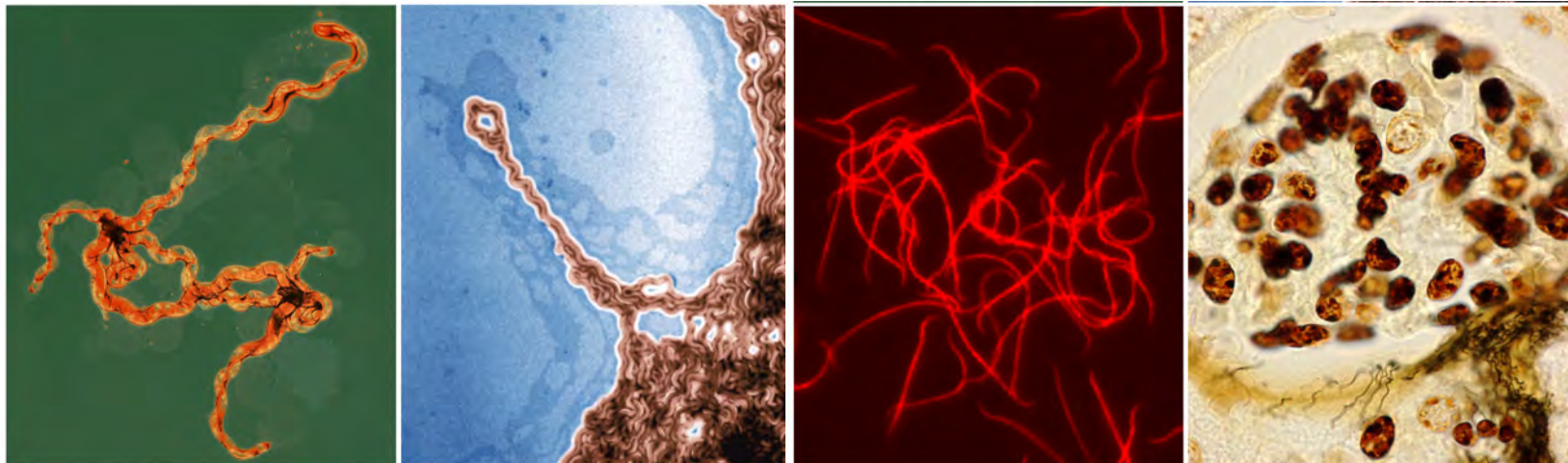


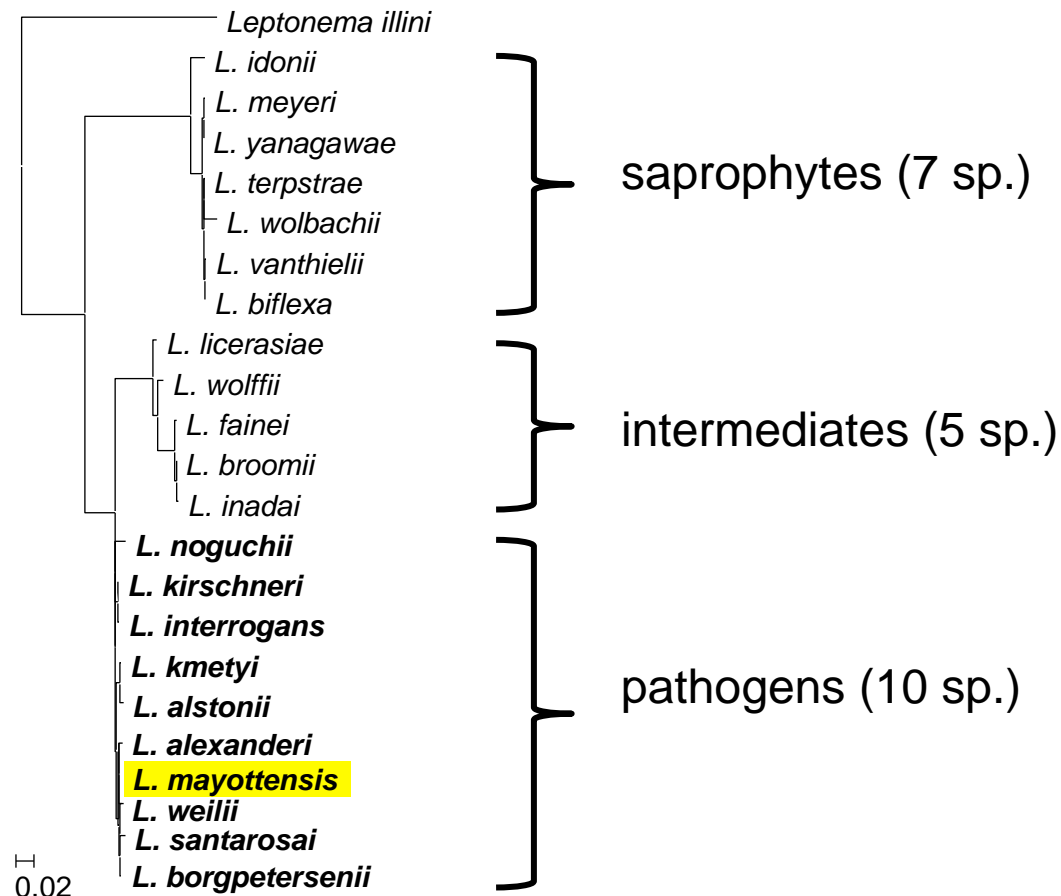
# Typing the agents of leptospirosis

## how and why



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# The genus *Leptospira*: 22 species and >300 serovars



**Experimental infection  
models (hamsters,  
guinea pigs, gerbils):**

➤ **non virulent**

➤ **non virulent**

➤ **virulent**

Phylogenetic tree using Maximum Likelihood based on the analysis of a ~1,300 bp sequence of the 16S rRNA gene of *Leptospira* spp. The scale bar represents the proportion of nucleotide substitutions

# Identification of the circulating agents

- There is a lack of information on *Leptospira* circulating strains worldwide
- Under estimation of the diversity of serovars/species



