

PACE-NET PLUS Workshop
Tahiti, 12-14 November 2014

How European laboratories can contribute to the surveillance of infectious diseases in the Pacific



Unité de Recherche sur les Maladies Infectieuses Tropicales Emergente
URMITE – UMR CNRS 7278, IRD 198, INSERM U1095

URMITE

- Centralized microbiology laboratory
- French reference center and WHO collaborative laboratory for Q fever, rickettsioses, bartonellosis
- French reference center for tularemia
- French reference center for the detection of bioterrorism agents

URMITE

Syndrome-based sampling

Dedicated sampling kits

- Pneumonia
- Tropical diseases
- Bone and joint infections
- Meningitis
- Encephalitis
- Endocarditis
- Pericarditis
- Uveitis
- MERS
- Ebola

Dont think, sample!



URMITE

Syndrome-based diagnosis

Pneumonia

- Serology *C. burnetii*, *C. pneumoniae*, *C. psittacii*, *L. pneumophila*
- Urinary antigen *Legionella pneumophila*, *Streptococcus pneumoniae*
- PCR *M. pneumoniae*, *C. pneumoniae*, *C. burnetii*, *B. pertussis*

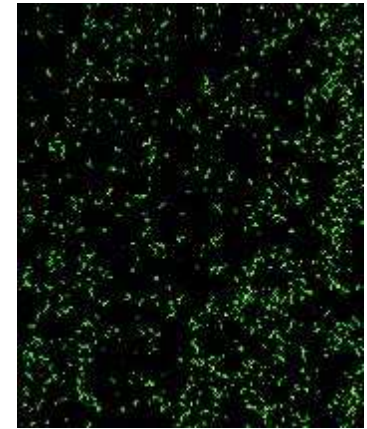
Sexually-acquired infections

- Serology *T. pallidum*, HIV, HCV, HBV
- ICT HIV
- PCR *T. pallidum*, *C. trachomatis*, *N. gonorrhoeae*, HIV

Meningitis

- PCR *N. meningitidis*, *S. pneumoniae*, *M. pneumoniae*, enterovirus, HSV1-HSV2

Always adapt assays to local epidemiology



Example of rickettsioses:

Europe, Mediterranean area: *R. conorii*, *R. aeschlimannii*,
R. sibirica mongolitimonae, *R. slovaca*, *R. raoultii*, *R. helvetica*, *R. felis*, *R.*
massiliae

Sub-saharan Africa: *R. conorii*, *R. africae*, *R. sibirica mongolitimonae*, *R.*
felis

Americas: *R. rickettsii*, *R. parkeri*, *R. africae*, *R. felis*, *R. massiliae*

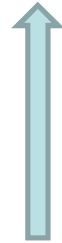
Asia: *R. sibirica*, *R. sibirica mongolitimonae*, *R. heilongjiangensis*, *R.*
helvetica, *R. conorii*, *R. honei*, *R. felis*

Australia: *R. australis*, *R. honei*, *R. felis*

URMITE

- 100,000 specimens per year
- Syndrome-based diagnosis
- Emerging microorganisms
 - Medical problem → sampling → culture → sequence → diagnostic tool design → tool evaluation → publication → test dissemination
- Numerous collaborations with medical teams worldwide
- Remote laboratories in Marseille, Senegal, onboard cruise and shipping boats

**Sending specimens to
references laboratories**



**Diagnosis and surveillance of infectious
diseases in remote areas**



On site testing

Sending specimens to reference laboratories

Technical constraints

- Reserved to culture-negative cases or unexplained syndromes
- Serum for serology assays (frozen at -20° C, room temperature, dried on blotting paper)
- EDTA blood for PCR (frozen at -20° C)
- Heparinized blood for culture (frozen at -80° C)
- Biopsies (-80° C for culture, -20° C for PCR)
- Swabs in case of inoculation eschar (-80° C for culture, room temperature for PCR)
- paraffin-embedded biopsies (room temperature for histology, immunohistochemistry and PCR)
- Arthropods (-80° C for culture, room temperature for PCR)

Remote area-adapted sampling

Dried blood on blotting paper

D DIAGNOSTIC LABORATORY IMMUNOLOGY, July 1999, p. 483-488
9/\$04.00+0
1999, American Society for Microbiology. All Rights Reserved.

Vol. 6, No. 4

Diagnosis of Rickettsial Diseases Using Samples Dried on Blotting Paper

FLORENCE FENOLLAR AND DIDIER RAOULT*

Eschar swabs

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 17, No. 1, January 2011

Identification of Rickettsial Infections by Using Cutaneous Swab Specimens and PCR

Yassina Bechah, Cristina Socolovschi, and Didier Raoult



Diagnosis of Rickettsioses from Eschar Swab Samples, Algeria

Nadjet Mouffok,¹
Cristina Socolovschi,
Anwar Benabdellah,
Aurelie Renvoisé,
Philippe Parola,
and Didier Raoult

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 17, No. 10, October 2011

Dried DNA in extraction columns (4 drops of blood ~200µL)



Examples of studies conducted on the diagnosis and surveillance of infectious diseases in foreign countries

Diagnosis of endocarditis:

