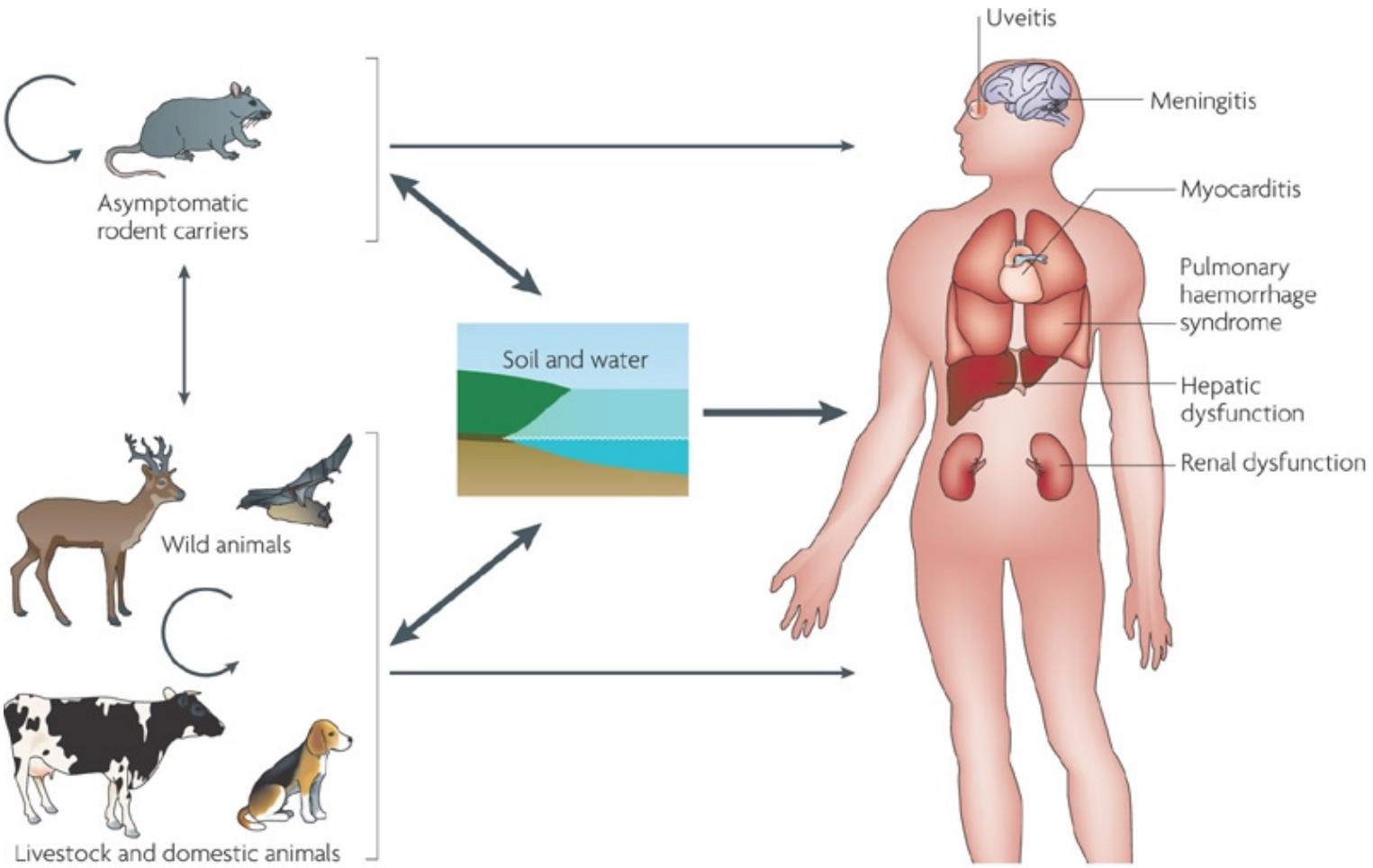
The cycle of leptospirosis



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

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

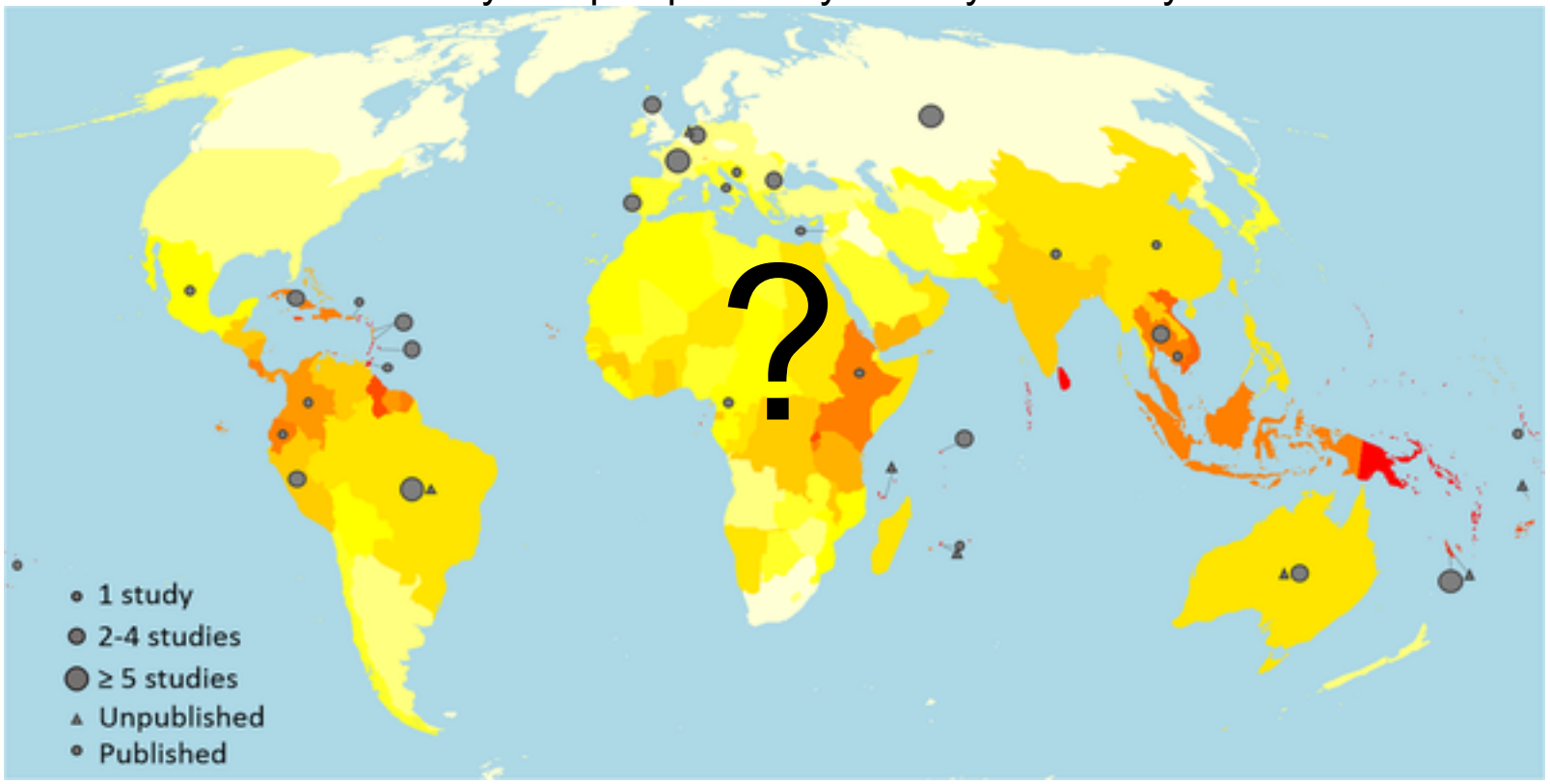
Emerging disease

- **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)

Global burden of leptospirosis

>1 million severe cases and 60,000 deaths due to leptospirosis / year

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.