# Identification of the circulating agents

- Identification of the circulating serovars (and not the species) is the first step towards:
  - (i) improving diagnostic tools
  - (ii) identifying animal reservoirs
  - (iii) generating control strategies (eradication of reservoirs, vaccine development, etc)

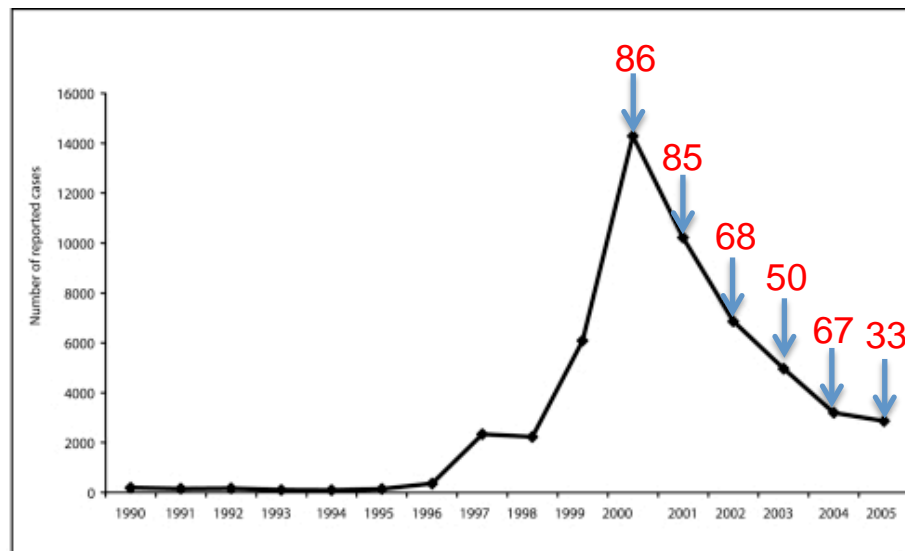
## Typical reservoir hosts of common leptospiral serovars

Reservoir hosts	Serovar (s)
Pigs	Pomona, Tarassovi
Cattle	Hardjo
Horses	Bratislava
Dogs	Canicola
Rats	Icterohaemorrhagiae, Copenhageni
Mice	Ballum, Arborea
Bats	Cynopteri, Wolfii

# Identification of the circulating agents

- Collection of local strains
  - Evaluation of the trends and emergence of clones

