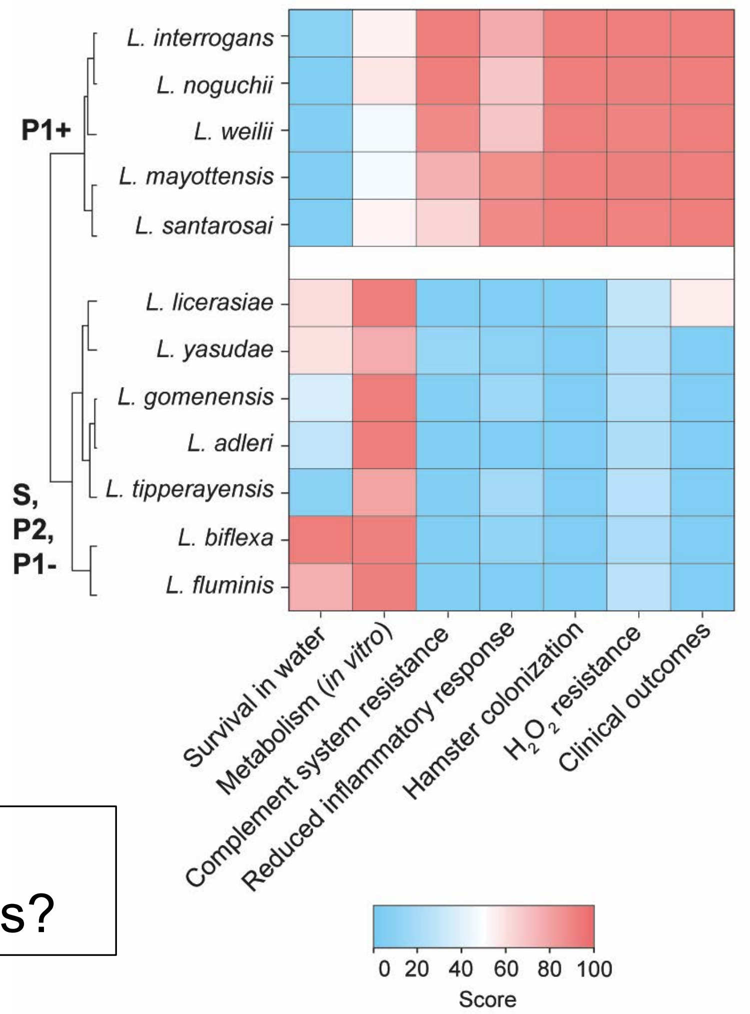
infection in hamsters





Only P1+ species share virulence properties



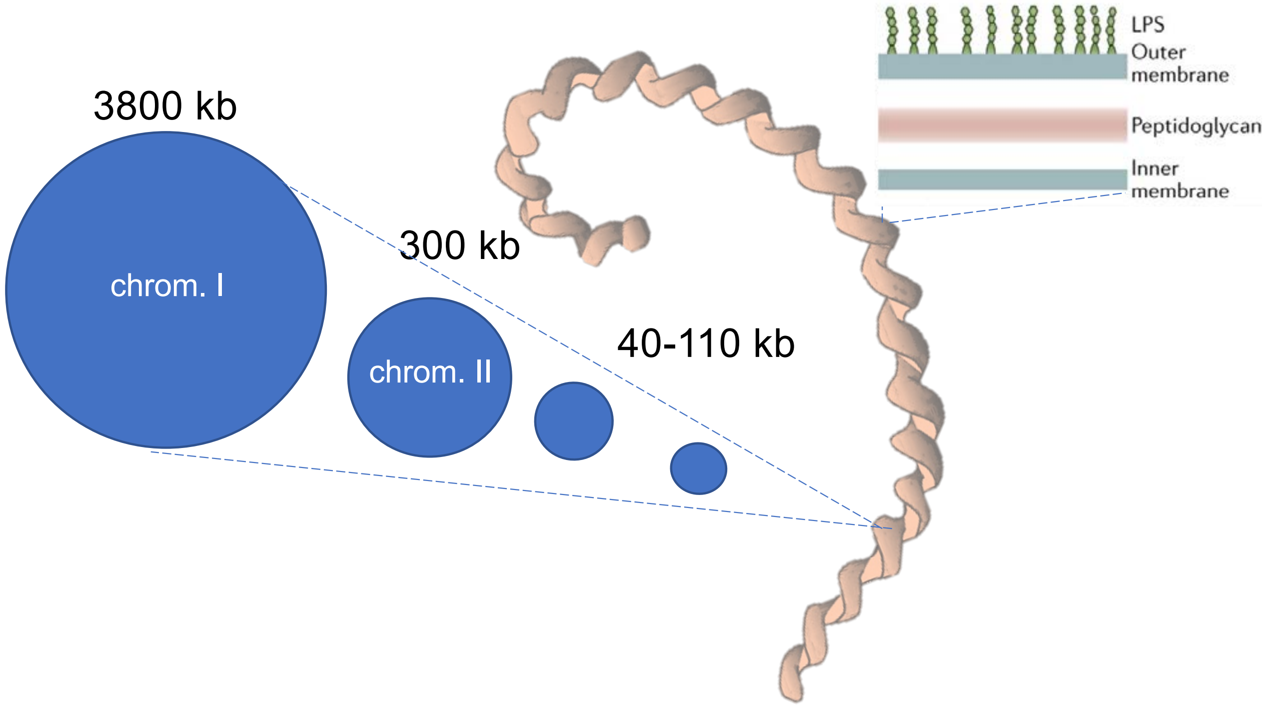


Giraud-Gatineau et al. 2023

- Are certain virulence traits specific to groups of species, individual species, serovars, or even strains?

Spirochetes make it differently

- Helical-shaped bacteria
- 2 chromosomes and plasmids
- Diderms
- Endoflagellar system
- Surface-exposed LPS



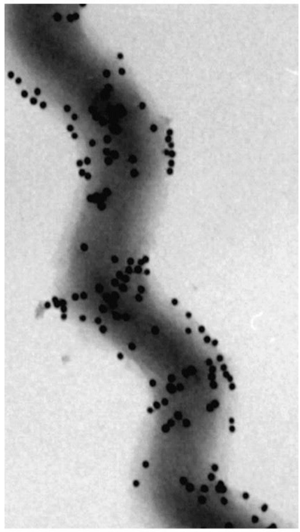
Pathogenic *Leptospira* :

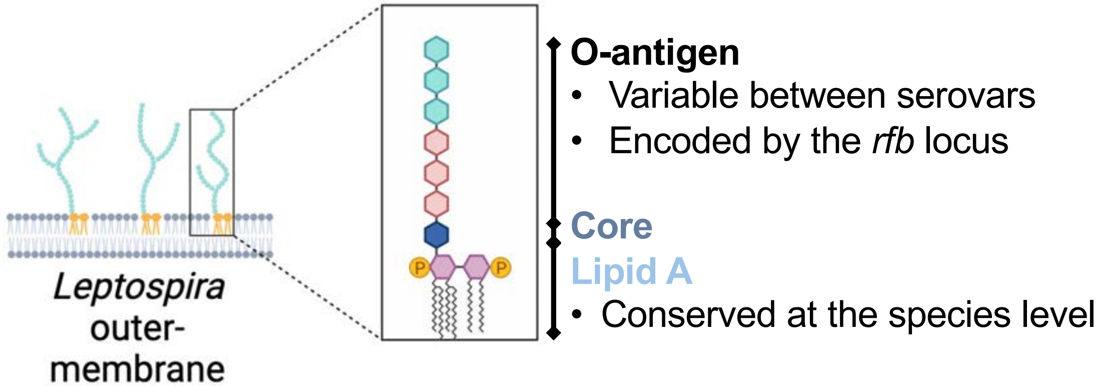
- Extracellular bacteria
- Stealth pathogens (escape complement killing and recognition by innate immune cells)
- No type III to type X secretions systems
- No pathogenicity islands or virulence plasmids
- Over-representation of genes of unknown function

The *Leptospira* lipopolysaccharide (LPS)

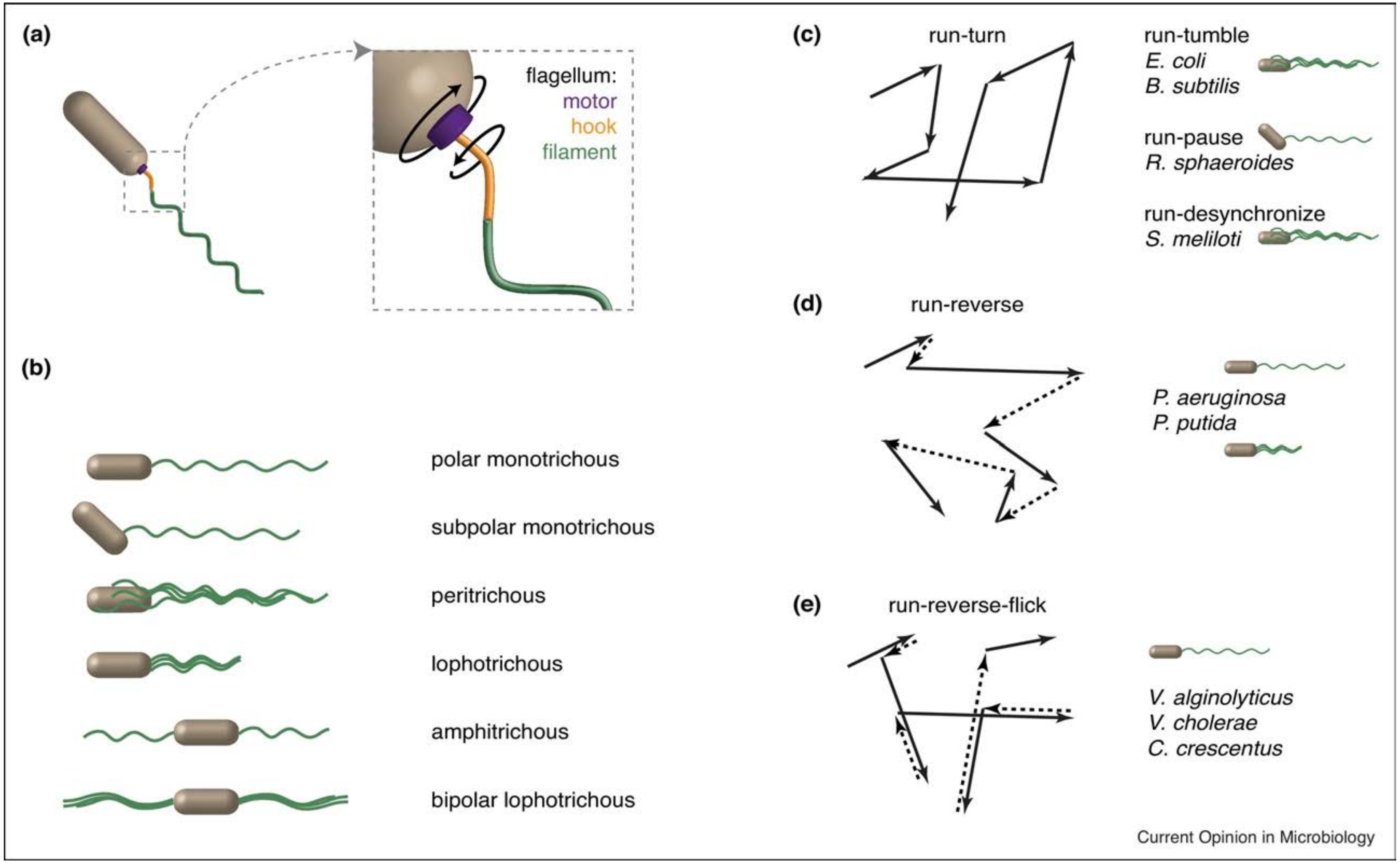
- A major surface-exposed antigen
- Not a human TLR4 agonist (Werts et al. 2001) but is recognized by mouse TLR4 (Nahori et al. 2005)
- Essential for virulence (Eshghi et al. 2009, Murray et al. 2010)
- Lipid A : low-toxicity, the atypical structure varies depending on the species and group of species (Que-Gewirth et al. 2004, Pětrošová et al. 2023)
- O-antigen: encoded by the the *rfb* locus, whose gene composition determines the serovar (Chinchilla *et al.* 2024, Nieves *et al.* 2022)