- Algeria: 2000 – 2003: 110 cases, zoonoses = 17.3% of IE (Benslimani *et al.* Emerg Infect Dis 2005;11:216-24)
- Brazil: 1998 – 2009, 51 patients with BCNE, zoonoses: 5.9% of BCNE (Lamas *et al.* Int J Infect Dis 2013;17:e65-6)
- India: 2005– 2006: 111 patients, zoonoses: 8% of IE (Balakrishnan *et al.* Emerg Infect Dis 2008;14:1168-9)
- Thailand: 2010-1012: 132 patients, zoonoses 11.4% of IE (Watt *et al.* Emerg Infect Dis 2014;20:473-6)

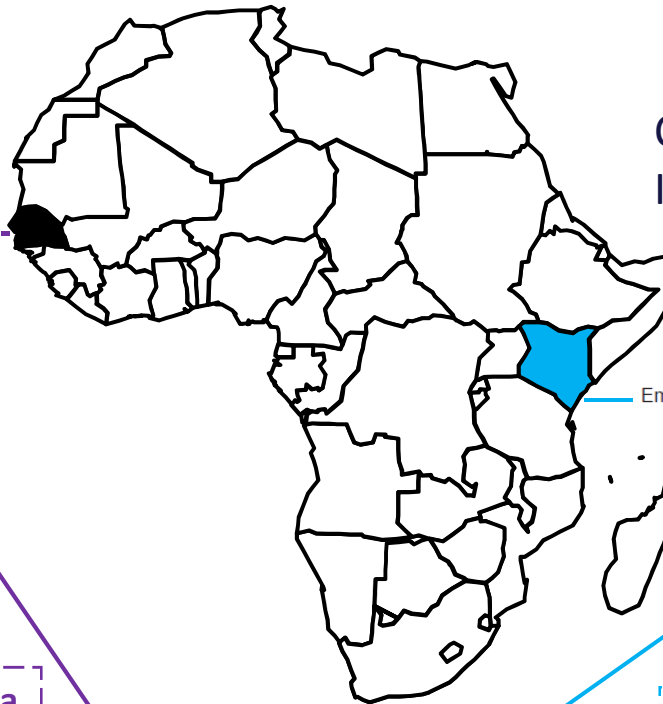
Study of unexplained fever in Senegal

R. felis : a common cause of bacteremia in sub-saharan Africa

***Rickettsia felis*-associated Unruptive Fever, Senegal**

Cristina Socolovschi, Oleg Mediannikov,
Cheikh Sokhna, Adama Tall, Georges Diatta,
Hubert Bassene, Jean-François Trape,
and Didier Raoult

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 16, No. 7, July 2010



Confirmed by a study conducted
In Kenya by another team

Human Infection with *Rickettsia felis*, Kenya

Allen L. Richards, Ju Jiang, Sylvia Omulo, Ryan Dare, Khalif Abdirahman, Abdile Ali,
Shanaaz K. Sharif, Daniel R. Feikin, Robert F. Breiman, and M. Kariuki Njenga

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 16, No. 7, July 2010

4.4% of cases of bacteremia

3.8% of cases

Prevalence of *R. felis* in non-malaria fever in sub-saharan Africa ~4%

Tropheryma whipplei

- Agent of the Whipple's disease, disease first described in 1907
- Chronic disease mainly observed in caucasian males of fifty years
- High prevalence of asymptomatic carriage in feces and saliva in Senegal

Rural Senegal			
	Population	Adults	Children
Feces	Prevalence	17.4%	48.2%
Saliva	Prevalence	1.7%	9.5%

- *T. whipplei* detected in the blood of **6.4%** of febrile patients for the first time

OPEN ACCESS Freely available online



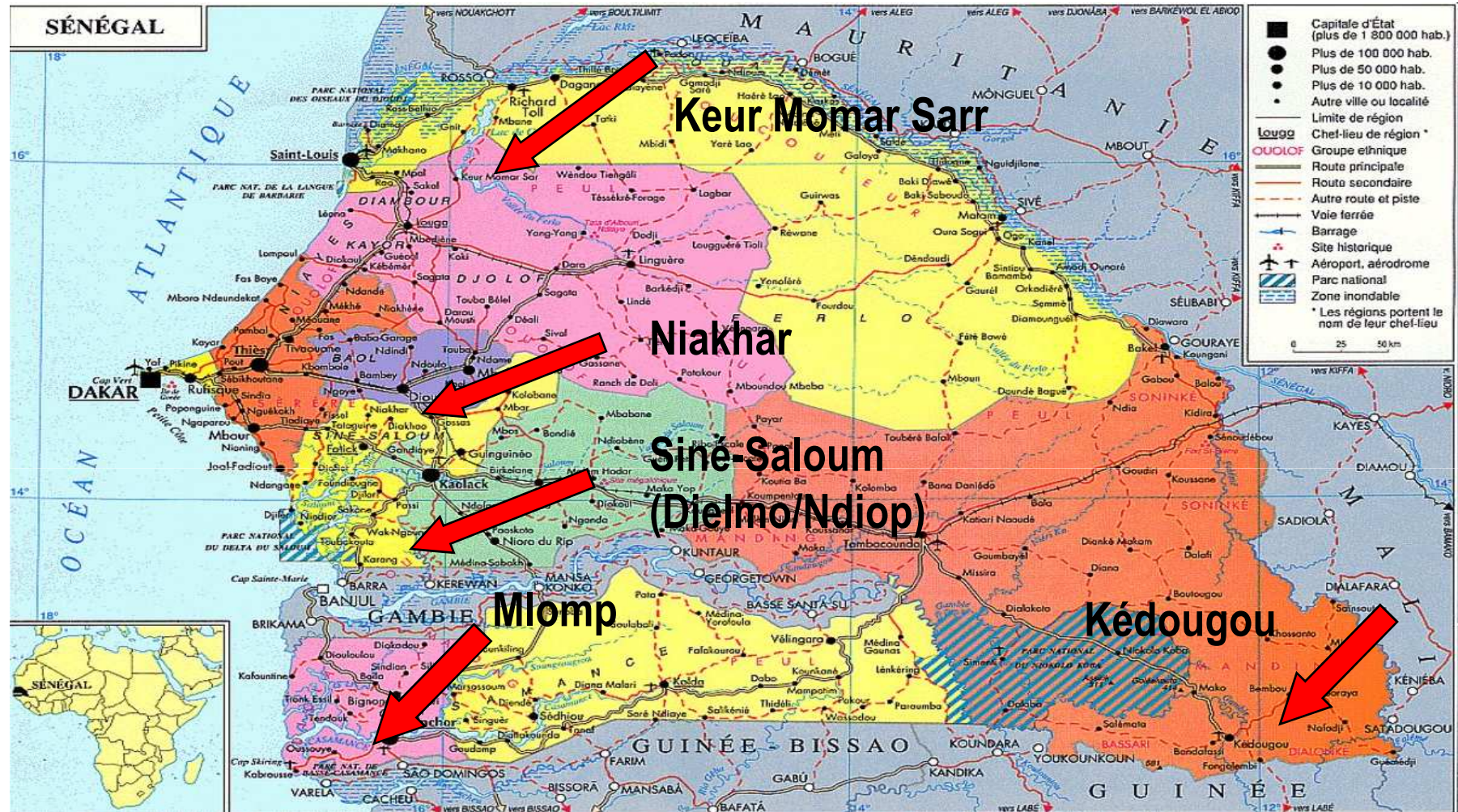
Tropheryma whipplei: A Common Bacterium in Rural Senegal