Costa *et al.* 2015

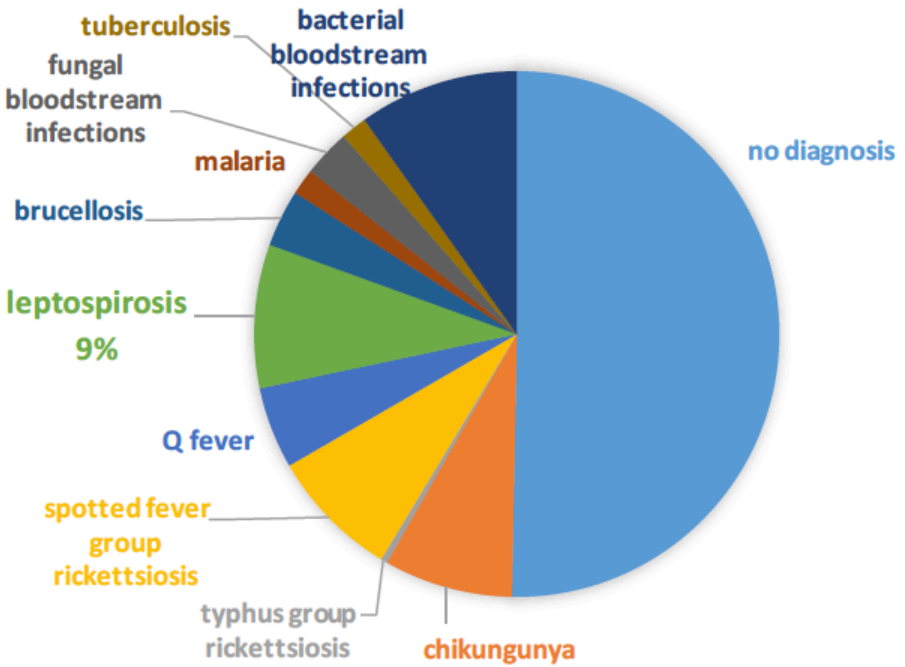
An under-recognized cause of acute fever

Leptospirosis in humans and animals

Allan *et al.* 2015

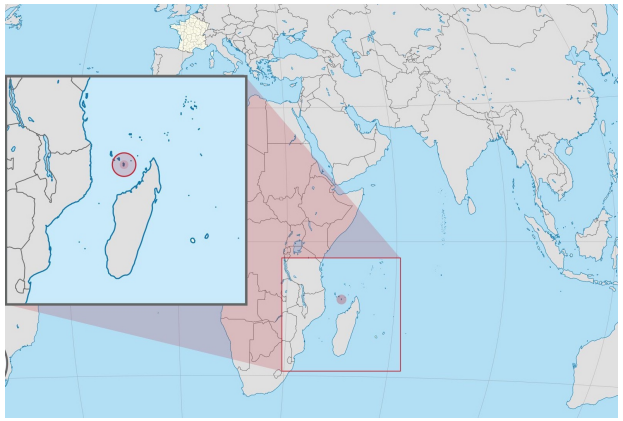
Cohort of 870 febrile cases in northern Tanzania

Malaria was the clinical diagnosis for 61%, but was the actual cause of fever in only 2%