L. interrogans coated with gold-labeled anti-LPS monoclonal antibodies (Haake et al. 2015)



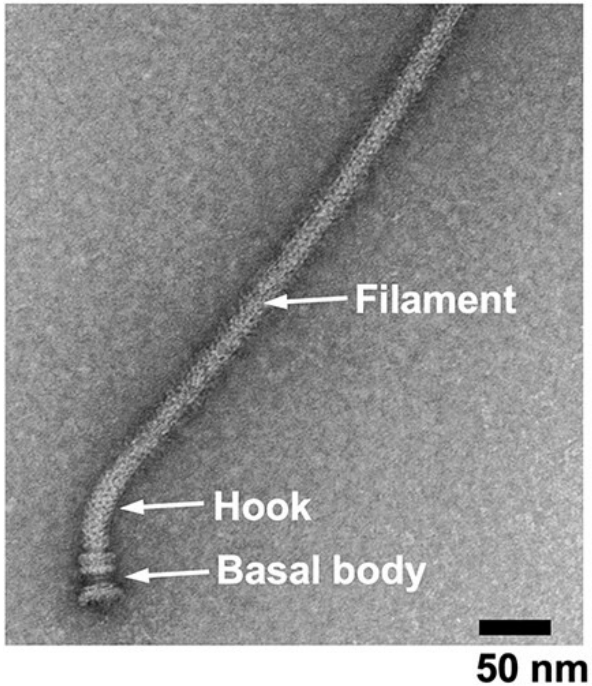


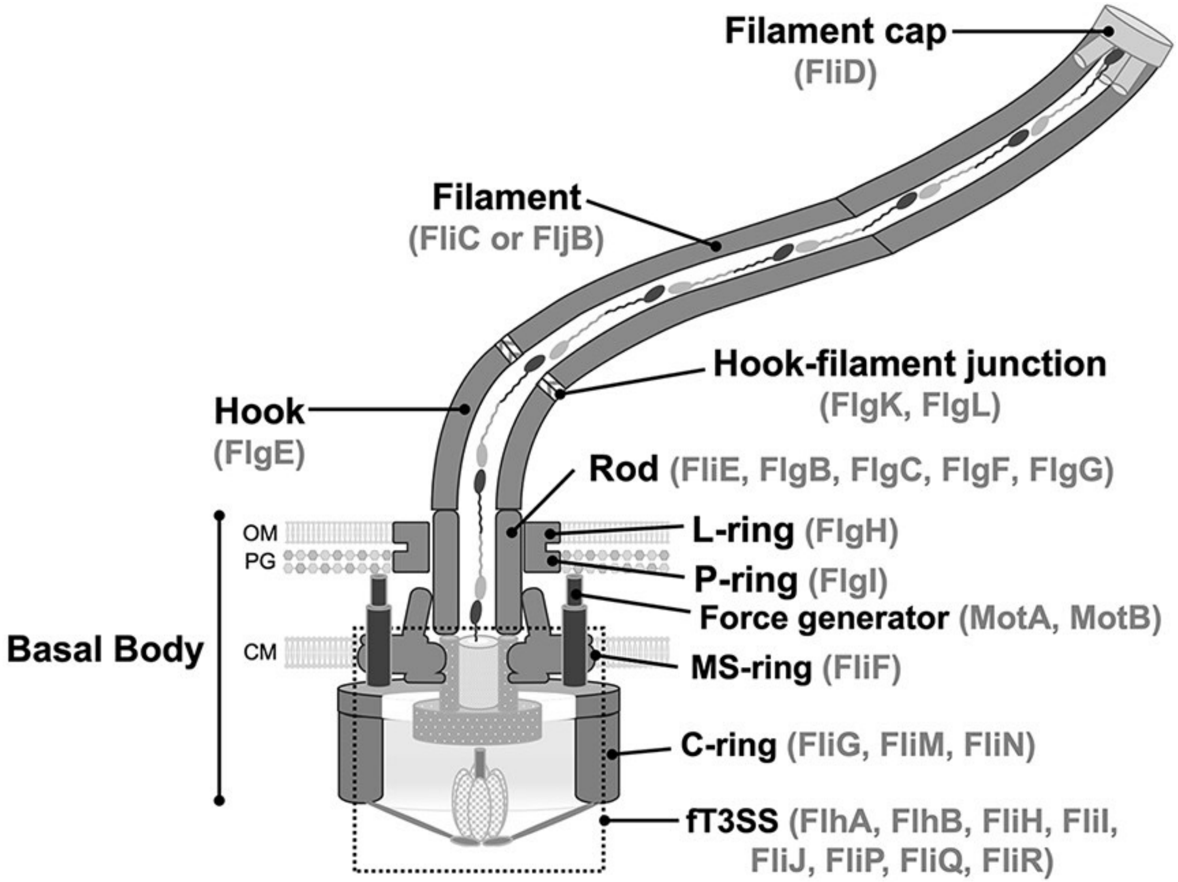
Basics of flagellar motility



Grognot et al. 2021

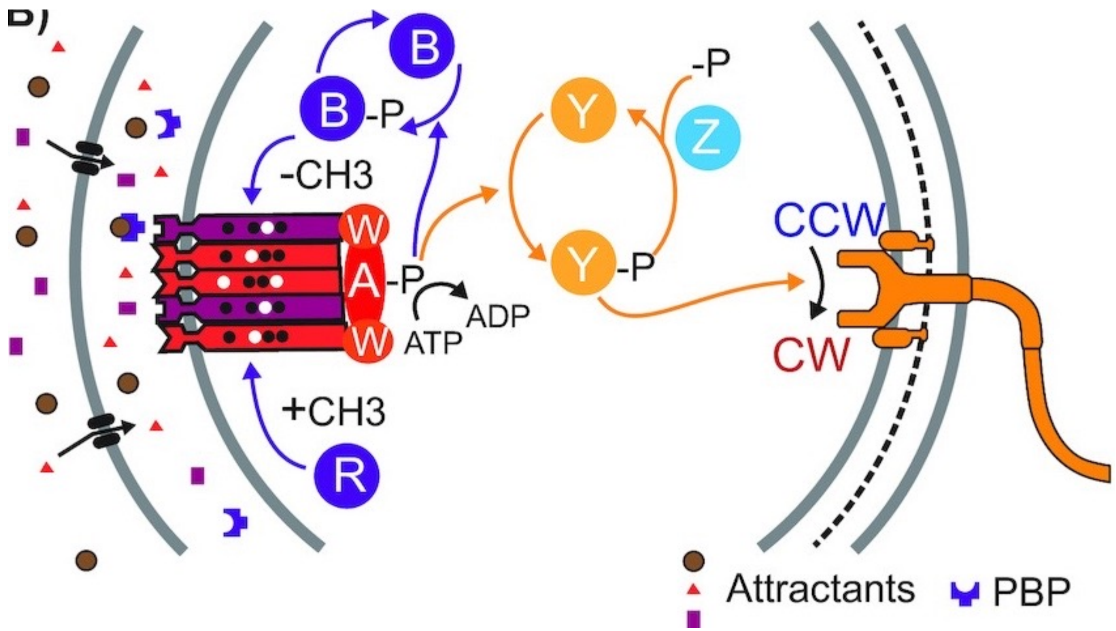
The model of the *Salmonella* flagellum