% of *Leptospira* isolated belonging to ST34

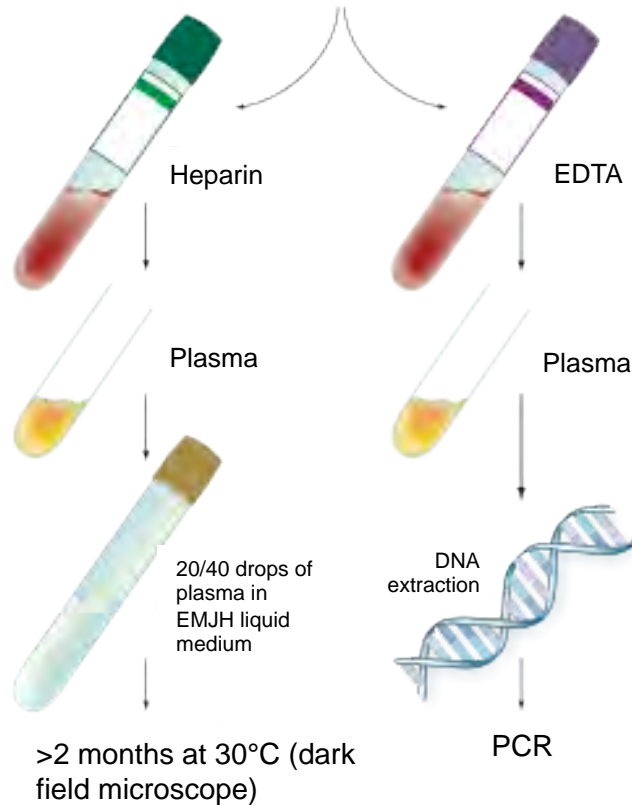


Thaipadungpanit et al. A dominant clone of *Leptospira interrogans* associated with an outbreak of human leptospirosis in Thailand. PLoS Negl Trop Dis. 2007 Oct 31;1(1):e56

- ST34 clone (*L. interrogans* serovar Autumnalis) was associated with the 1998–2003 outbreak of leptospirosis in northeast Thailand
  - Successful clone: selective advantage in the host or environment, increased virulence ?

# Identification of *Leptospira* by PCR, serology, and/or culture

**Blood from patients with acute febrile illness**  
(first week after the onset of symptoms)



Molecular (MLVA, PFGE, etc) and serological (MAT) typing  
➤ species, serogroup, serovar, genotype

**Convalescent blood samples**  
(second week after the onset of symptoms)



Serological (MAT) typing  
➤ serogroup

# Microscopic Agglutination test / MAT (Martin & Pettit, 1918)

*Martin L, Pettit, A. 1918. Sero-diagnostic de la spirochaetose icterohaemorrhagique. Bull. Mem. Soc. Med. Hop.42: 672-675*

Reference test for the serodiagnosis of leptospirosis

- Living antigens (cultures of representative serogroups and local strains)
- Evaluation of the degree of agglutination between each antigen and the patient serum

*Levett PN. 2003. Usefulness of serologic analysis as a predictor of the infecting serovar in patients with severe leptospirosis. Clin Infect Dis. 36:447-52*

*Smythe LD, Wuthiekanun V, Chierakul W, Suputtamongkol Y, Tiengrim S, Dohnt MF, Symonds ML, Slack AT, Apiwattanakorn A, Chueasuwanchai S, Day NP, Peacock SJ. 2009. The microscopic agglutination test (MAT) is an unreliable predictor of infecting Leptospira serovar in Thailand. Am J Trop Med Hyg. 81:695-7*

- MAT correctly determined the infecting serovar in < 50% of cases, indicating that this assay is a poor predictor of infecting serovars.



World map with Mayotte and the French West Indies



- Culture isolation from patients and animals
- Direct amplification from blood (human) and kidneys (animals)
- Strain identification by serological and/or molecular techniques (PFGE, MLST, MLVA, sequencing of 16S rRNA and secY)



# French West indies, Caribbean sea

## Martinique and Guadeloupe

- Common geological environments
- ~400,000 inhabitants
- Similar levels of urbanization
- Economy depends on tourism and agriculture (sugar cane and bananas)
- The climate is tropical with a dry and reany season



Burden of leptospirosis in 2002-2008 and results of the 2011 incidence study (S. Cassadou, Cire Antilles Guyane)

	Average no per year	Average annual rate/100,000 inhabitants
Guadeloupe 2002-2008	99.4	22.5
Martinique 2002-2008	54.8	13.9
Guadeloupe 2011	267 [183-351]	69.4 [47.6-91.1]
Martinique 2011	240 [144-337]	60.6 [36.3-85.0]