Alpha Kabinet Keita¹, Hubert Bassene², Adama Tall³, Cheikh Sokhna², Pavel Ratmanov^{1,4}, Jean-François Trape², Didier Raoult^{1,2}, Florence Fenollar^{1,2*}

December 2011 | Volume 5 | Issue 12 | e1403

Addition of other study areas in Senegal

Other climatic zones and habitats

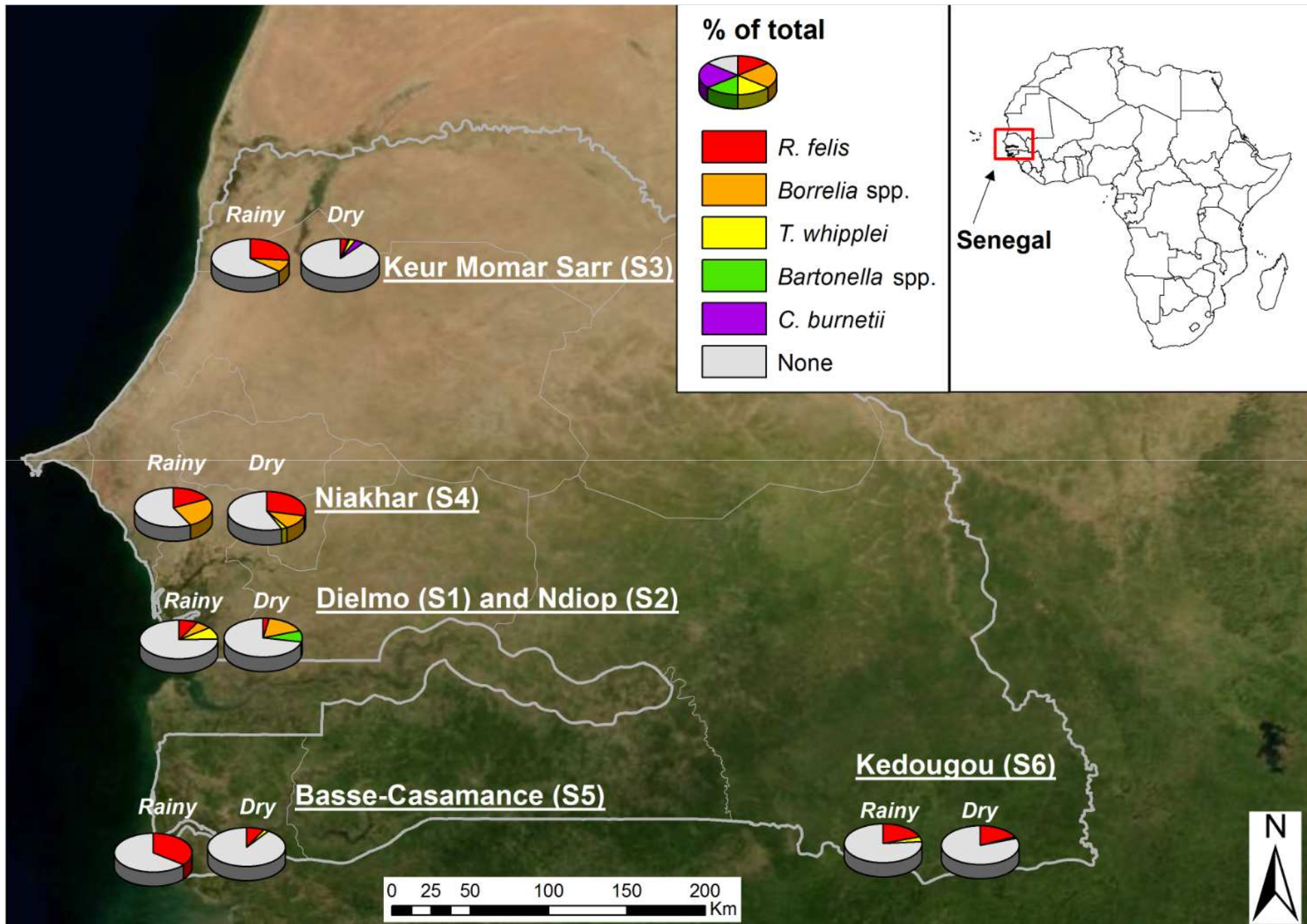


Mediannikov O, Socolovschi C, //, Raoult D. Common epidemiology of *R. felis* infection and malaria, Africa. *Emerg Infect Dis.* 2013;19:1775-83.

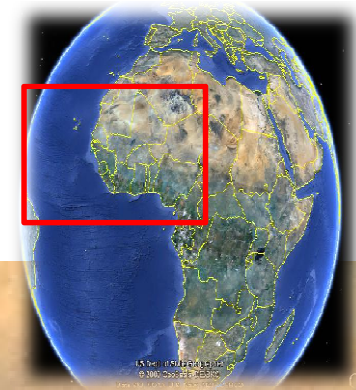
Diatta G, Mediannikov O, Sokhna C, //, Raoult D. Prevalence of *B. quintana* in patients with fever and head lice from rural areas of Sine-Saloum, Senegal. *Am J Trop Med Hyg.* 2014;91:291-3.