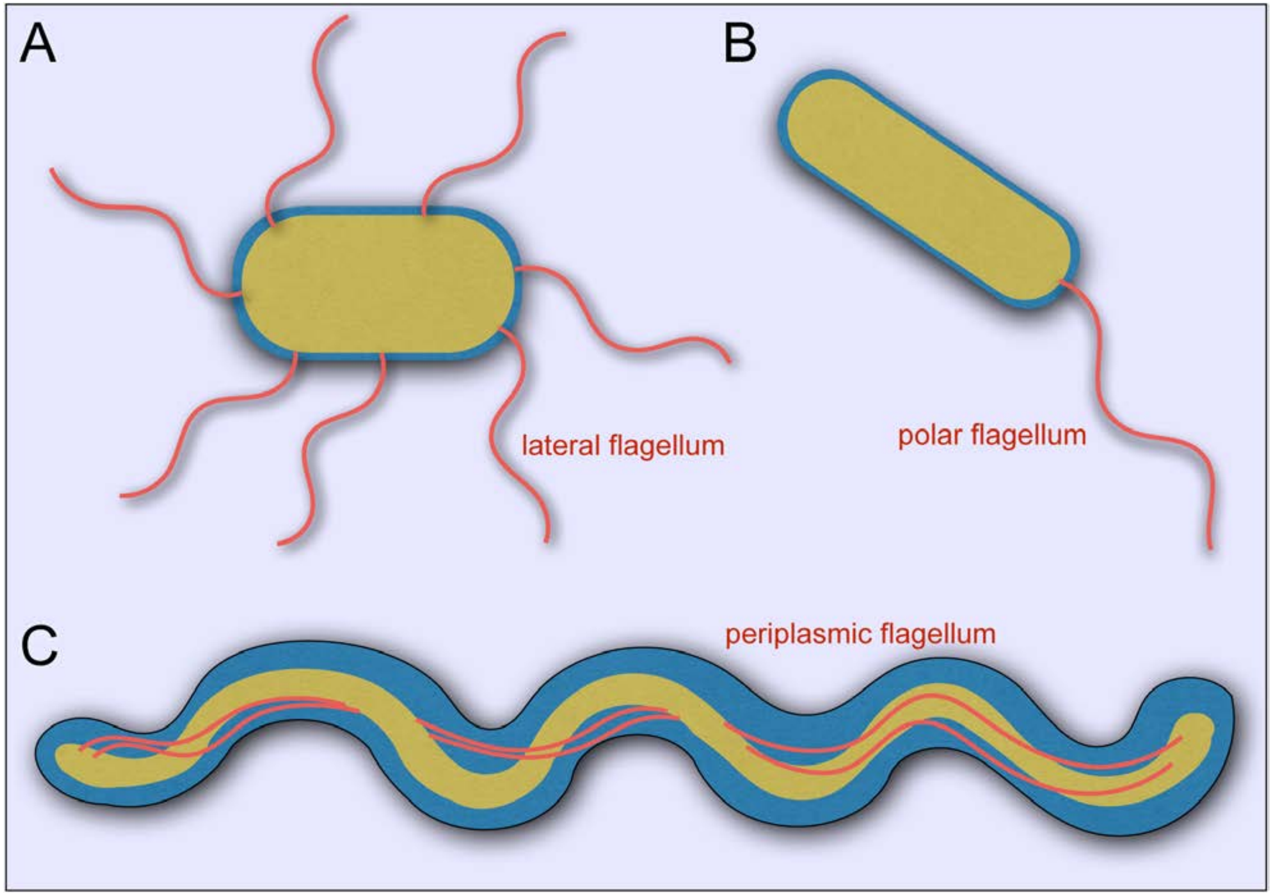
Minamimo et al. 2023

Chemotactic signaling pathway and flagellar motility



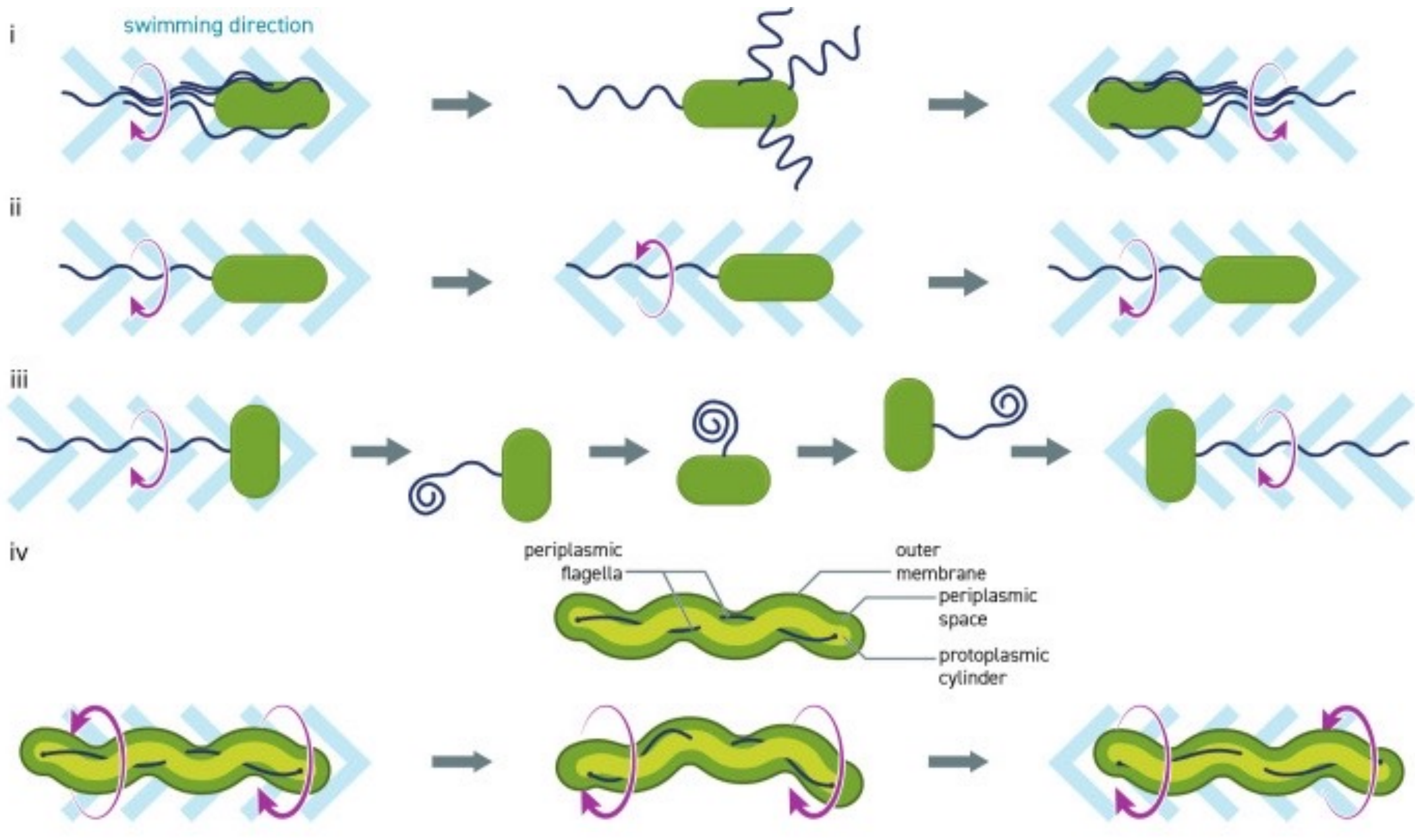
Colin et al . 2021

Bacteria flagella

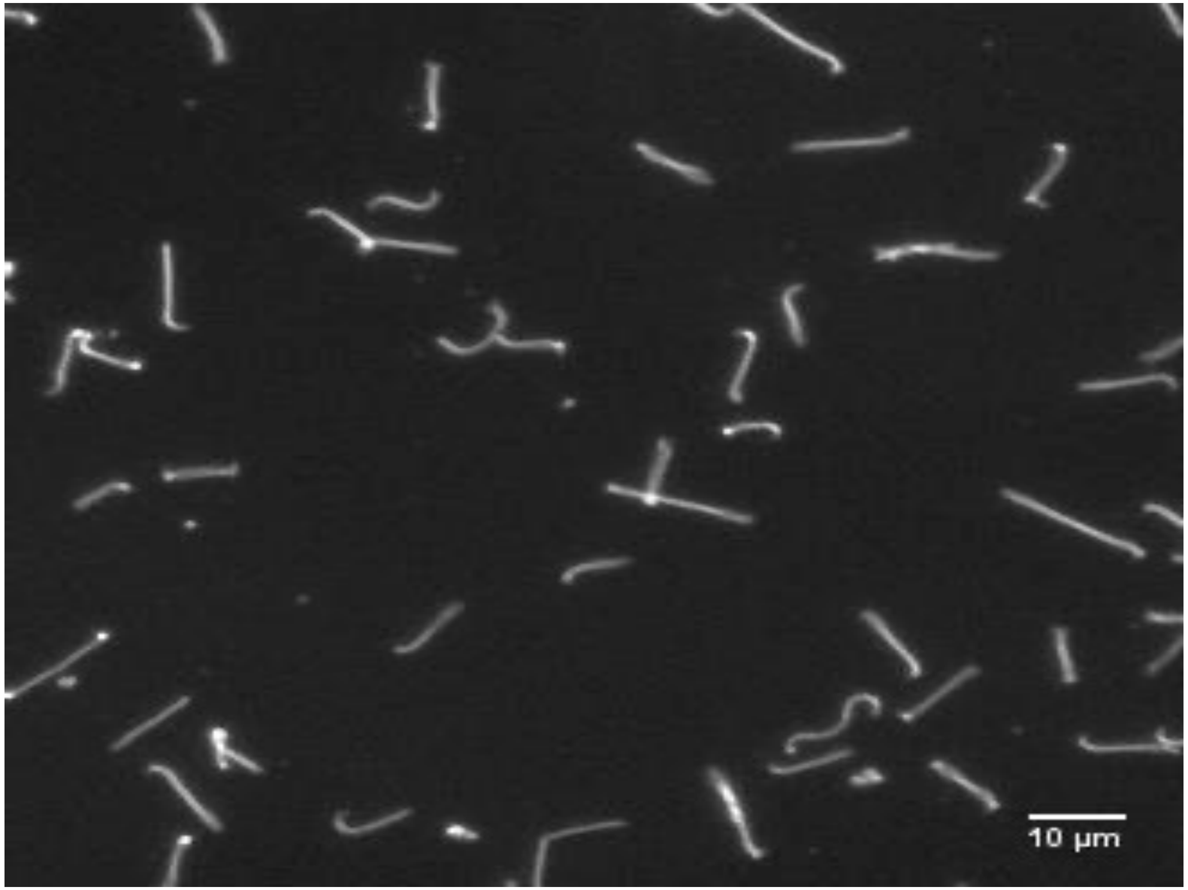


Chang et al. 2019

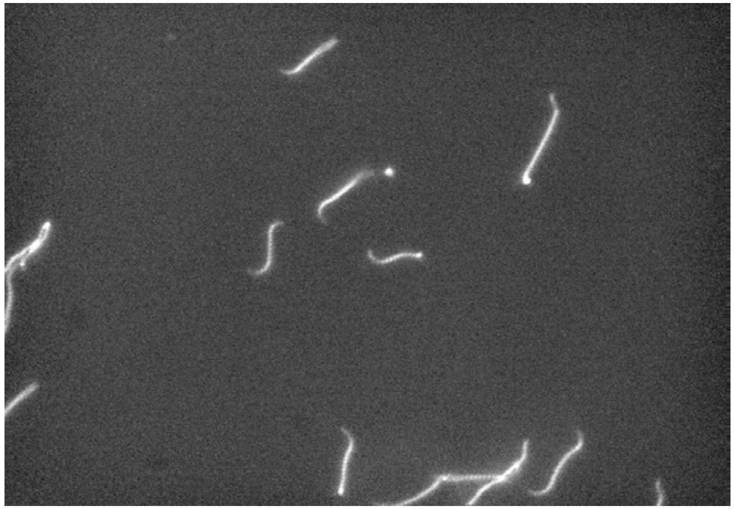
Flagella patterns and associated swimming behaviour