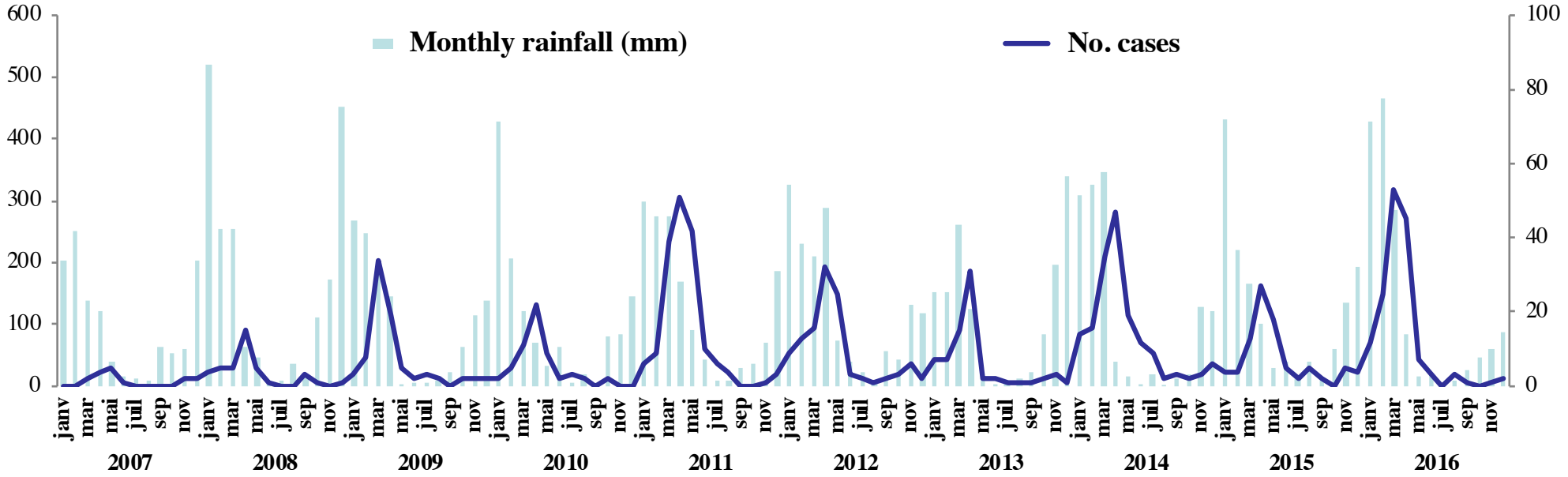


Crump *et al.* 2013

Annual Rainfall-Associated Epidemics of Leptospirosis



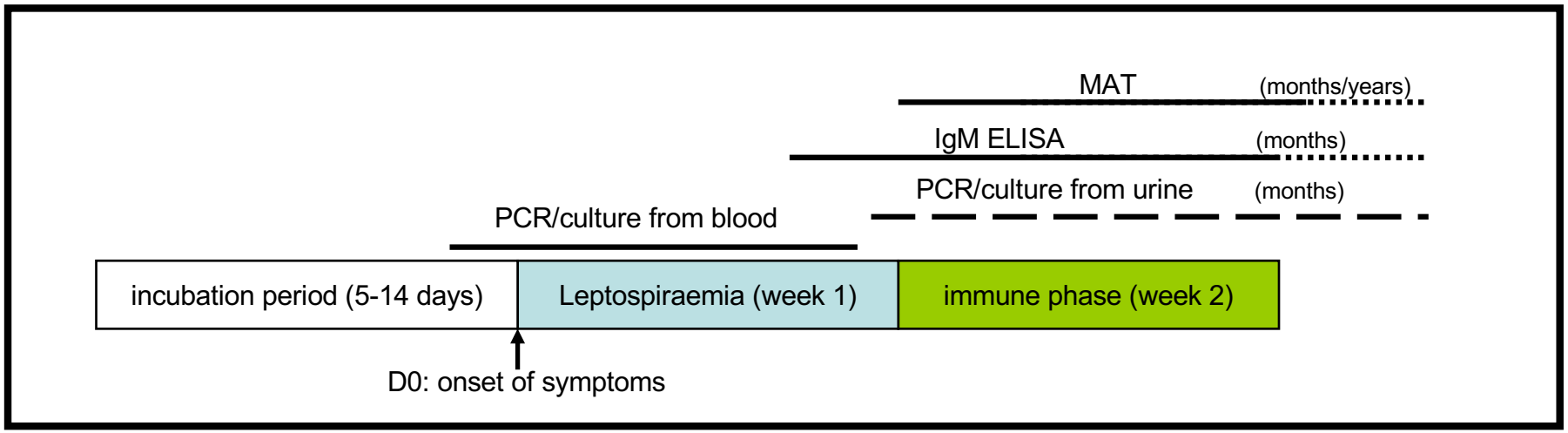
- 101st French Department
- Indian Ocean
- ~200,000 inhabitants



