Institut Pasteur

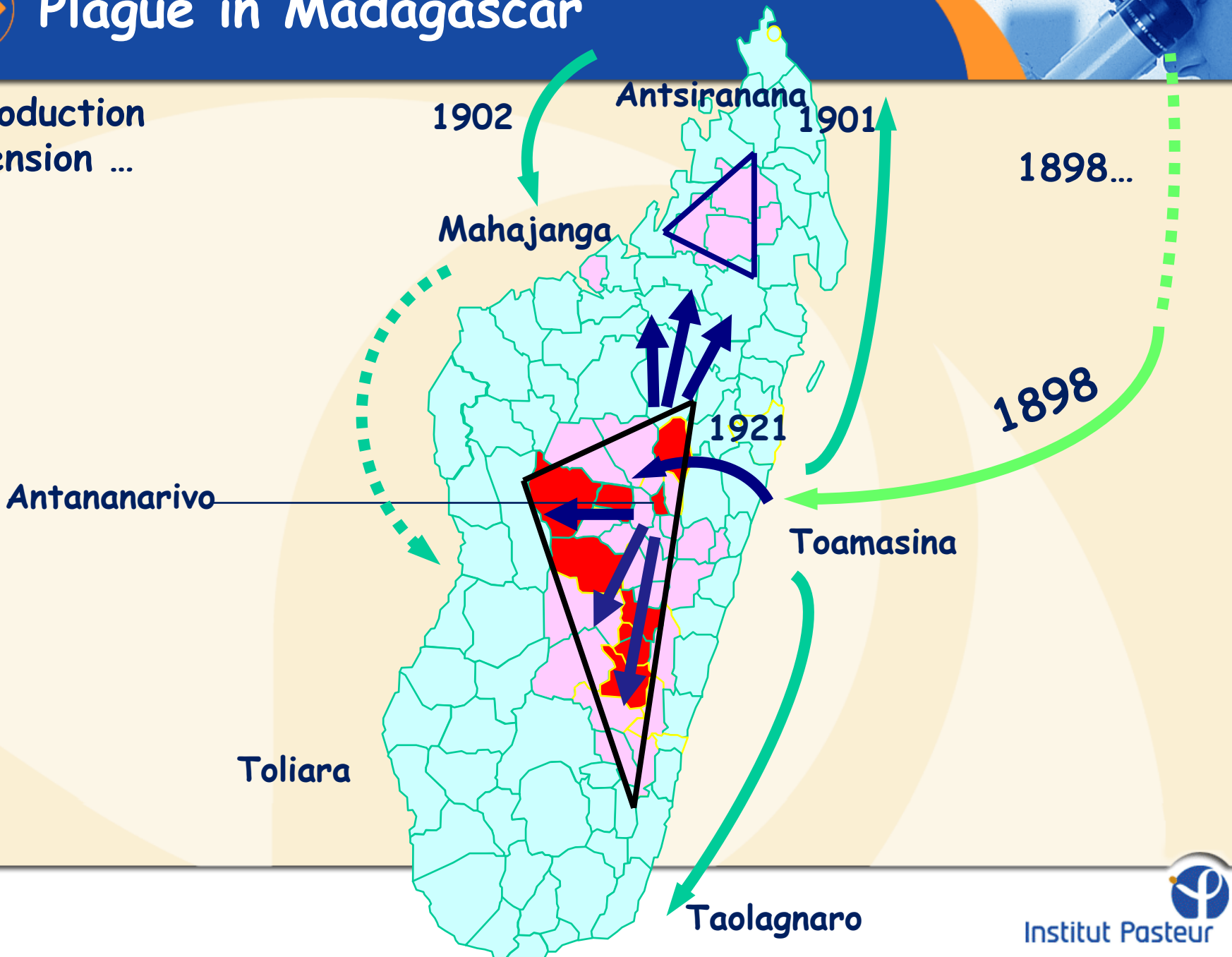
PLAGUE RAPID TESTS: Development, implementation, use at primary health care level