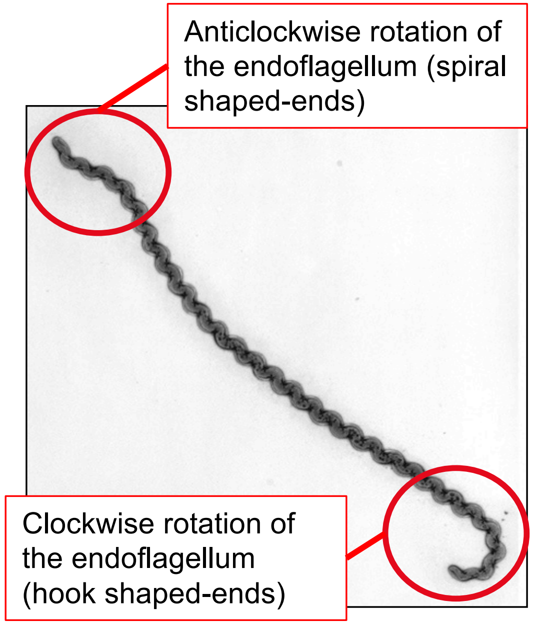


Armitage 2024

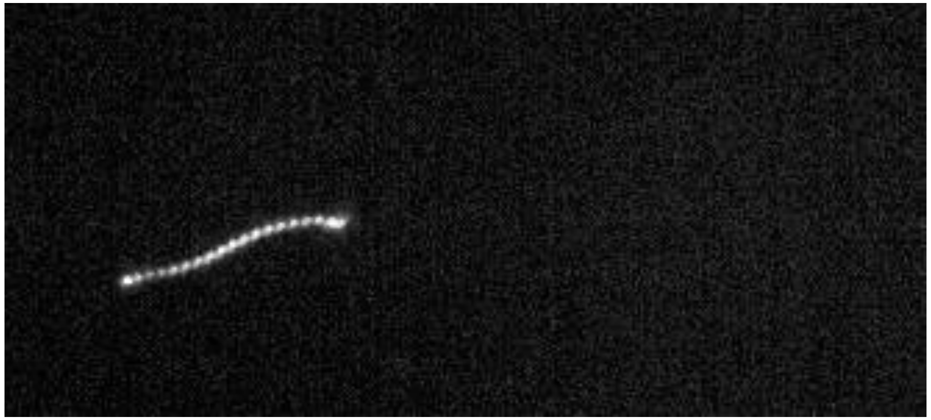

10 µm

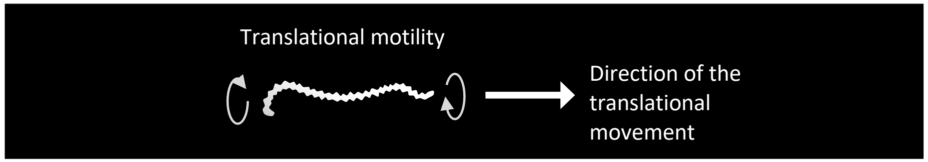
A bacteria with corskrew motility



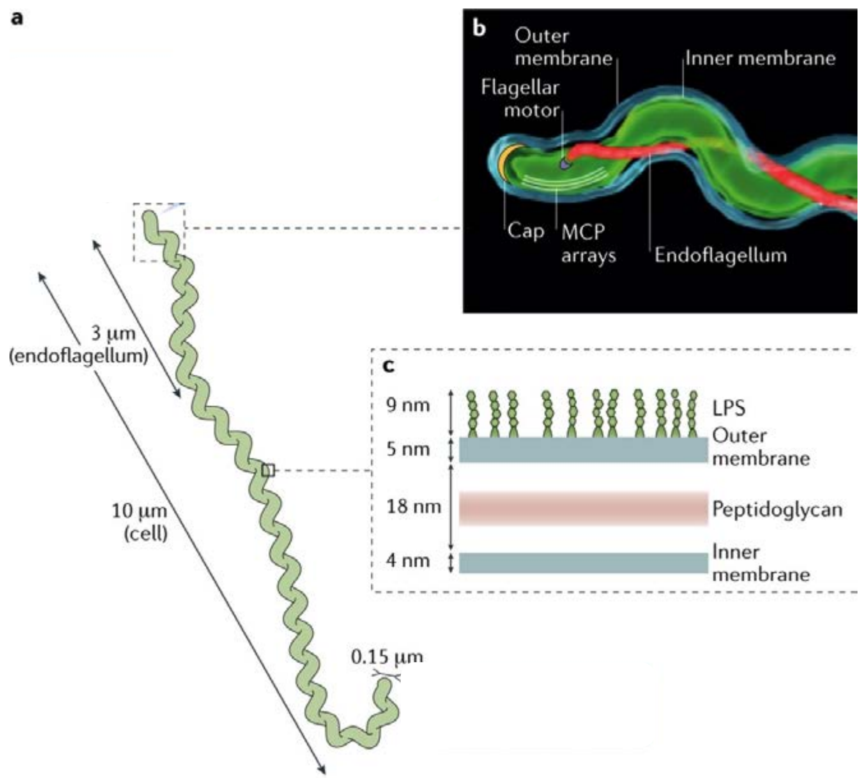


A bacteria with corskrew motility





What does the agent of leptospirosis look like ?



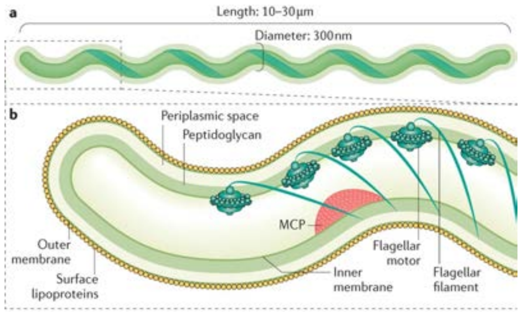
Picardeau 2017

Comparison of the cell structure, the periplasmic flagella, motility modes and adhesins associated with motility among spirochete species

Spirochete	Number of flagella	Morphology	Motility mode	Adhesion molecules associated with invasion and motility
Borrelia	~20	Flat-wave, planar	Swimming	DbpA/B, BBK32, P66, VlsE, OspC
Brachyspira	~20	Helical	Swimming	–
Treponema	~4–6	Flat-wave (*T. pallidum*), helical (*T. denticola*)	Swimming and crawling (*T. denticola*)	Msp
Leptospira	2	Helical	Swimming crawling	LigA, LenA

Strnad et al. 2024

***Borrelia* spp.**



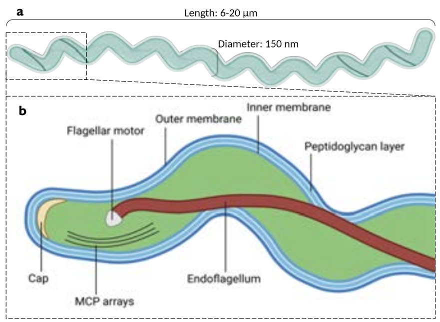
Flat wave

8-11 endoflagella/cell end

Long and thin

diderm bacteria

***Leptospira* spp.**



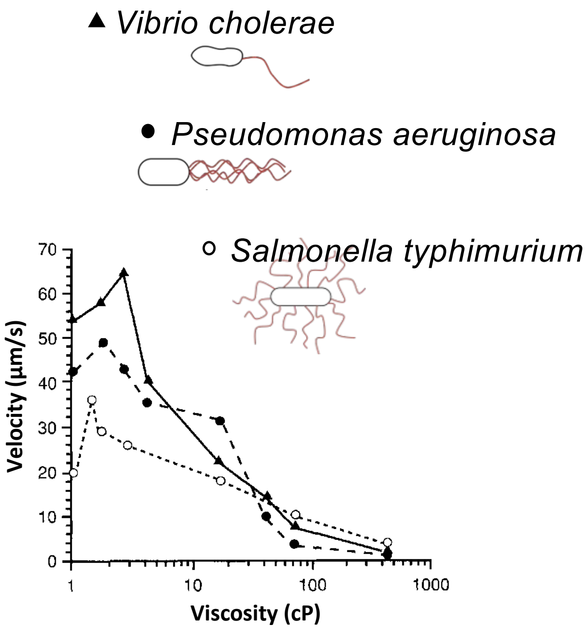
Helical-shaped

1 endoflagellum/ cell end

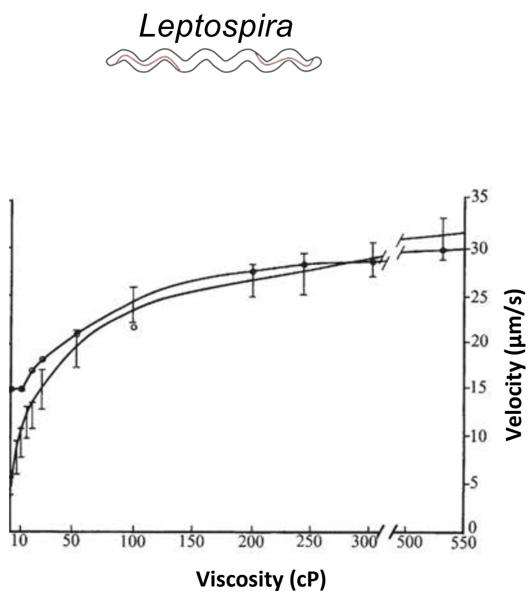
Steere et al, 2016

Velocity of *Leptospira* increases in viscous environments

Effect of viscosity on velocity of exoflagellated and endoflagellated bacteria

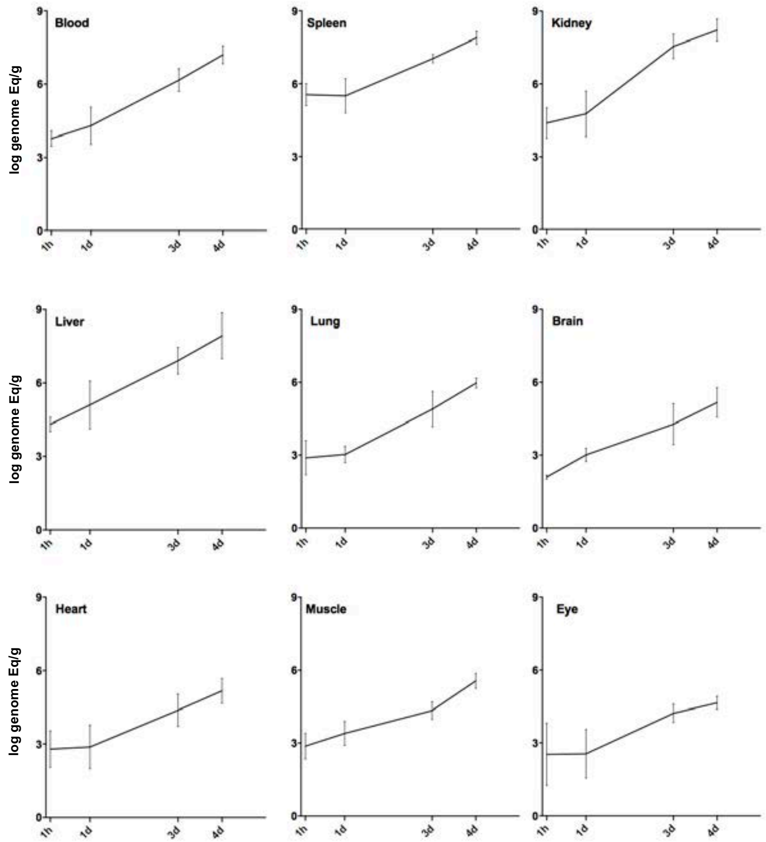


Shigematsu *et al.* J Med Microbiol1988



Kaiser *et al.* Nature 1975

Leptospira rapidly disseminate hematogenously to all tissues



L. interrogans

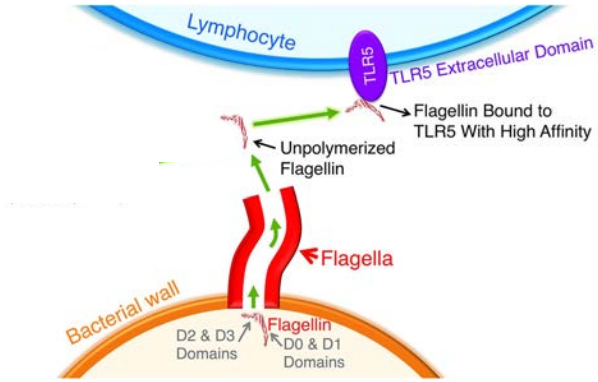
- Intraperitoneal infection
- Animals were euthanized at different time points
- Each hamster was perfused with a saline solution
- Organs collected
- DNA extraction
- Determination of burden by qPCR