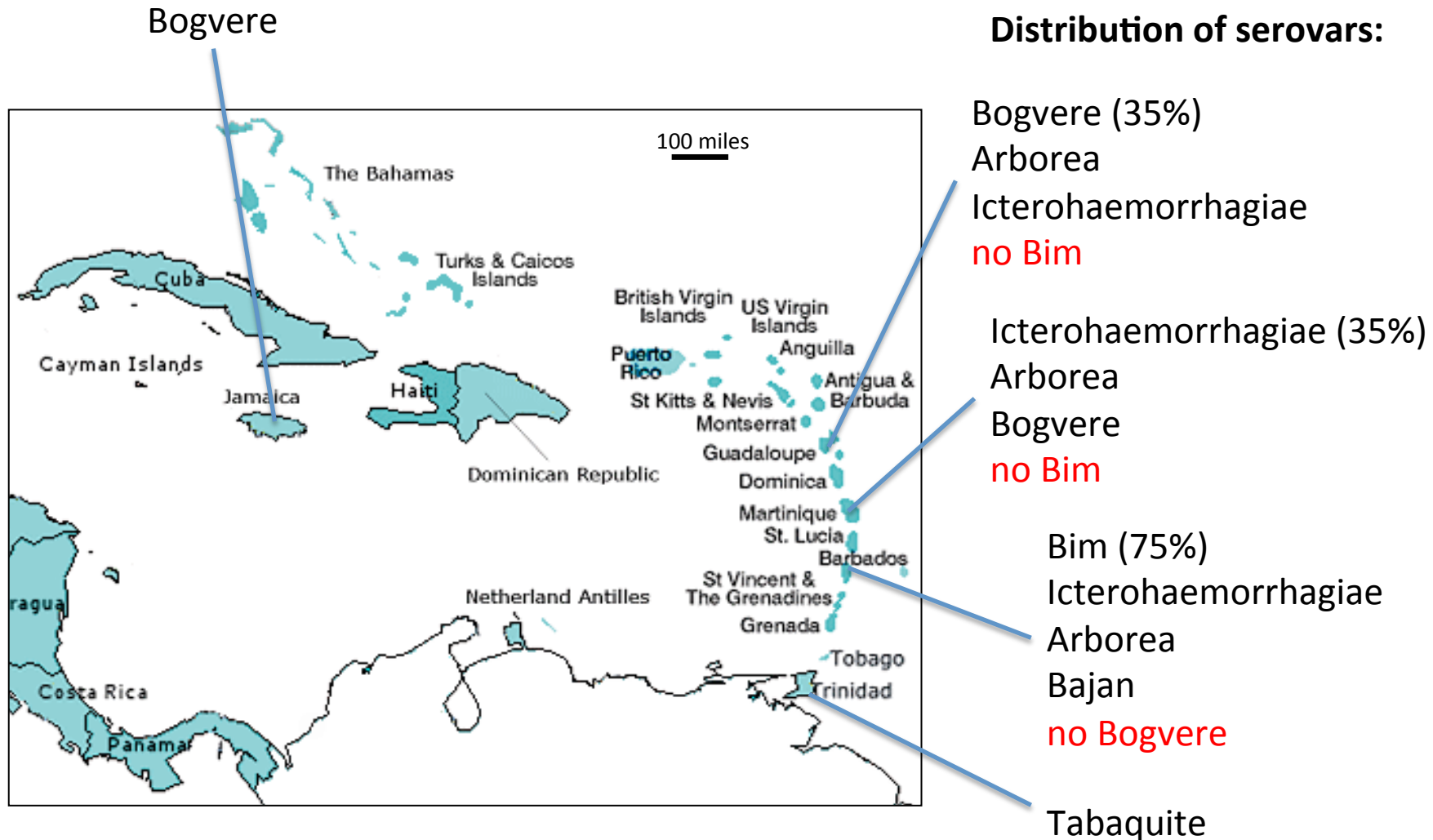
# Serovar diversity of pathogenic *Leptospira* circulating in the French West Indies

Molecular and serological features of >150 *Leptospira* isolates from Guadeloupe and Martinique:

- 13 genotypes (5 species, including *L. kmetyi*)
  - serovars Icterohaemorrhagiae/Copenhageni (~30%)
  - serovar Arborea (~20%)
  - serovar Bogvere (~20%)
  - serovars Tabaquite and Bajan and other unknown serovars
- 
- Rodents, opossums, mongoose, bats, pigs, cattle, toads and dogs have been found hosts of pathogenic *Leptospira* species



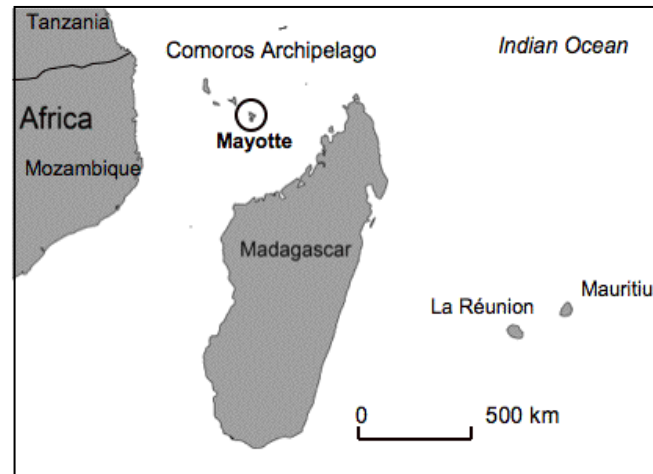
# Circulating strains in the Caribbean islands