Pagès et al. 2017

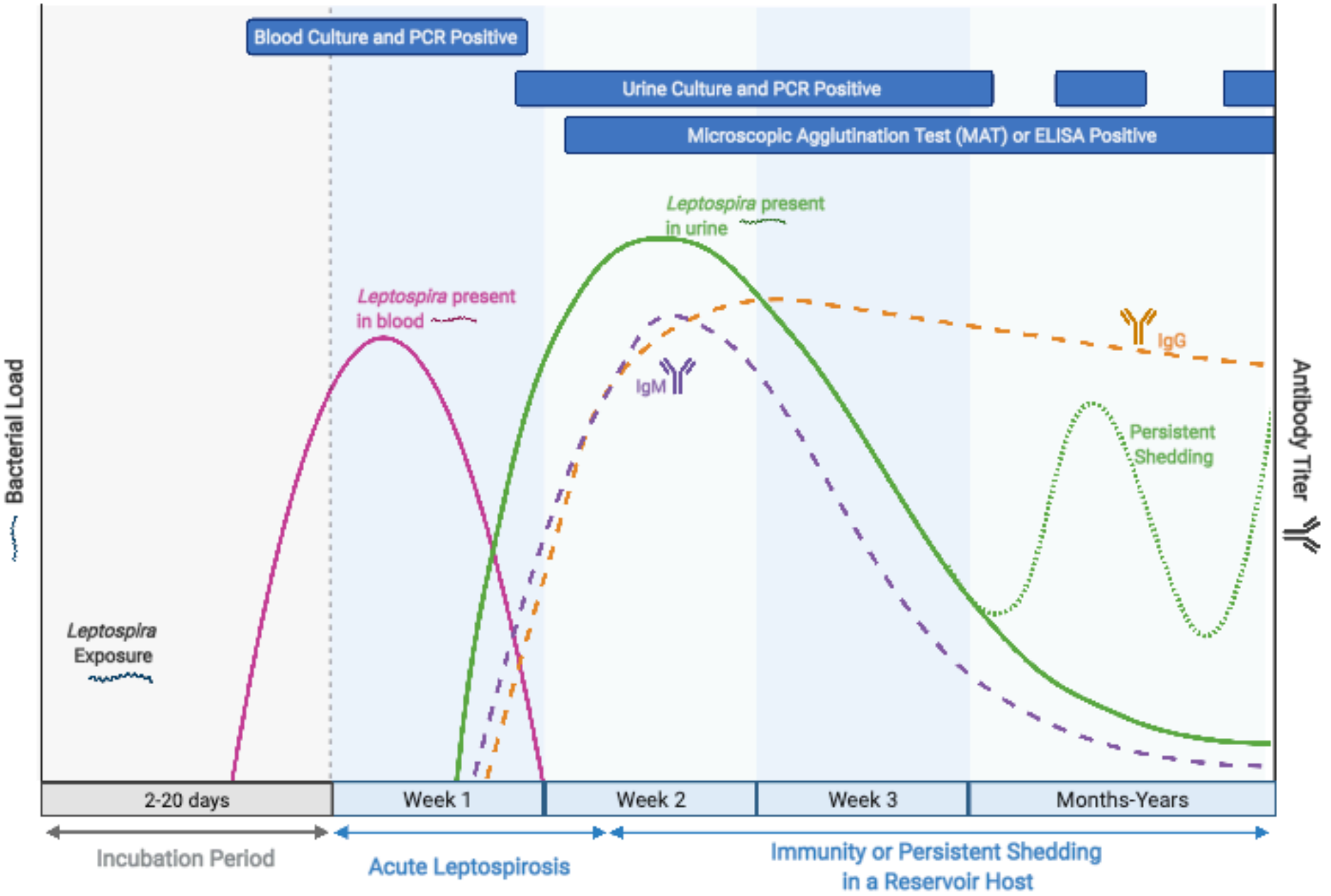
Challenges for Diagnosis and Treatment

- Misdiagnosis common, symptoms mimick other febrile illnesses (malaria, dengue, etc)
- Antibiotherapy need to be initiated early
- Low sensitivity of diagnostic tests:
  PCR (blood, urine)
  serology (Microscopic Agglutination Test, ELISA)
- Lack of standardization and access in resource-poor settings
- Diagnostics complicated by large heterogeneity of agents and epidemiology



MAT, microscopic agglutination test ; ELISA, enzyme-linked immunosorbent assay ; PCR, polymerase chain reaction.