Mediannikov O, Socolovschi C, //, Raoult D. *B. crocidurae* infection in acutely febrile patients, Senegal. *Emerg Infect Dis.* 2014;20:1335-8.

Angelakis E, Mediannikov O, Socolovschi C, //, Raoult D. *C. burnetii*-positive PCR in febrile patients in rural and urban Africa. *Int J Infect Dis.* 2014;28:107-110.



Development of other collaborations in sub-saharan Africa



Diagnosis of infections in islands

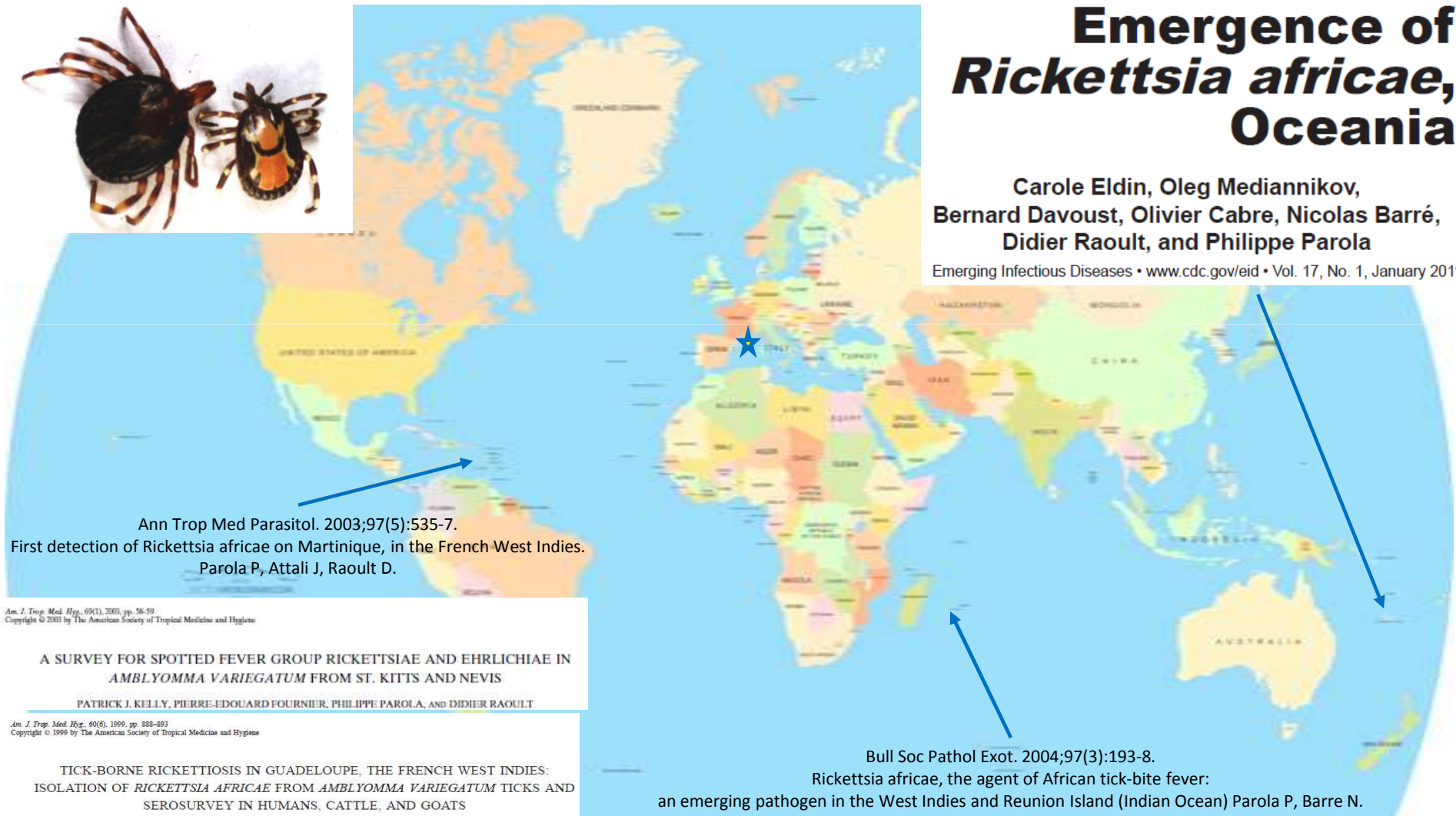
West Indies, Reunion, New Caledonia



Emergence of *Rickettsia africae*, Oceania

Carole Eldin, Oleg Mediannikov,
Bernard Davoust, Olivier Cabre, Nicolas Barré,
Didier Raoult, and Philippe Parola