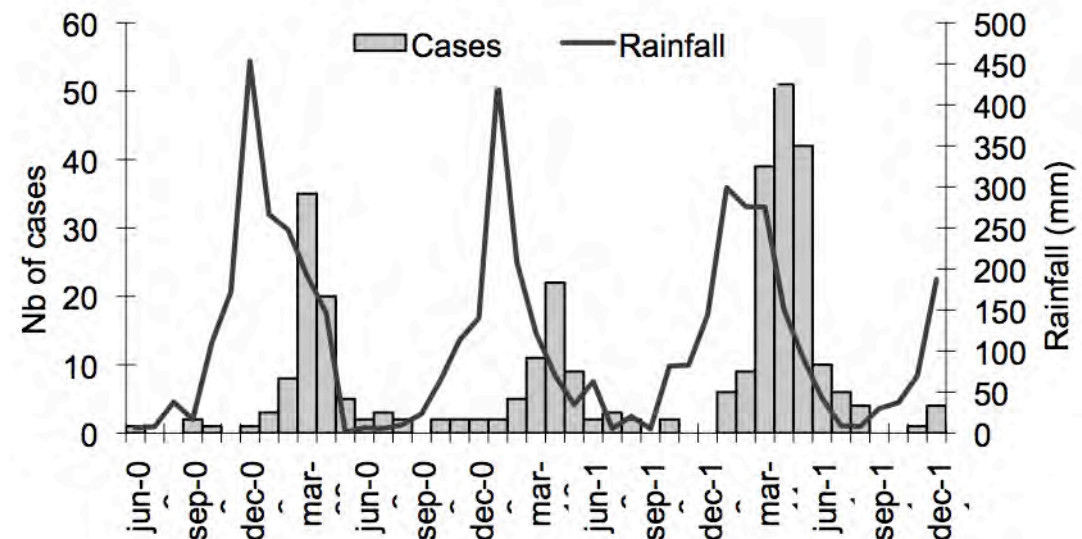
- Different distribution of the predominant pathogenic leptospiral serovars
- Correlation with distribution of the animal reservoirs in these islands ?

# Mayotte, Indian Ocean

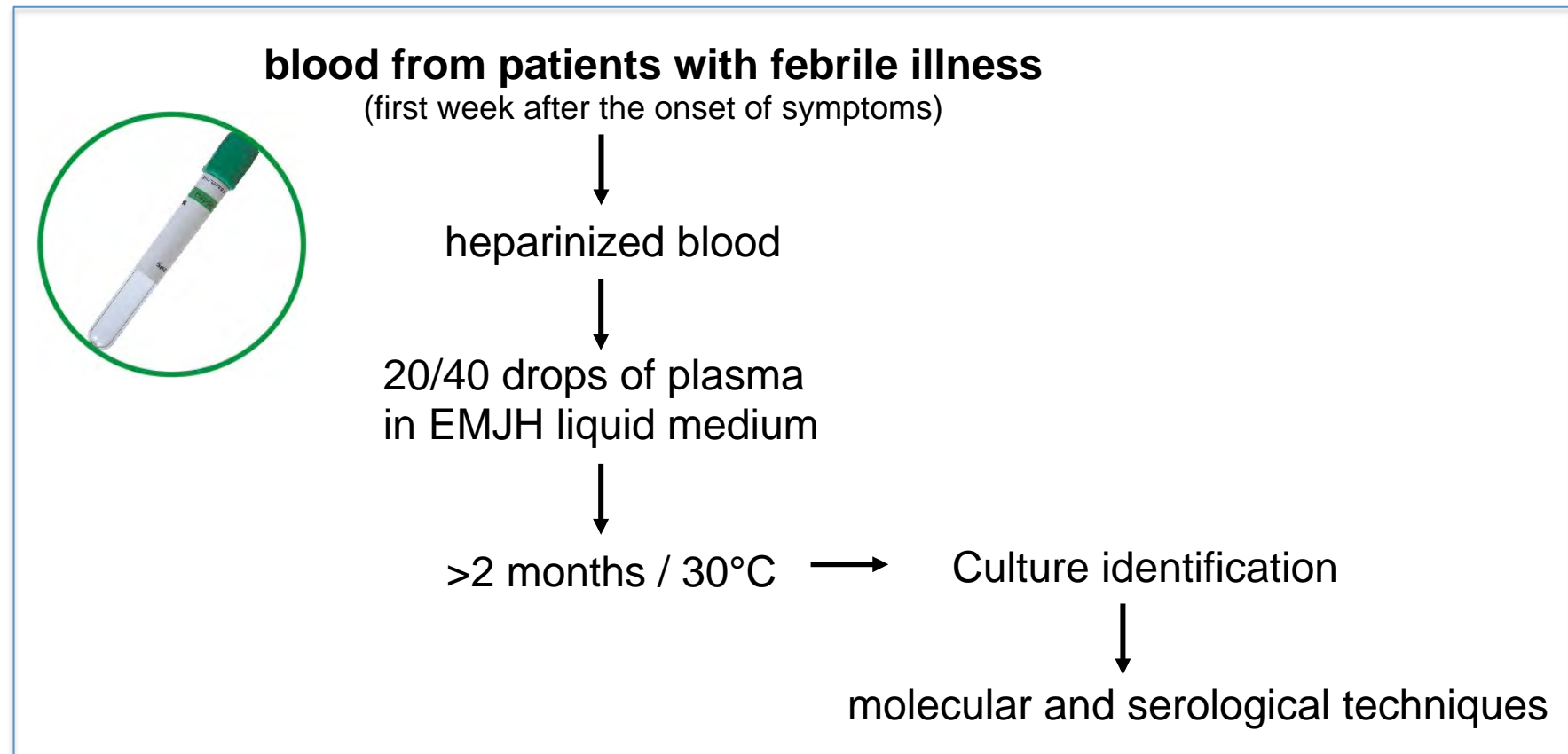
- ~200,000 inhabitants
- 53% <20 years old
- unemployment is high
- 25% immigrants from the Comoro Islands
- most households lack proper sanitation
- proximity with animals (dogs, sheep, cattle, or goats)