Wunder *et al.* 2016

Leptospira escape early host detection during the infection

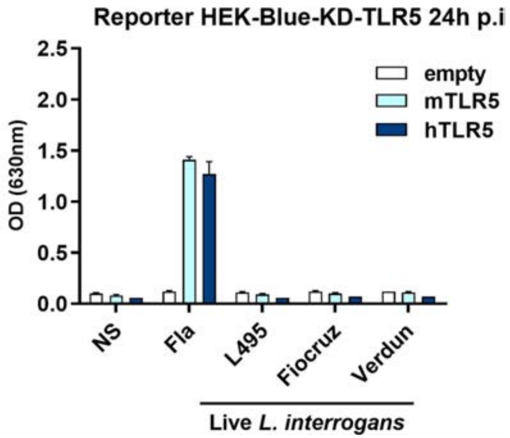
- Live *Leptospira* do not signal through TLR5

Exoflagellated bacteria



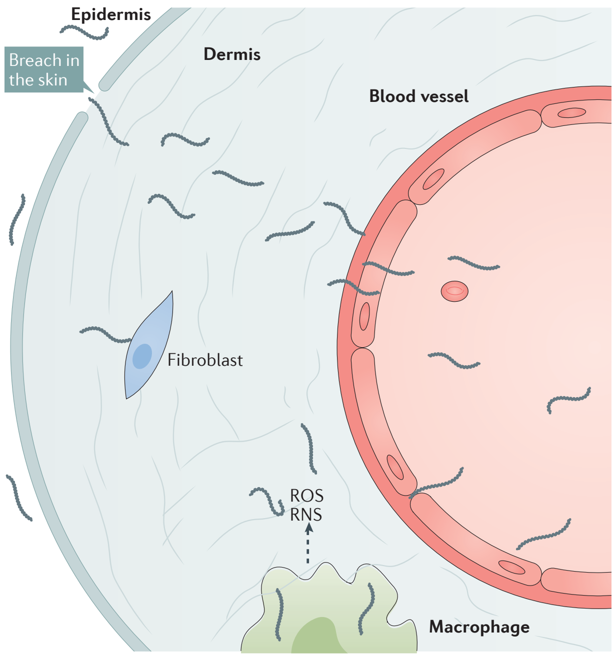
Lu and Swartz 2016 *Sci Rep*

Leptospira



Holzapfel … Werts. 2020. Front Immunol

Motility is essential in host infection



Stages of the infection process :

Pathogen entry and dissemination
- **Chemotaxis**
- **Motility**
- **Tissue penetration**
- Adhesion
- Inhibition of wound repair and blood clotting

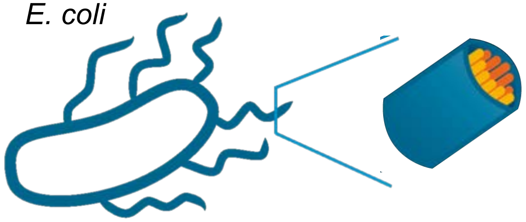
Pathogen adhesion
- Adhesion to extracellular matrix
- Adhesion to host cells

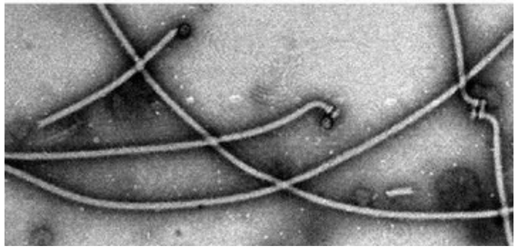
Persistance
- Nutrient acquisition
- **Immune evasion**
- Resistance to stress conditions

Damage host tissues
- Inflammation
- Vascular damage
- Lung haemorrhage
- Renal failure
- Jaundice
- Autoimmunity-uveitis

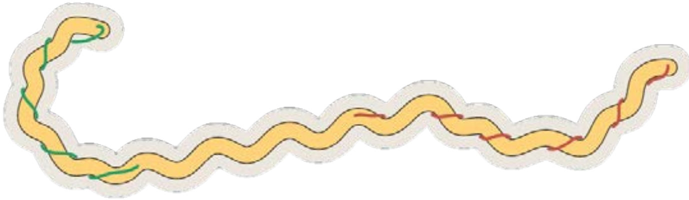
Renal colonisation
- **Traverse tissue barriers**
- Adhesion
- Transmission to new host

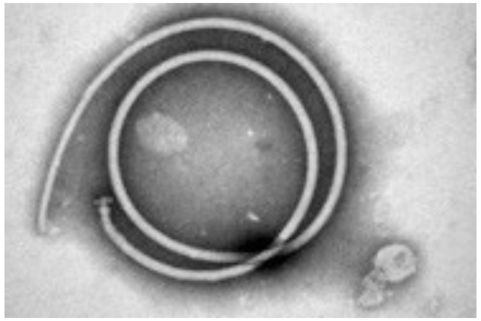
Exoflagellated vs endoflagellated bacteria





Purified flagella



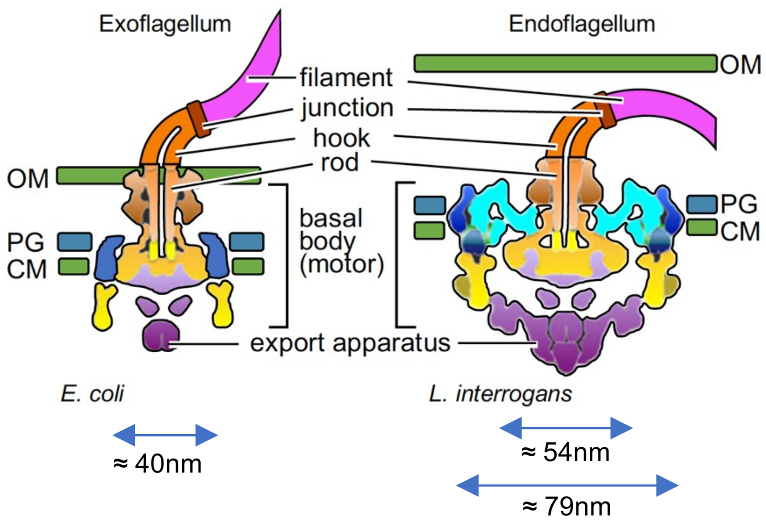


Purified flagella (supercoiled)

The endoflagellum, a complex nanomachine

In situ flagellar motor structures determined by cryo-ET and subtomogram averaging (adapted from Zhao *et al.* 2014)

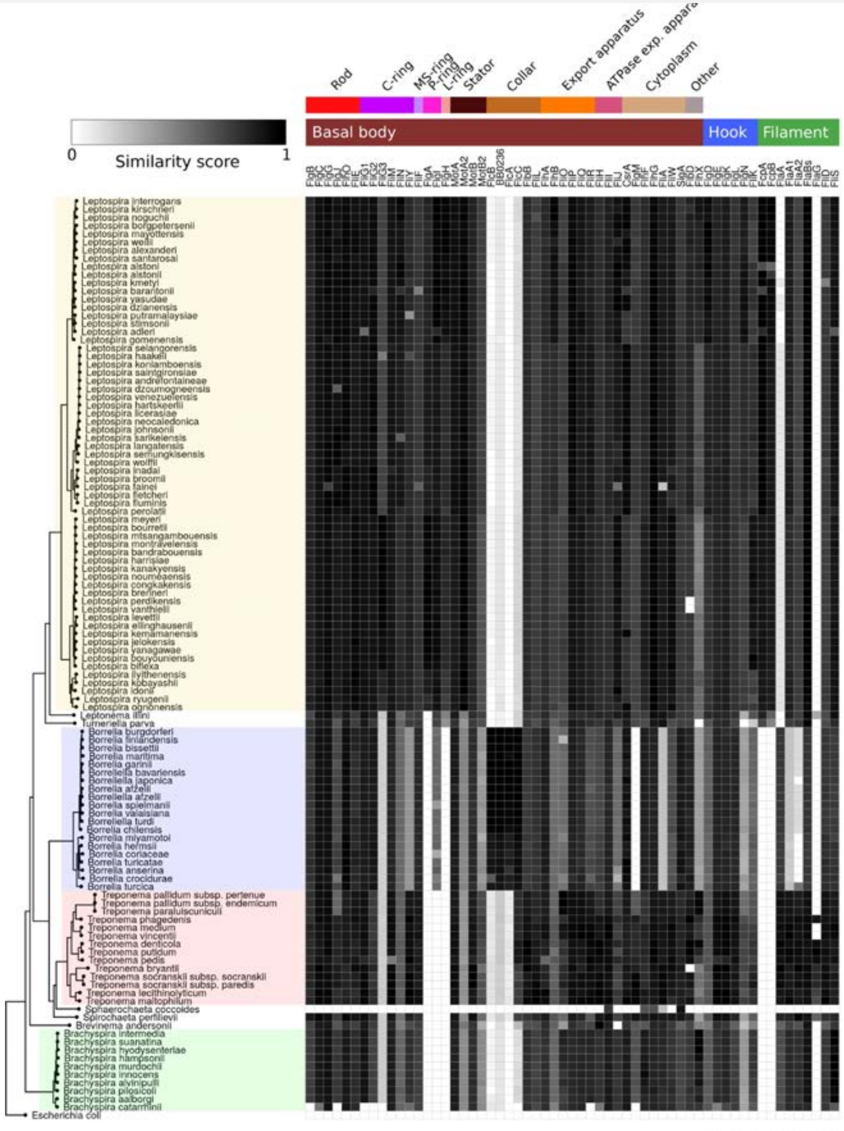
Filament:
- Gram positive and negative bacteria: a single flagellin protein
- Spirochetes : multi-protein complex