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 17, No. 1, January 2011



Ann Trop Med Parasitol. 2003;97(5):535-7.
First detection of *Rickettsia africae* on Martinique, in the French West Indies.
Parola P, Attali J, Raoult D.

Am. J. Trop. Med. Hyg., 69(1), 2003, pp. 58-59
Copyright © 2003 by The American Society of Tropical Medicine and Hygiene

A SURVEY FOR SPOTTED FEVER GROUP RICKETTSIAE AND EHRlichIAE IN *AMBLyOMMA VARIEGATUM* FROM ST. KITTS AND NEVIS

PATRICK J. KELLY, PIERRE-EDOUARD FOURNIER, PHILIPPE PAROLA, AND DIDIER RAOULT

Am. J. Trop. Med. Hyg., 60(6), 1999, pp. 888-893
Copyright © 1999 by The American Society of Tropical Medicine and Hygiene

TICK-BORNE RICKETTSIOSIS IN GUADELOUPE, THE FRENCH WEST INDIES: ISOLATION OF *RICKETTSIA AFRICAe* FROM *AMBLyOMMA VARIEGATUM* TICKS AND SEROSURVEY IN HUMANS, CATTLE, AND GOATS

PHILIPPE PAROLA, GUY VESTRIS, DOMINIQUE MARTINEZ, BERNARD BROCHIER, VERONIQUE ROUX, AND DIDIER RAOULT

Bull Soc Pathol Exot. 2004;97(3):193-8.
Rickettsia africae, the agent of African tick-bite fever:
an emerging pathogen in the West Indies and Reunion Island (Indian Ocean) Parola P, Barre N.

Diagnosis of infections in islands

French Polynesia

- Collaboration with D. Musso (Institut Malardé)
- Search for zoonotic and arthropod-borne infections in Polynesia
- Study of specificities in the human gut microflora of Polynesians

Musso et al. *BMC Infectious Diseases* 2014, 14:255
<http://www.biomedcentral.com/1471-2334/14/255>



RESEARCH ARTICLE

Open Access

Absence of antibodies to *Rickettsia spp.*, *Bartonella spp.*, *Ehrlichia spp.* and *Coxiella burnetii* in Tahiti, French Polynesia

Didier Musso^{1*}, Julien Brout², Philippe Parola³, Didier Raoult³ and Pierre-Edouard Fournier³

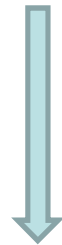
Dubourg G, Lagier JC, Robert C, Armougom F, Hugon P, Metidji S, Dione N, Dangui NP, Pfliegerer A, Abrahao J, Musso D, Papazian L, Brouqui P, Bibi F, Yasir M, Vialettes B, Raoult D. Culturomics and pyrosequencing evidence of the reduction in gut microbiota diversity in patients with broad-spectrum antibiotics. *Int J Antimicrob Agents*. 2014;44:117-24.

Hugon P, Lagier JC, Robert C, Lepolard C, Papazian L, Musso D, Vialettes B, Raoult D. Molecular studies neglect apparently gram-negative populations in the human gut microbiota. *J Clin Microbiol*. 2013;51:3286-93.

Sending specimens to
references laboratories

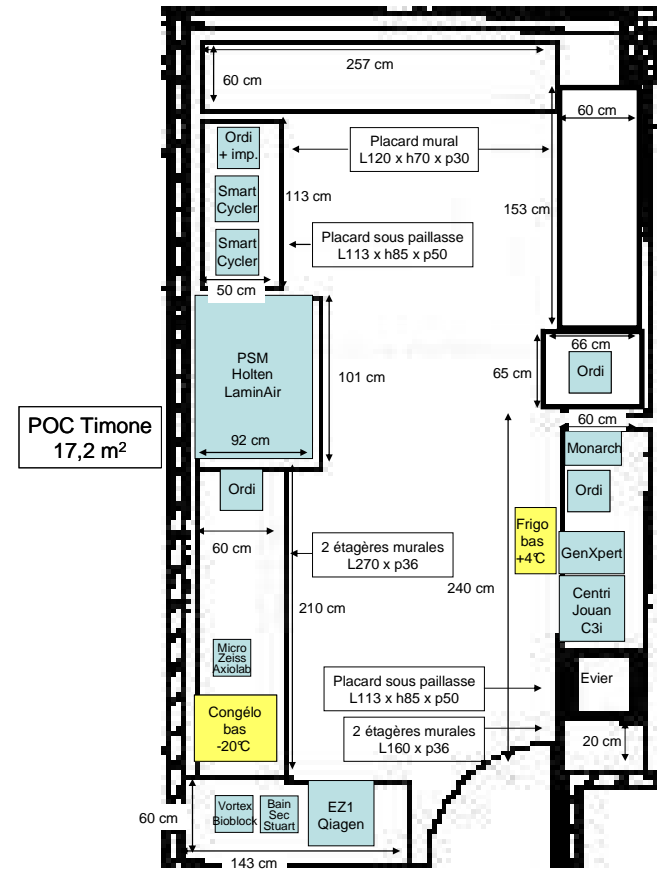


**Diagnosis and surveillance of infectious
diseases in remote areas**



On-site testing

Development of point of care laboratories



A small space in a strategic place

Point-of-care (POC) Aims

Providing microbiology results within health care time

- Answering three questions :
 - Isolation?
 - Hospitalization?
 - Treatment?
- Syndrome-based sampling and testing
- Immunochromatography assays and RT-PCR