Monthly rainfall (in mm) and number of cases of leptospirosis, 2009-2011



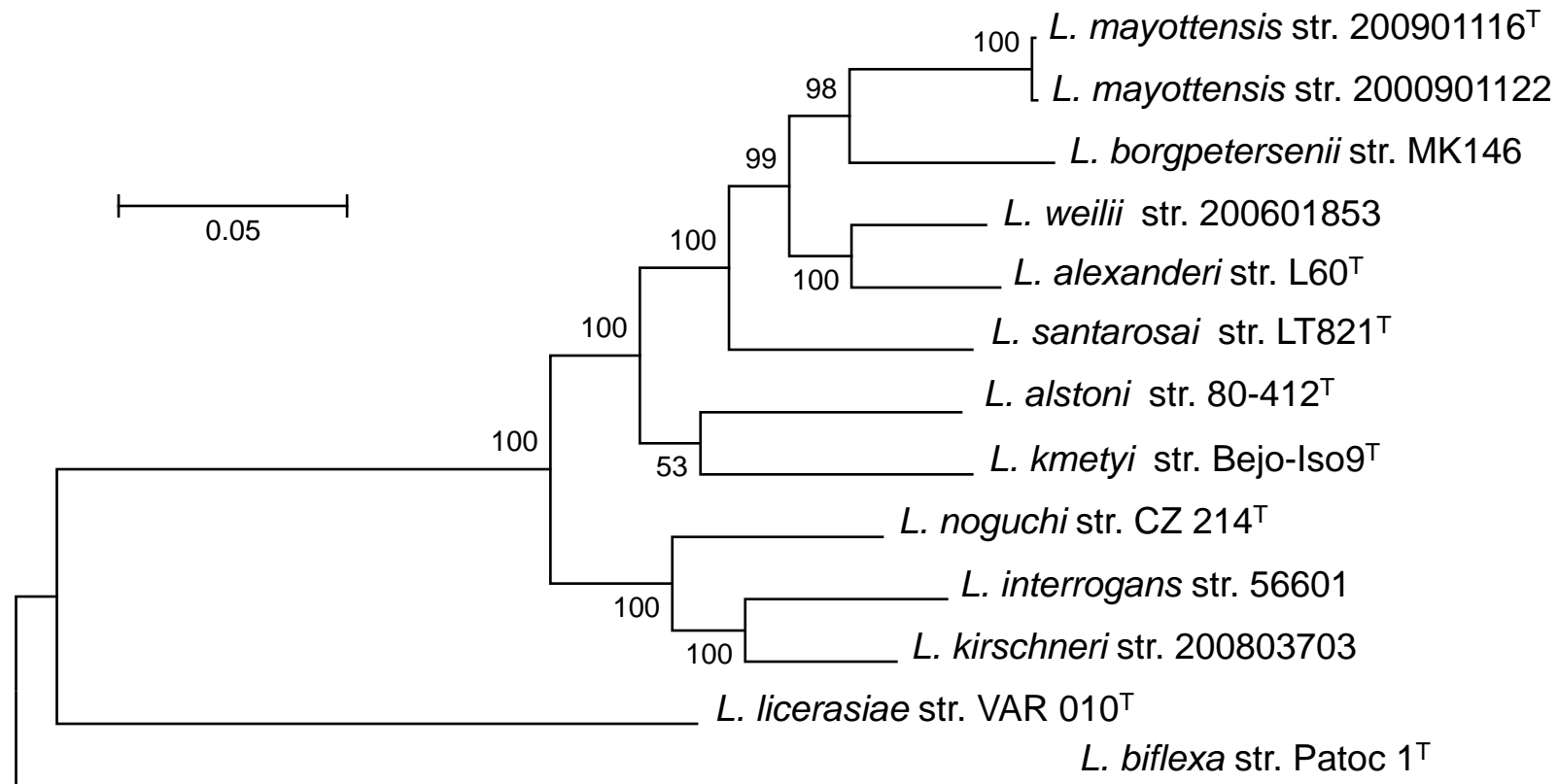
# Molecular and serological features of >200 *Leptospira* isolates