Hook:
- Heavily cross-linked in spirochetes

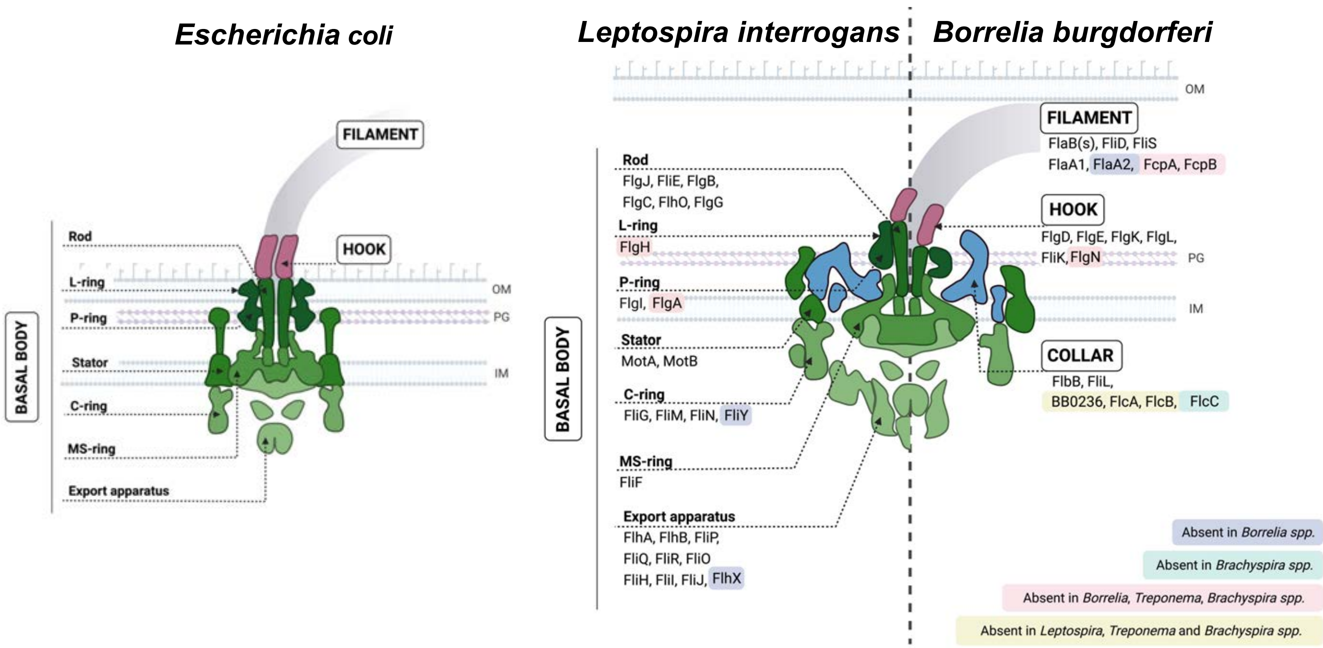
Basal body:
- Larger and more complex in spirochetes as compared with other bacteria

Distribution of flagellar protein-encoding genes in spirochetes



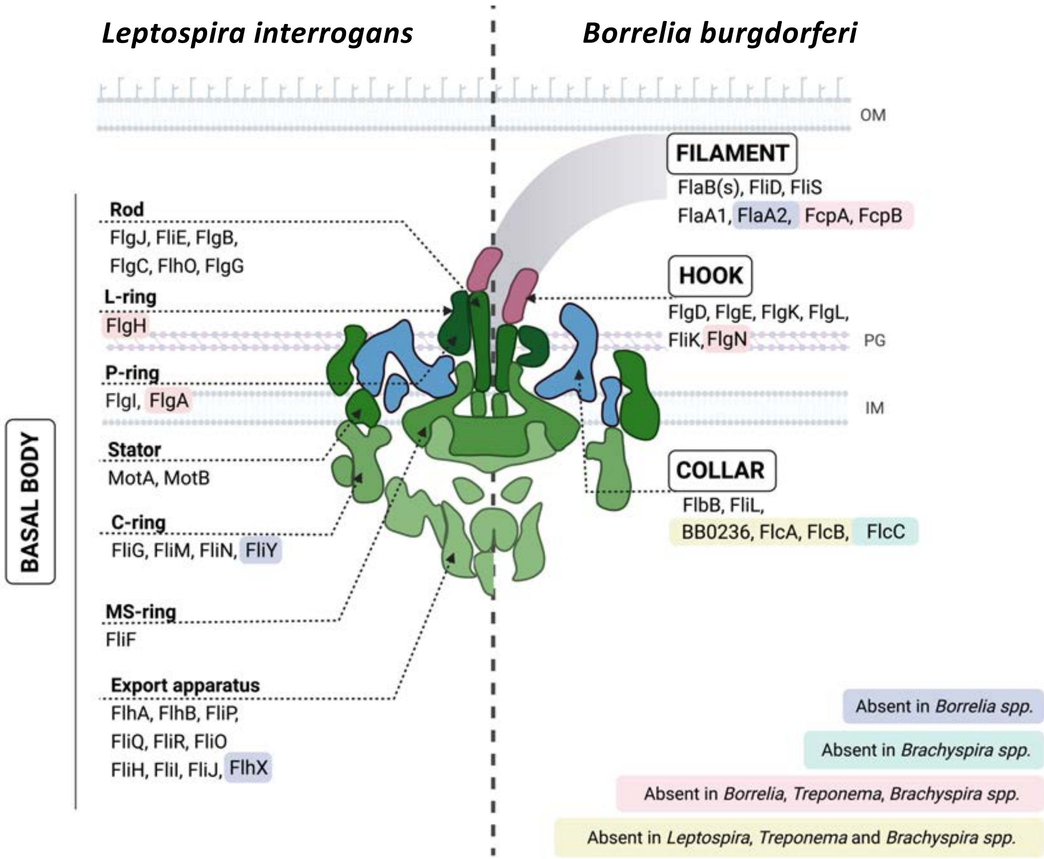
San Martin et al. 2023

The endoflagellum, a complex nanomachine



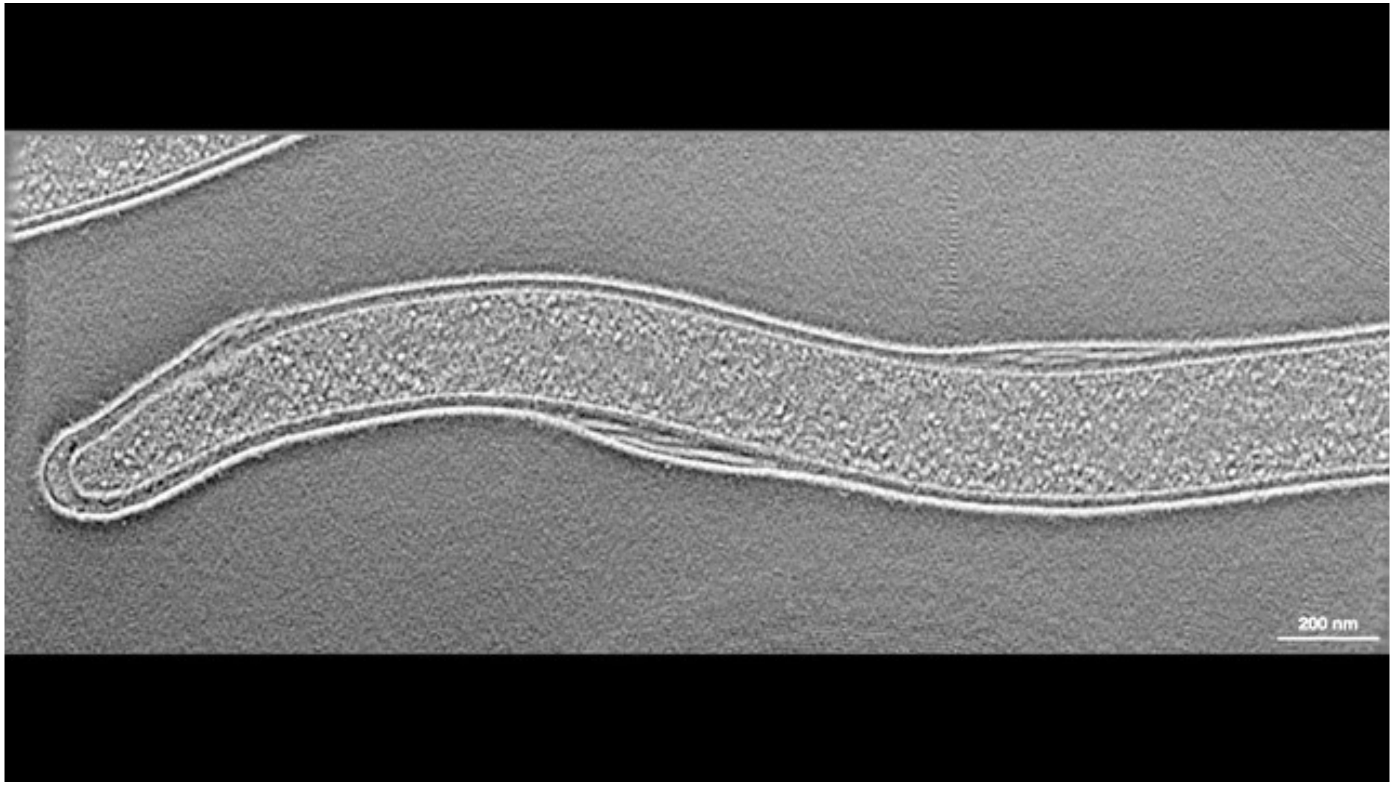
San Martin, Fule et al, 2022

The endoflagellum, a complex nanomachine



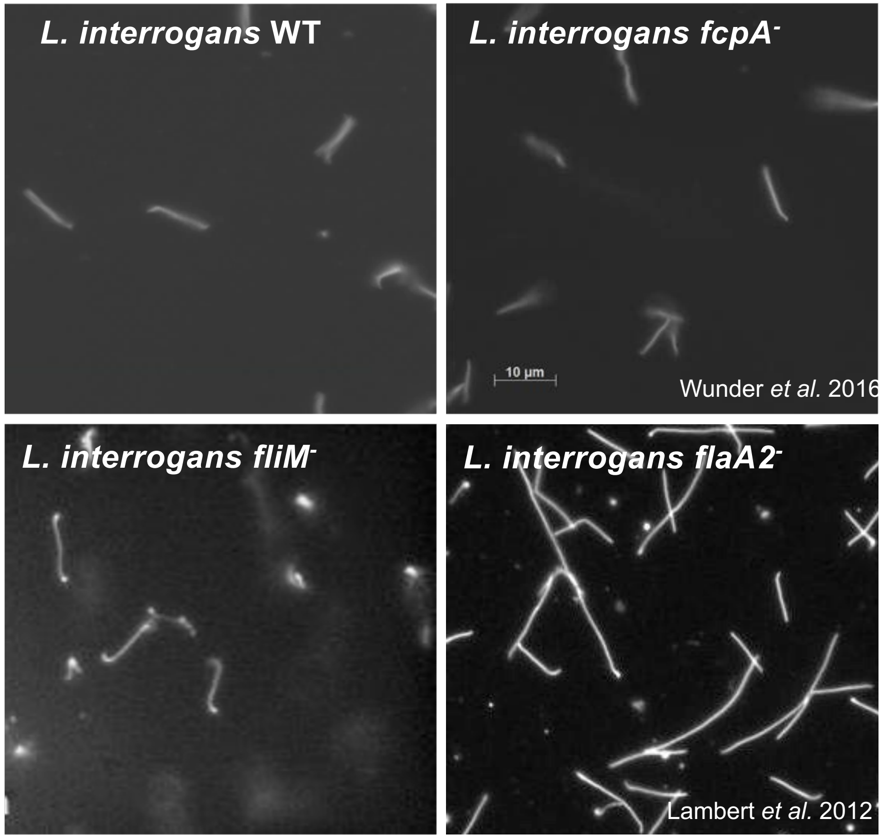
San Martin, Fule et al, 2022

Visualization of the modular collar structure and its capacity to accommodate diverse membrane curvatures in *B. burgdorferi* cell