Open 7/7, 24/24



Syndrome-based analyses

POC laboratory

PNEU

Pneumonia

- Flu ICT
- VRS ICT
- Mycoplasma pneumoniae PCR
- Whooping-cough (B. pertussis) PCR
- Coxiella burnetii PCR
- Staphylococcus aureus (ICU only) PCR
- Pneumocystis jiroveci PCR
- Legionella urinary antigen
- Pneumococcal urinary antigen
- Procalcitonin

MENIN

Meningitis

- Cytology
- Enterovirus PCR
- Herpes virus PCR
- Varicella zoster virus PCR
- Meningococcus PCR
- Pneumococcus PCR
- Cryptococcus ICT

GYNECO

Gynecology

- HIV ICT
- Group B streptococcus PCR
- Atopobium vaginae PCR
- N. gonorrhoea ICT

GASTRO

Gastro-enteritis, diarrhoea

- Rotavirus – adenovirus ICT
- Norovirus ICT
- Clostridium difficile - Helicobacter pylori ICT

ANG

Pharyngitis

- Group A streptococcus ICT
- Infectious mononucleosis MNI test

Tropical fever

Tropical fever

- Malaria PCR
- Malaria ICT
- Dengue ICT

Other

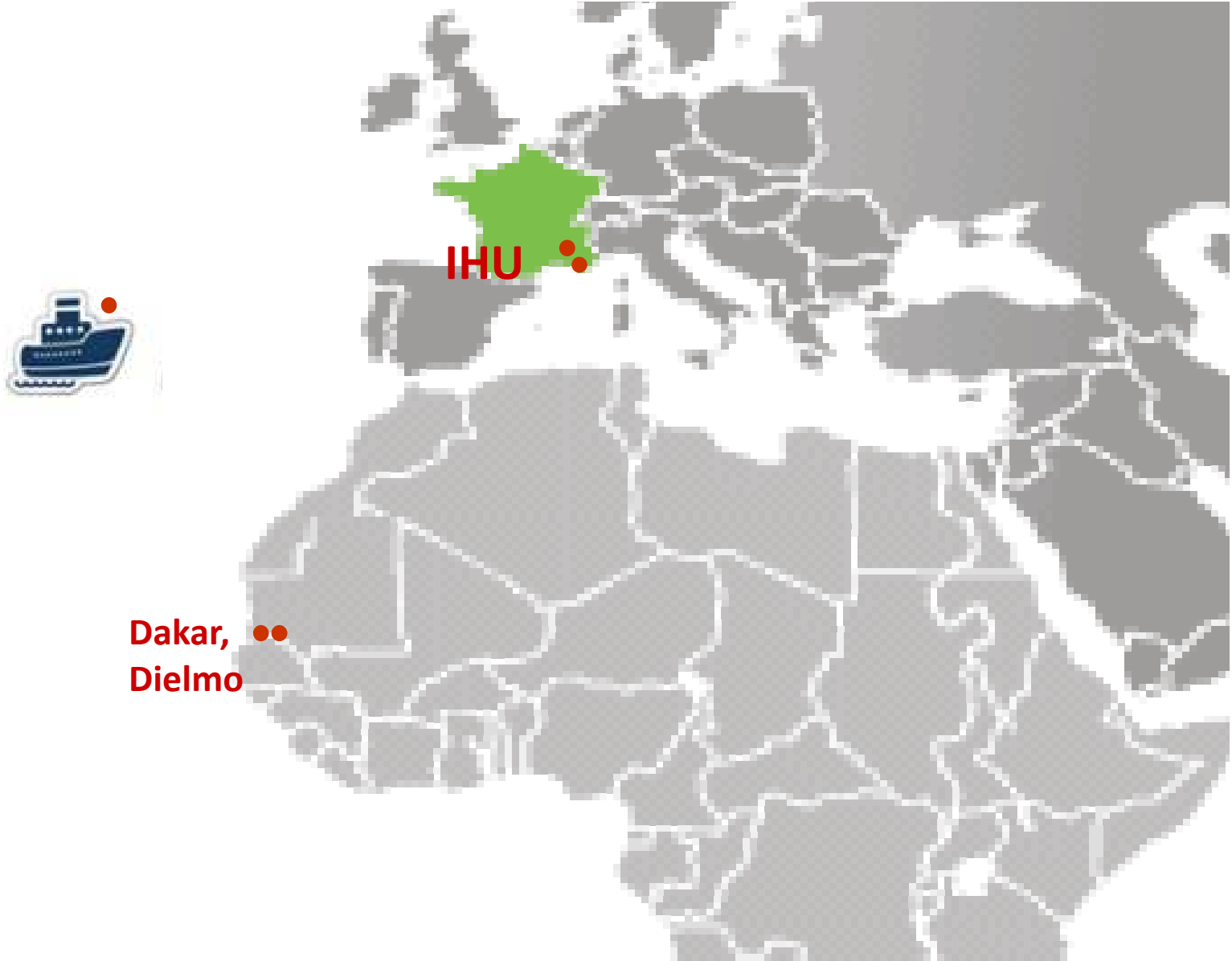
Others

- Blood exposure accident = HIV
- Tetanus toxin ICT
- Procalcitonin

French certification (COFRAC)