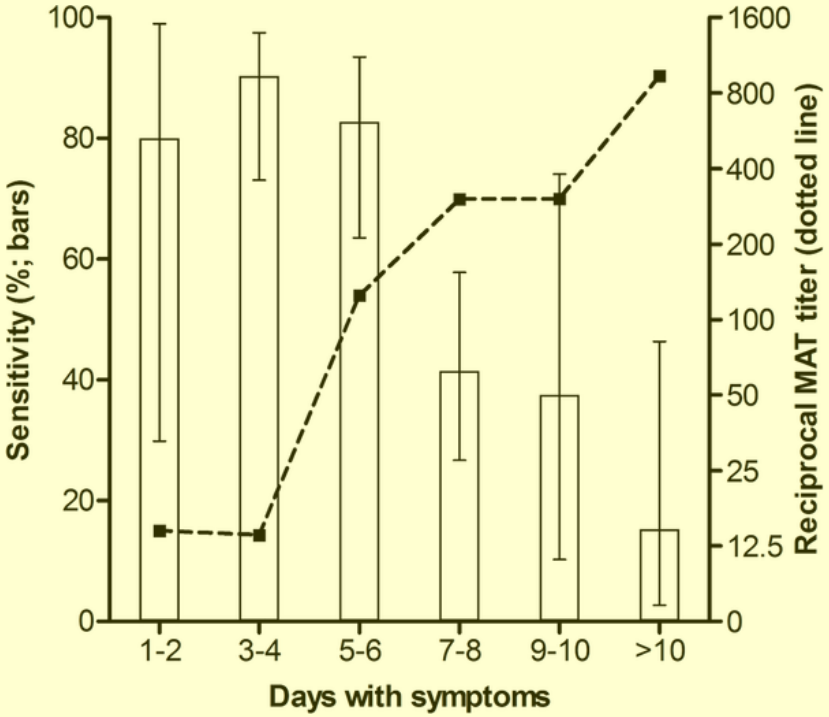
Kinetics of leptospirosis and diagnosis



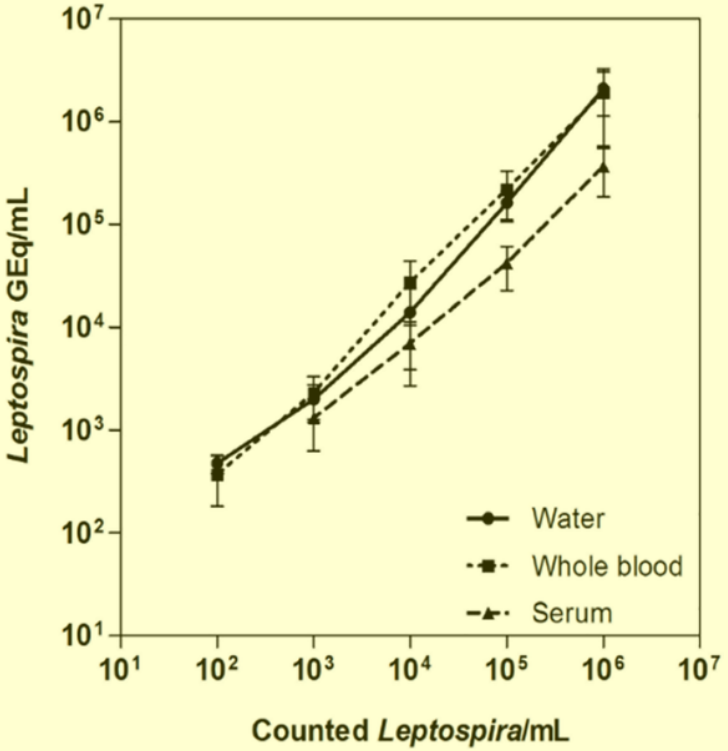
Syles *et al.* 2022

Real-time PCR assay on blood for early diagnosis of leptospirosis

Positivity of the real-time PCR assay according to days after onset of symptoms



Effect of different matrices on the accuracy of the real-time PCR test



- Higher sensitivity for samples from the first week after the onset of symptoms
- Higher sensitivity on blood in comparison to serum