Chang et al. 2021

Non-motile mutants in *L. interrogans*



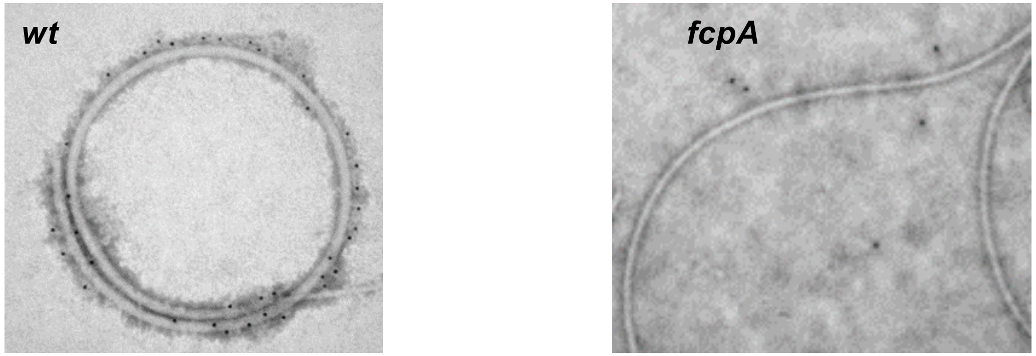
- Screening of libraries of random mutants for growth deficiency
- Target mutagenesis
- Isolation of spontaneous non-motile variants

➢ Identification of novel flagellar proteins FcpA and FcpB (Wunder *et al.* 2016, 2018)

FcpA is necessary for the formation of the flagellar sheath

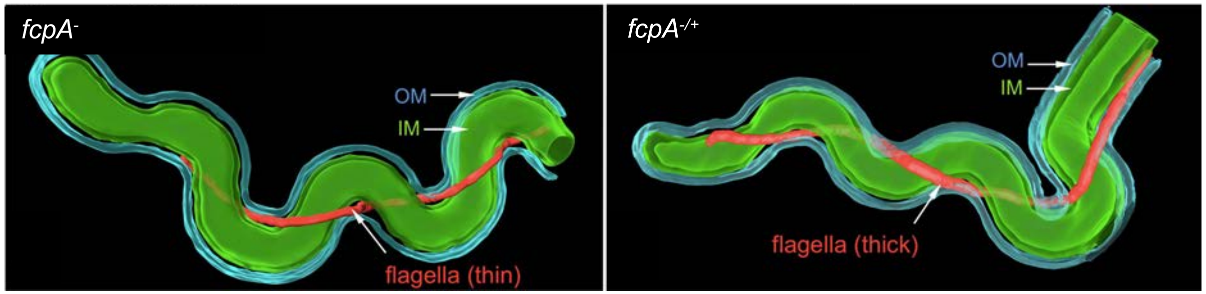
- Anti-FcpA antibodies specifically labelled the surface of PFs

immuno-electron microscopy



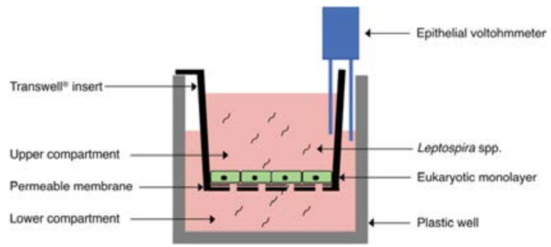
- Diameters of PFs from *fcpA*$^{-}$ strains (mean 16.27 ± 2.88 nm) were significantly smaller than those from wt strains (mean 22.8 ± 2.01nm).

Cryo-electron tomography

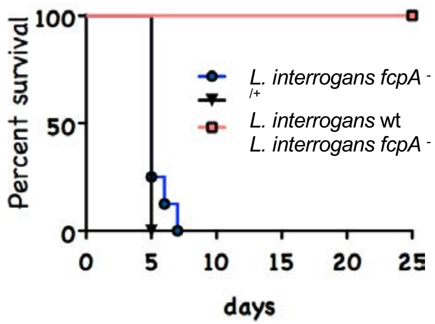


Wunder *et al.* 2016

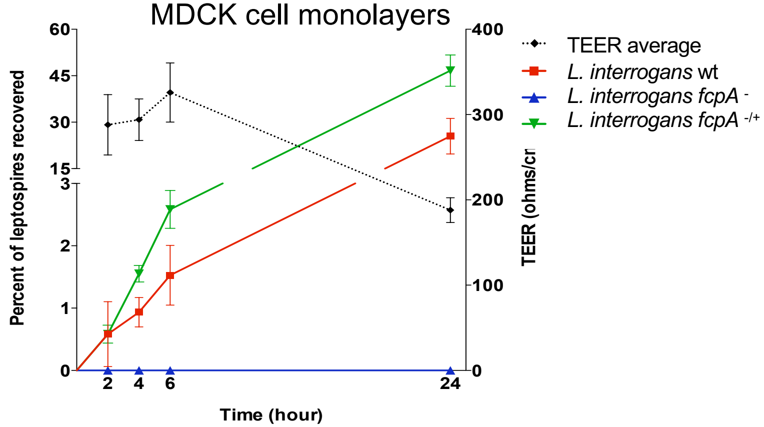
Virulence and translocation of the *fcpA* mutant



Virulence assays by IP injection of 10^6 bacteria in hamsters



Translocation across polarized MDCK cell monolayers

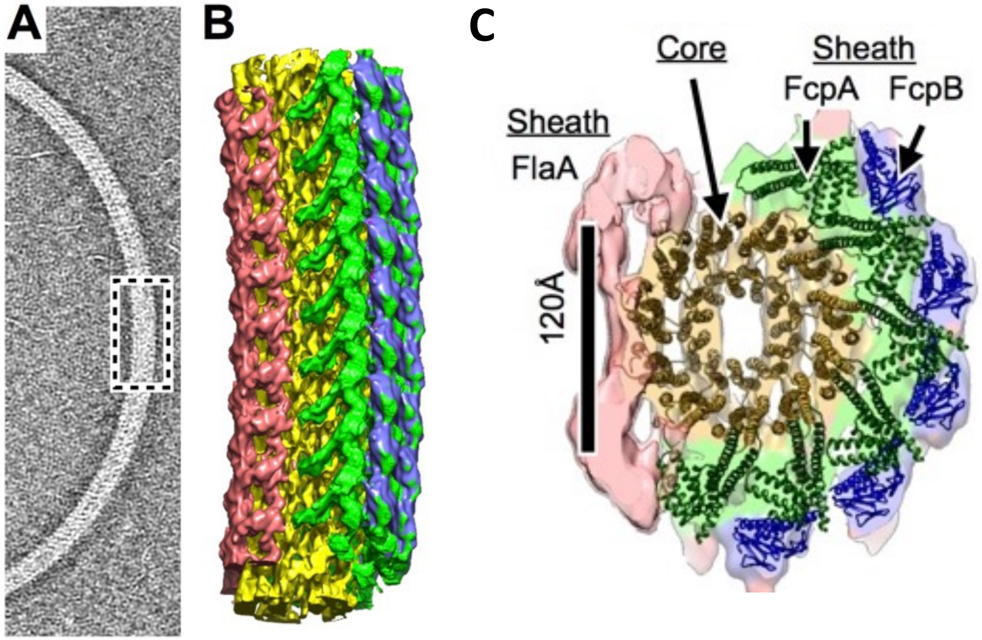


Wunder *et al.* 2016

- the *fcpA*- mutant is attenuated in motility and virulence

Asymmetric architecture of the flagellar filament

Molecular model of the flagellar filament enabled by new cryo-ET averaging methods and two new X-ray structures of sheath proteins with novel folds



X-ray structures of sheath proteins FcpA and FcpB with novel folds

(*A. Buschiazzo, IP Montevideo*)

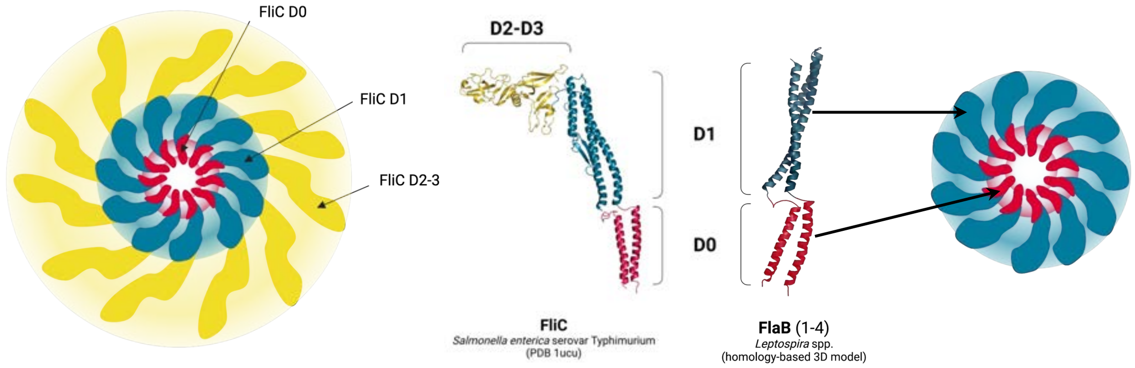
Gibson *et al.* 2020

Cryo-ET (*C. Sindelar, Yale university*)

Gibson *et al.* 2020

Cross-section of the flagellar filament

Salmonella enterica *Leptospira biflexa*



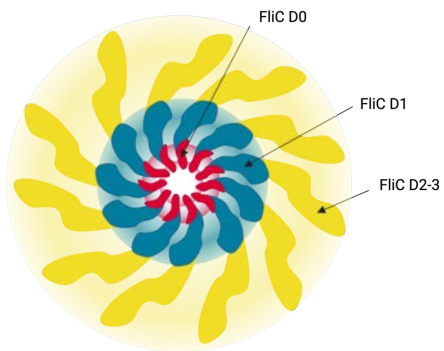
11 protofilaments of FliC (D0, D1, D2, D3)

core: 11 protofilaments of FlaBs (D0, D1)

Yonekura et al, 2003; San Martin, Fule et al, 2022

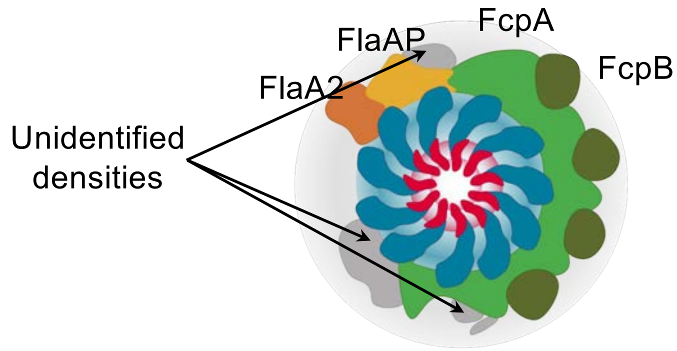
Cross-section of the flagellar filament

Salmonella enterica



11 protofilaments of
FliC (D0, D1, D2, D3)