- 16 new sequence types (ST)
- serogroup Mini is predominant (>70%)
- serogroup Icterohaemorrhagiae is not present
- identification of a new serovars and species

# ***Leptospira mayottensis* sp. nov., a pathogenic *Leptospira* species isolated from humans**

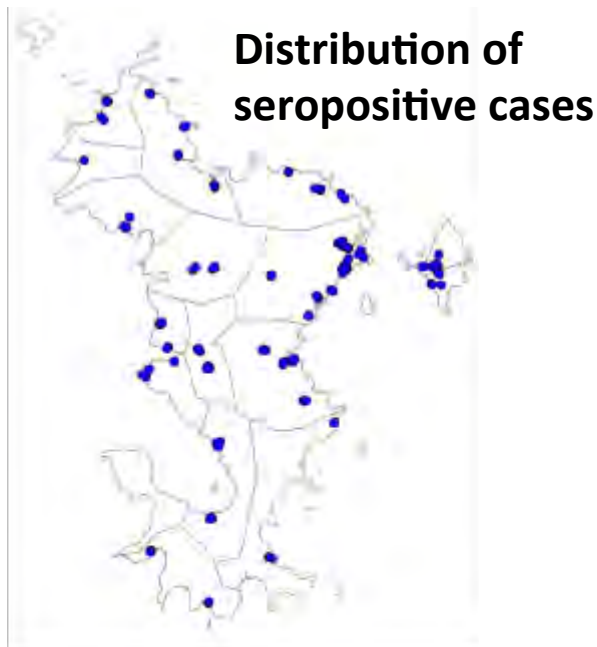
- Sequencing of genomes of two representative strains (JCVI)



Phylogenetic tree of concatenated sequences of genes *rpoB*, *recA*, *fusA*, *gyrB*, *leuS* and *sucA*

# Seroprevalence study in Mayotte, 2011

- Blood samples were collected from 1414 participants in March 2011
- Questionnaire data were used to explore associations between seropositivity and risk factors (demographics, and exposures at home, work, and during recreation)
- 14.9% of participants were seropositive
- Global seroprevalence in Mayotte: 16.5% (IC95% 13.6-19.7)
  - Seroprevalence in La Réunion <1% in 2006 (Desvars *et al.* 2011)
- Serogroups: Mini (74.7%), then Pyrogenes, Grippotyphosa, Pomona, etc



## Risk factors:

- Sex (males)
- Age
- Living in Comoros islands >10 years
- Contact with animals (goats, zebus)
- Proximity to water source or open sewers
- Poor housing (floor type)
- Contact with soil/water
- Farmers/unemployed



# *Leptospira* serogroup and species distribution in animals

A total of 292 animals were sampled between march-may 2007: 156 rats, 50 brown lemurs, 49 flying foxes, 29 domestic dogs, and 8 stray dogs