Riediger et al. (2017) Rapid, actionable diagnosis of urban epidemic leptospirosis using a pathogenic Leptospira lipL32-based real-time PCR assay. PLOS Neglected Tropical Diseases 11(9): e0005940.

ELISA IgM

- Highly variable performance of the kits (Serion, PanBio, etc) according to the local epidemiology
- Some serovars (Australis and Grippotyphosa) are not well-detected
- Early detection from ELISA IgM in comparison to MAT

Sensitivities of three serological tests assays according to DPO
(using qPCR as gold standard for the diagnosis of acute leptospirosis) :

Number of patients	DPO	ELISA Serion	ELISA Pasteur-Hb	GenBio Immunodot
n=40	0-5	**32** (22-50)	**30** (18-45)	**42** (29-58)
		p<0.05	p<0.05	NS
n=21	6-10	**86** (65-95)	**76** (55-89)	**67** (45-83)
		NS	NS	NS
n=16	11-15	**94** (72-99)	**93** (72-99)	**94** (72-99)
		NS	NS	NS
n=26	>15	**92** (75-98)	**96** (80-99)	**92** (75-98)
		NS	NS	NS

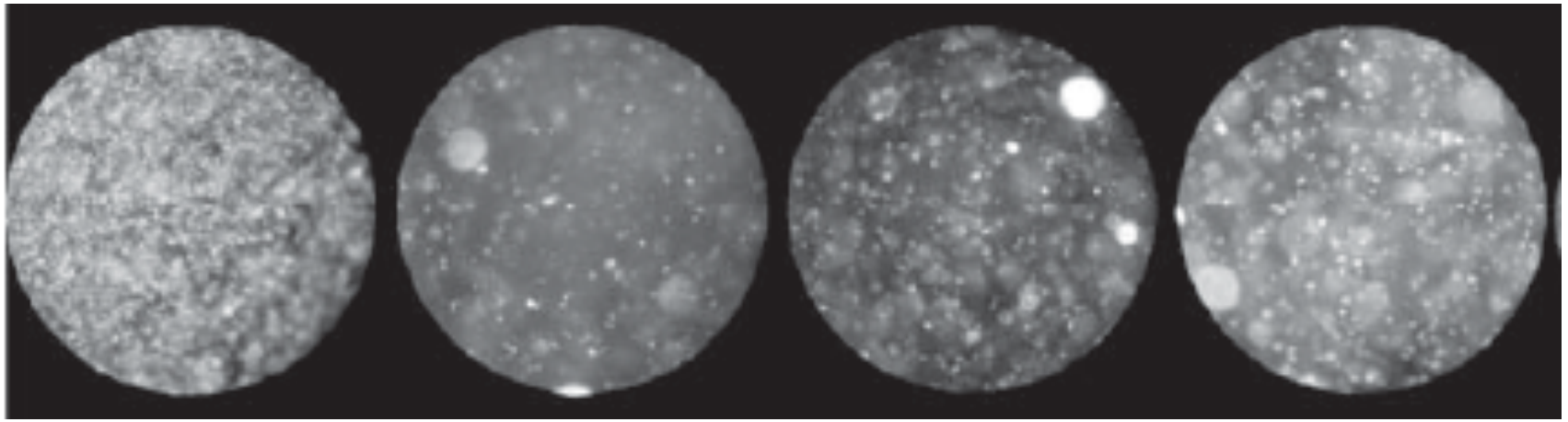
Significantly different results between two consecutive DPO intervals are indicated by p<0.05