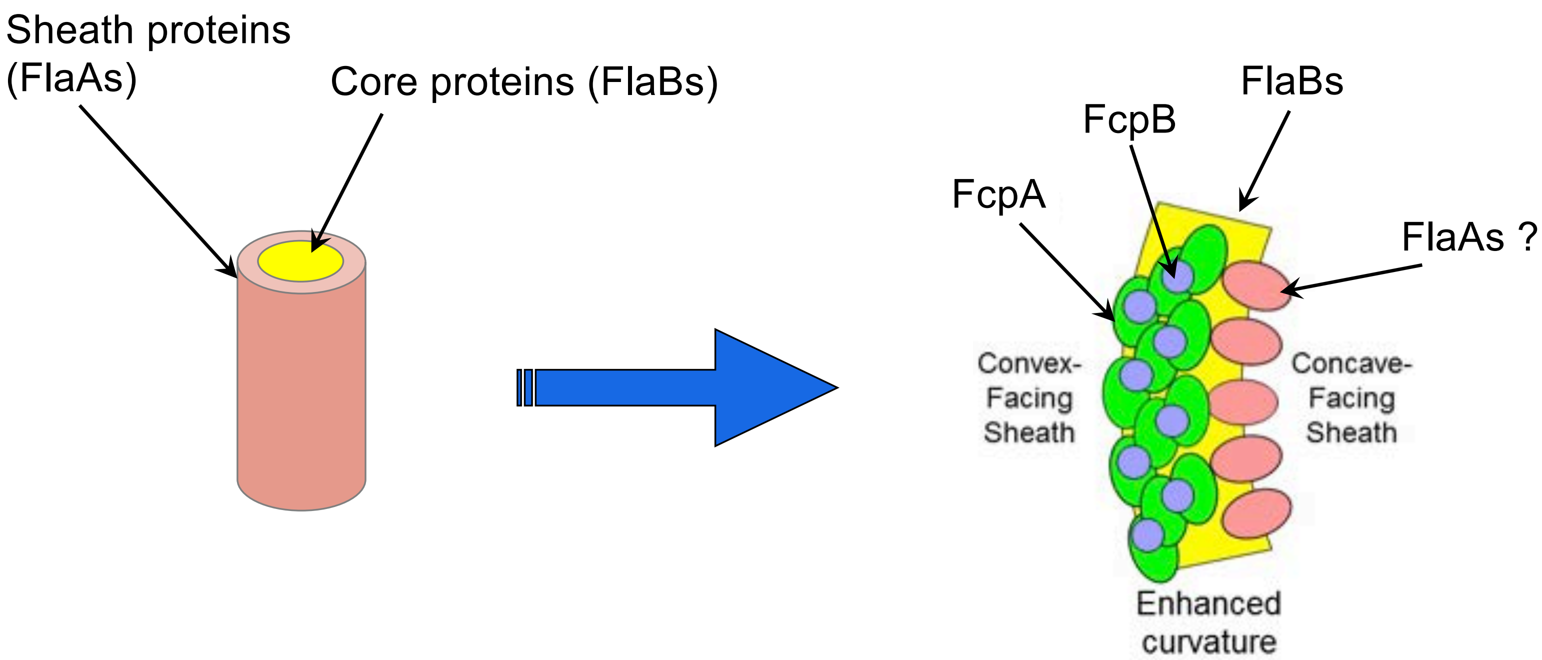
Leptospira biflexa



core: 11 protofilaments of FlaBs (D0, D1)
sheath: FlaA, FcpA, FcpB, flaAP, etc

Yonekura et al, 2003; San Martin, Fule et al, 2022

3D model of the flagellar filament

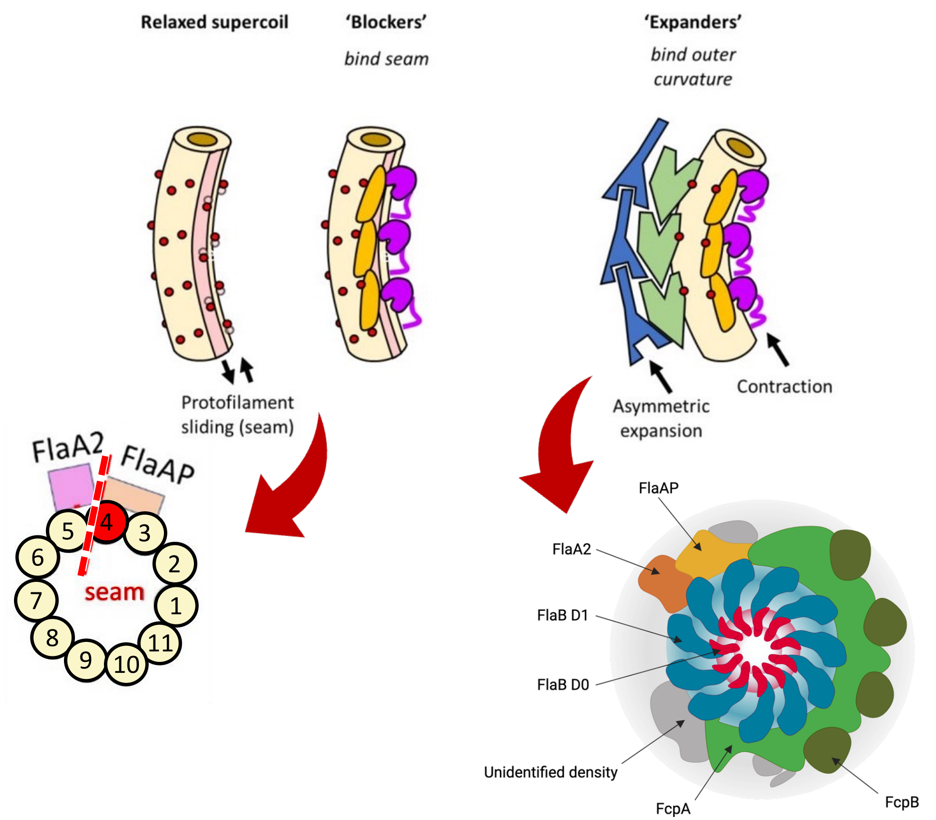


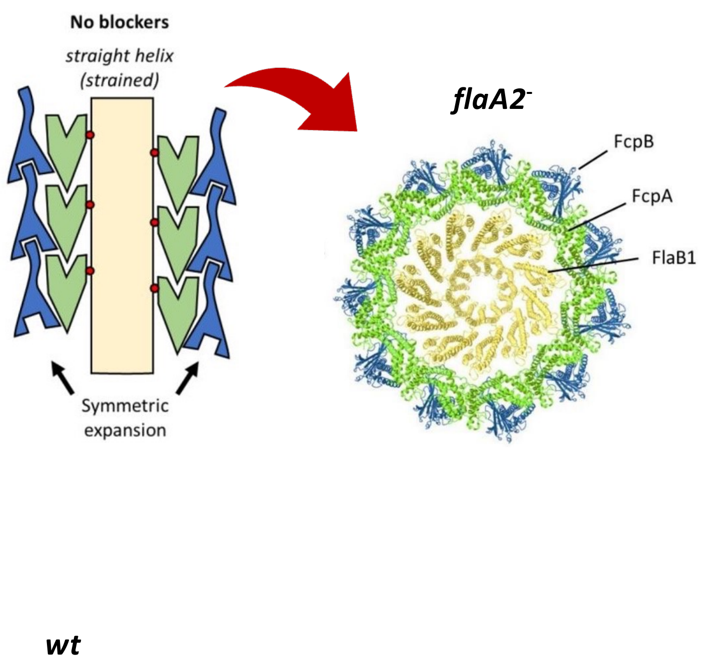
« simple » model

more complex model

What are the determinants of the flagellar-sheath curvature ?

Asymmetry in the core assembly (i.e. preferential positioning of FlaB isoforms) could contribute to curvature selection by recruiting specific sheath proteins

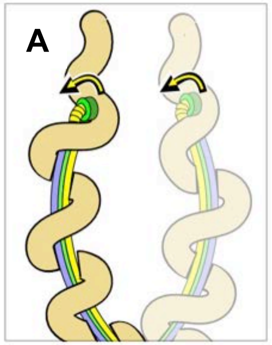




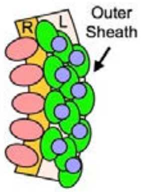
Brady et al., 2022 (bioRxiv)

Model for sheath-dependent flagellar motility in *Leptospira* spp

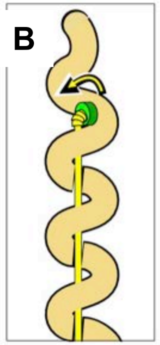
Wild-type



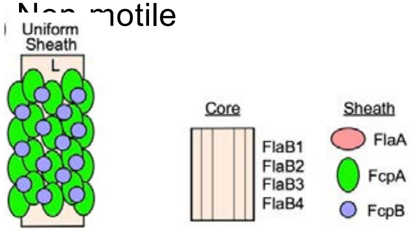
- Asymmetric architecture of the filament
- Supercoiled filaments
- Hook- and spiral-shaped ends
- Translational motility



flaA mutant



- Symmetric architecture of the filament
- Relaxed filaments
- Filament rotation does not induce body deformations
- Non-motile



- Filament curvature is due to asymetrical binding of sheath proteins
- Filament curvature is essential for intimate interaction between the flagella and cell body in the periplasmic space

The endoflagellum in *Leptospira*

- A simple model of Spirochetes (*T. pallidum* is not cultivable, *B. burgdorferi* possess 9-11 endoflagella)
- A more complex organization than expected : there is more than just FlaA and FlaB
- The filament is highly asymmetric (determinants of asymmetry ?)
- Filament/cell body interactions to be studied
- Enable the spirochetes to rapidly cross connective tissues and barriers (and to escape the recognition by the innate immune system)
- A novel mechanism of bacterial motility
- Use of non-motile mutant as live vaccines

- How do filament properties affect translational motility ?
- What are the evolutionary mechanisms that enable the flagellum to be located in the periplasm ?
- How the direction of flagella rotation is controlled and coordinated ?

Leptospirosis : knowlegde gaps and limitations

Knowledge gaps	Limitations
What is the true burden ? Cost in livestock ?	Misdiagnosis common
What are the drivers for the re-emergence ?	Low sensitivity of diagnostic tests
What is the relevance of environmental P1-/P2 in Public health ? host reservoirs ?	Limited epidemiological informations
What could be the effective control measures ?	Culture isolation is fastidious
Early detection of disease	Neglected disease (not in the WHO list, limited funding)
Bacterial ecology poorly understood	No robust and cross-protective vaccines
What are the mechanisms of host adaptation?	Complex prevention (environment, reservoir, humans, etc)
What are the key genetic and phenotypic changes undergone by P1+ ?	Genetic manipulations in pathogens is challenging
	Mechanisms different from those found in model bacteria

Unité « Biologie des Spirochètes »

Centre National de Référence de la Leptospirose
Centre Collaborateur OMS de la Leptospirose
Pasteur International Unit « Leptospirosis Pasteur Network »

Funding:

Santé publique France

NIH

ANR AGENCE NATIONALE DE LA RECHERCHE

Institut Pasteur PTR ACIP

Institut Pasteur de Nouvelle-Calédonie

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