- June 2014
- Principle of remote microbiology laboratory
- Three tests :
 - immunochromatography, *Legionella pneumophila*
 - immunochromatography, *Streptococcus pyogenes*
 - immunochromatography, *Streptococcus pneumoniae*

Localisation of the five URMITE POCs



Technology transfer

Establishment of a laboratory Point-Of-Care (POC) in Dielmo, Senegal:

Immuno-chromatographic tests

Molecular assays: Real-Time PCR assays

Constraints

Reagents

Ready-to-use and resistant to unstable transport conditions
Preparation of lyophilized ready-to-uses PCR mixes
Regenerated in the laboratory

Room (9 m²)

Specifically prepared and well constructed
Thermo-isolated (double brick wall)
Air conditioned (tropicalised air conditioner)
Protected from dust and insects (SAS)

Electricity

In the absence of electricity, a diesel electric generator should be used
Solar power: Not usually sufficient for the functioning of DNA extractor
and thermal cycler

Personnel

Specifically trained

Choosing a study area

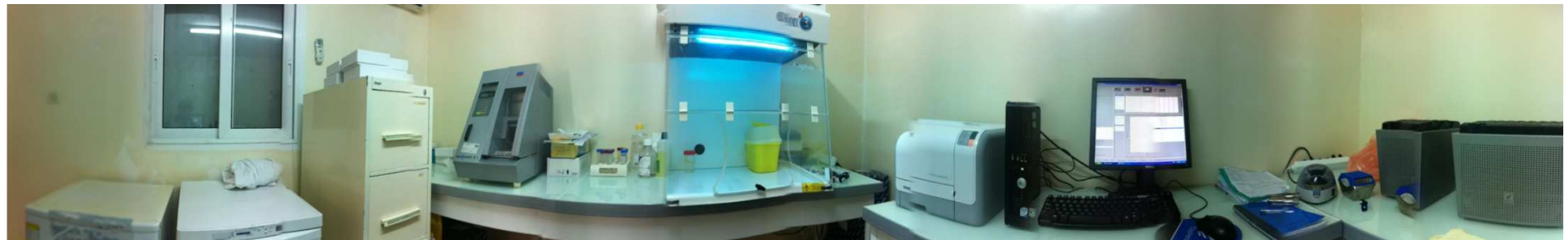
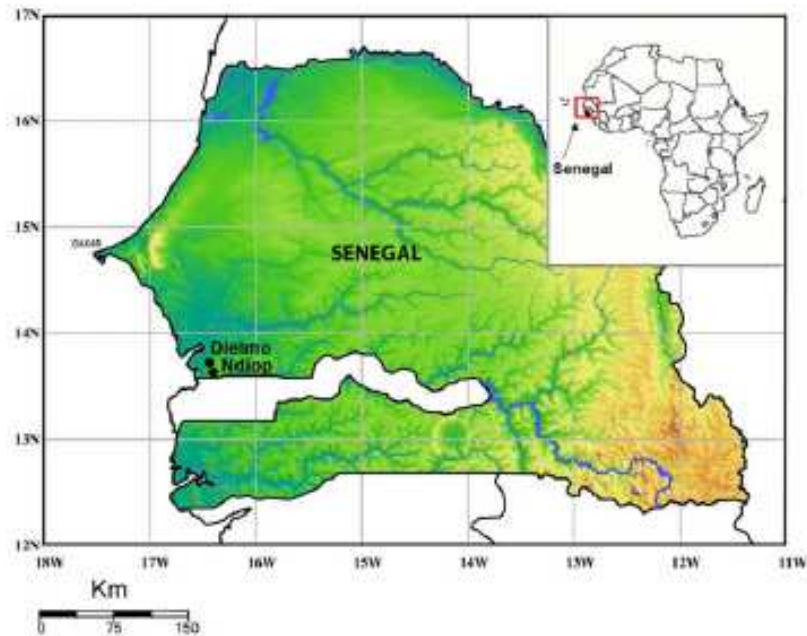
Select an area with existing medical infrastructure

→ The Dielmo field research station

- Build in 1990 in Dielmo, Senegal, IRD and IPD
- Malaria Surveillance Project
- Dispensary, laboratory and 10 huts
- A nurse, 2 technicians, 3 investigators, open 7 days 7, 24 hours 24
- Approval of the ethics committee Senegal
- Specific files for febrile episodes

→ Functional and experienced station





Sokhna C, Mediannikov O, Fenollar F, Bassene H, Diatta G, Tall A, Trape JF, Drancourt M, Raoult D. Point-of-care laboratory of pathogen diagnosis in rural Senegal. *PLoS Negl Trop Dis*. 2013;7:e1999.

Mediannikov O, Socolovschi C, Million M, Sokhna C, Bassene H, Diatta G, Fenollar F, Raoult D. Molecular identification of Pathogenic Bacteria in Eschars from Acute Febrile Patients, Senegal. *Am J Trop Med Hyg*. 2014: 13-0629.