➤ Similar distribution of species and serogroup between humans and animals

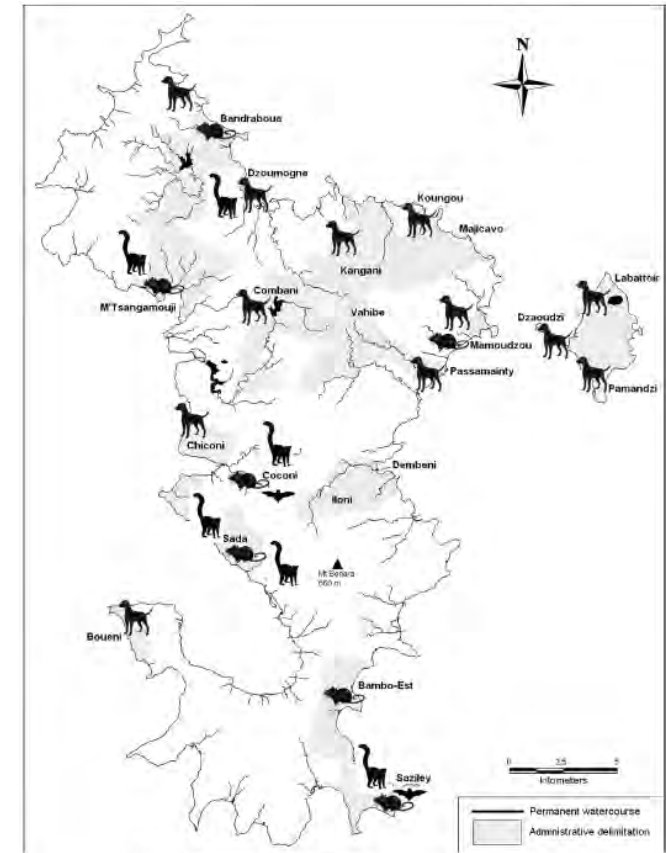


FIGURE 1. Cartography of Mayotte and sampling sites (S. Girard, ADEM-GESAM/GRAD).

Relative proportions of infecting serogroups in patients and wild non-vaccinated animals (MAT), and relative proportions of *Leptospira* species in patients and rats (16S rRNA sequencing of kidneys)

Species (n = number studied)	Microscopic agglutination test (MAT) (No. positives by serogroup/total No. of positives)				16S rRNA sequencing (No. of strains identified by species/No. strains studied)			
	Mini	Pyrogenes	Grippityphosa	Pomona	<i>L. borgpetersenii</i>	<i>L. interrogans</i>	<i>L. kirschneri</i>	<i>L. borgpetersenii</i> group B
Lemurs (N = 50)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	nd	nd	nd	nd
<i>Pteropus</i> bats (N = 49)	0/5 (0%)	4/5 (80%)	1/5 (20%)	0/5 (0%)	nd	nd	nd	nd
Non-vaccinated dogs (N = 15)	6/13 (46.2%)	1/13 (7.7%)	0/13 (0%)	0/13 (0%)	nd	nd	nd	nd
Rats*	7/14 (50%)	1/14 (7.1%)	3/14 (21.4%)	0/14 (0%)	9/20 (45%)	7/20 (35%)	2/20 (10%)	2/20 (10%)
Humans (N = 94) <sup>3</sup>	66/94 (70.2%)	17/94 (18.1%)	8/94 (8.5%)	3/94 (3.2%)	49/94 (52.1%)	8/94 (8.5%)	22/94 (23.4%)	15/94 (15.9%)

\*MAT: 125 rats studied; 16S partial sequences: 20 rats.  
nd = not done.

# Epidemiology of leptospirosis in Mayotte, Martinique and Guadeloupe

- Underestimation of the disease (better surveillance > higher incidence)
- Underestimation of the diversity of serovars and genotypes (new serovars/ genotypes/ species)
- High diversity of reservoirs
- MAT is an unreliable predictor of infecting serogroups (cross-reactions between serogroups)
- No strict association between serovar and reservoir
- Similar distribution of serovars between humans/animals (not only in rodents)
- Circulating strains in Mayotte differs from what is observed in other countries (including La Réunion island)

# Perspectives

- Identification of the agent (and diagnosis) by next-generation sequencing (enrichment of bacterial DNA/human DNA)
  - Human: serum, plasma, urine, cerebrospinal fluid (Waggoner et al. 2015. AJTMH)
  - Animals: kidneys, spleen (16S-metagenomics)
- RNA-based PCR assays (Backstedt *et al.* PLoS One 2015; Wagonner *et al.* 2014 PLoS One)
- Development of a LPS-based molecular methods
- Need to revise the nomenclature of *Leptospira* spp.: use of genovar instead of serovar ?

- Unité Biologie des Spirochètes
- Centre National de Référence de la Leptospirose
- Centre Collaborateur OMS pour la Leptospirose





# The Institut Pasteur International Network

32 Institutes spanning all five continents



- Zoonosis with a worldwide distribution
- Many Institutes are located in endemic areas