Courdurie et al. PLoS NTD 2017

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

- Reference test for the serodiagnosis of leptospirosis
- Living antigens (cultures of representative serogroups)
- Evaluation of the degree of agglutination between each antigen and the patient serum

➢ identification of the infecting serogroup (but not always a good predictor)



PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Genetic diversity of *Leptospira* isolates in Lao PDR and genome analysis of an outbreak strain

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Panel of strains for Laos

	strain	alias	species	serovar	serogroup	country
1	U I9931	201801754	L. borgpetersenii		Javanica	Laos
2	UI 12823	201801169	L. interrogans		Canicola	Laos
3	Akiyami A	Ag2	L. interrogans	Autumnalis	Autumnalis	
4	UI 14721	201801179	L. interrogans		Bataviae	Laos
5	UI29382	201801758	L. interrogans		Autumnalis	Laos
6	LNT 2340	201801198	L. weilii		Mini	Laos
7	UI 14535	201801177	L. weilii		Celledoni	Laos
8	UI 27845	201801194	L. interrogans		Australis	Laos
9	UI 8434	201801158	L. interrogans		Grippotyphosa	Laos
10	UI 23152	201801193	L. interrogans		Bataviae	Laos
11	SV 725	201801206	L. weilii		Hebdomadis	Laos
12	SV 865	201801207	L. interrogans		Pomona	Laos
13	SV719	201801765	L. interrogans		Icterohaemorrhagiae	Laos
14	Salinem	Ag14	L. interrogans	Pyrogenes	Pyrogenes	
15	UI 14346	201801175	L. interrogans		Grippotyphosa	Laos
16	M 84		L. borgpetersenii	Sejroë	Sejroë	
17	Verdun		L. interrogans	Icterohaemorrhagiae	Icterohaemorrhagiae	France

Panel of strains for Malawi, Zimbabwe and Mozambique

	isolate	alias	species	serovar	serogroup	country
1	202001908	RM 1	kirschneri	Sokoine	Icterohaemorrhagiae	Tanzania
2	202001909	Njenga	borgpetersenii	Kenya	Ballum	Kenya
3	202001911	Lambwe	kirschneri	Unknown	Autumnalis	Kenya
4	202001912	Kwale	borgpetersenii	Unknown	Pyrogenes	Kenya
5	TE 1992	None	interrogans	Lora	Australis	Tanzania
6	201401327	LNA18	borgpetersenii	arborea	Ballum	Madagascar
7	202001913	TOA 13	interrogans	Kuwait	Canicola	Madagascar
8	201501070	None	borgpetersenii	Unknown	Pomona	Mayotte
9	201600670	None	kirschneri	Unknown	Grippotyphosa	Mayotte
10	201700941	None	interrogans	Unknown	Pyrogenes	Mayotte
11	Wijnberg	Ag 10	interrogans	Copenhageni	Icterohaemorrhagiae	
12	M 84	Ag 15	borgpetersenii	Sejroë	Sejroë	
13	Ballico	Ag 1	interrogans	Australis	Australis	
14	Hond Itrecht IV	Ag 4	interrogans	Canicola	Canicola	
15	Hebdomadis	Ag 9	interrogans	Hebdomadis	Hebdomadis	
16	Pomona	Ag 13	interrogans	Pomona	Pomona	
17	200801925	Ag 20	borgpetersenii	ND	Mini	
18	201501056		borgpetersenii	ND	Mini	Mayotte

FIEBRE

Diagnosis of leptospirosis:

- ELISA on all serum samples (D0, D28, control) and MAT on ELISA positive samples
- PCR on plasma samples (D0)

Case definition (to be discussed):

Confirmed cases :

- seroconversion or ≥ 4-fold titer rise in paired sera by MAT
- Positive PCR on acute plasma samples (D0)

Probable cases :

- MAT titer >100 (Africa) or ≥800 (Laos) on single serum samples

- Complete the PCR (Malawi, Zimbabwe)
- Sequencing of PCR products to identify circulating genotypes
- Analyze serological data using case definition