Equipment

Working tables and chairs

A portable meteorological station

Laboratory equipment:

- One freezer at -20° C
- One refrigerator $+4^{\circ}$ C
- A safety cabinet designed for PCR rooms
- A heating block for Eppendorf tubes
- A manual polyvinyl pump
- A portable centrifuge for 1.5 ml tubes
- A vortex
- A Qiagen BioRobot EZ1 Workstation for DNA extraction
- Two Smart cycler II units (Cepheid) supplied with computers, printers and mini-centrifuges

Total cost of the POC estimated at **158,000 euros** (incl. all taxes and shipping)



DNA Extraction (EZ1 QIAGEN)



Preparation of PCR mixes for qPCR



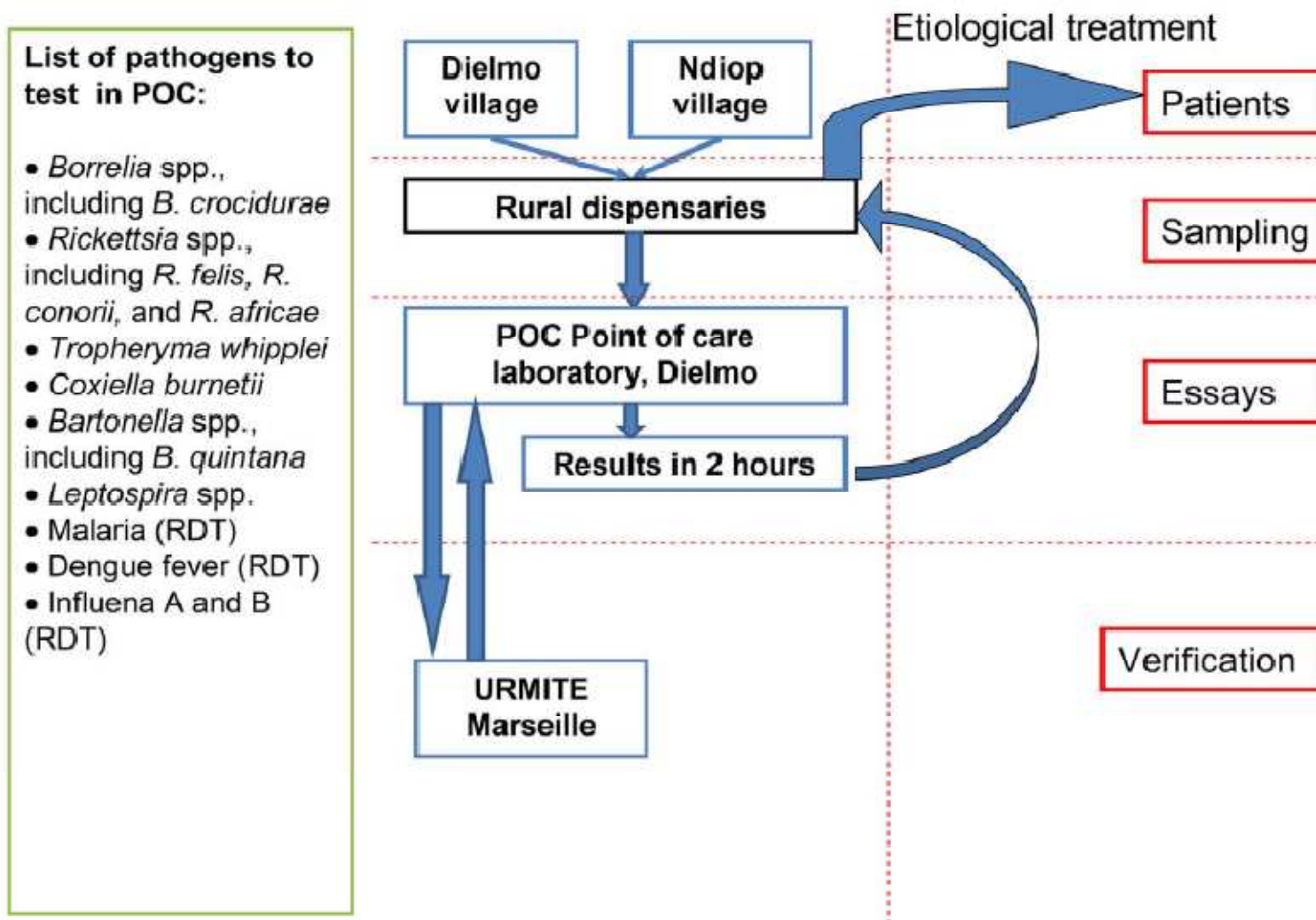


Figure 3. General scheme of the POC with a list of the pathogens diagnosed.

Senegal POC output

- November 1st, 2013 – October 31, 2014 : 576 specimens referred to the POC
- Diagnoses made: malaria = 159 (27.6%), flu A = 45 (7.8%), borreliosis = 19 (3.3%), *R. felis* bacteremia = 20 (3.4%), Q fever = 11 (1.9%), trench fever = 10 (1.7%), *T. whipplei* bacteremia = 4 (0.7%)

Summary of key points

- European laboratories can help establish the repertory of infectious diseases in the remote areas
- Working in remote areas imposes minimal constraints
- Focus collaborations with areas equipped with medical infrastructure
- Always assess the feasibility of assay choice by making a preliminary study => then, expand the study on a larger scale
- Possibility of technology transfer
- Applicable to the Pacific area