International meeting of Latin American experts on plague

Lima Peru, january 22-24, 2013

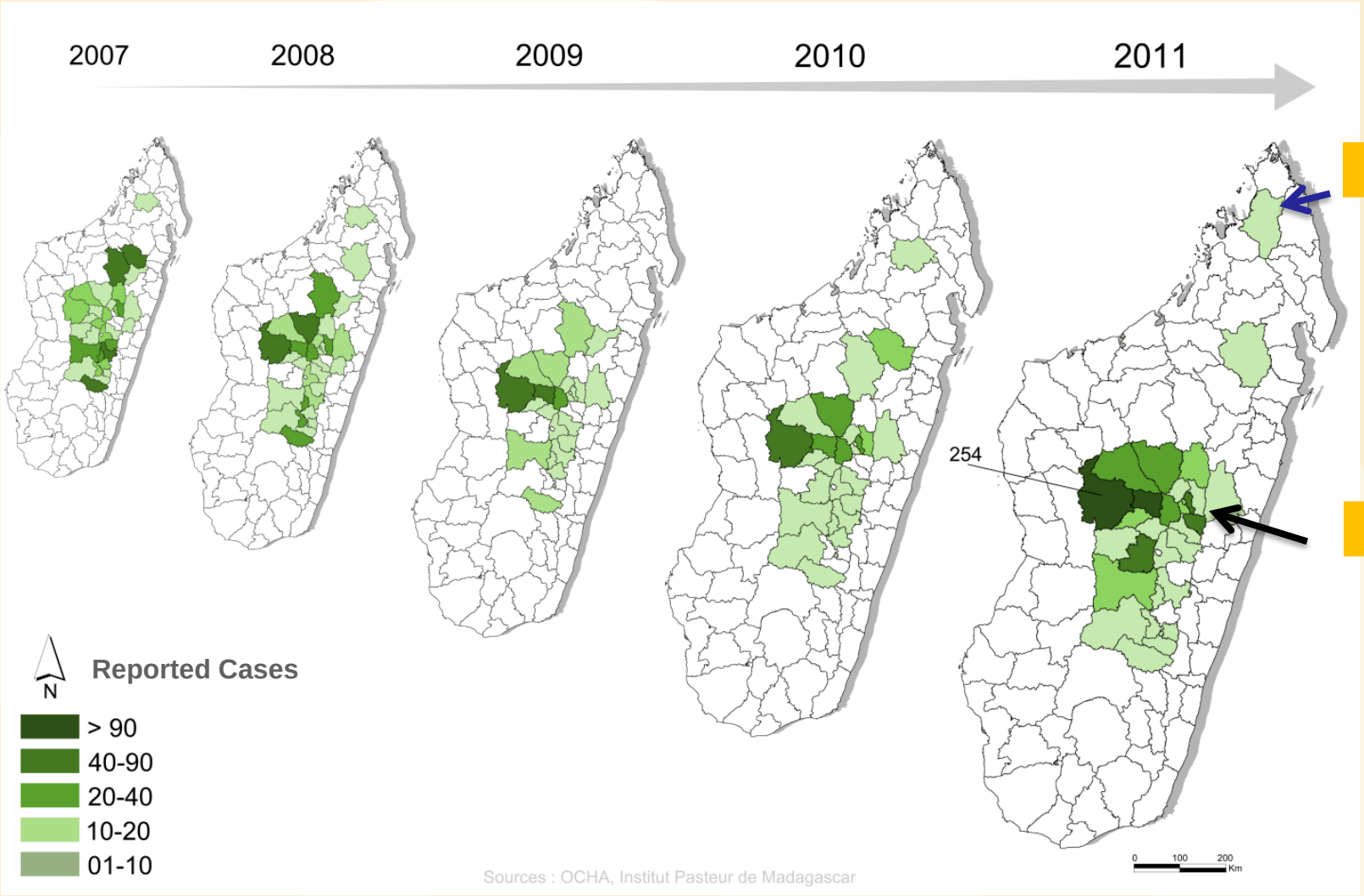
Plague in Madagascar

Introduction
Extension …



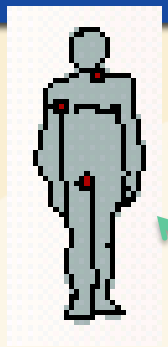
Institut Pasteur

Geographical distribution

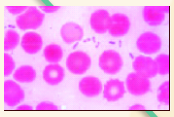


Institut Pasteur

Rural cycle



Xenopsylla cheopis



Yersinia pestis

Rattus rattus

Synospsyllus fonquerniei

Clinical Diagnosis… Primary level

- **Bubonic Plague: sudden onset of fever, chills, headache, very painful swelling of lymph nodes, severe malaise, prostration (incubation 5-6d, without treatment = death in <1 week)**

- **Pneumonic Plague: fever, cough with blood stained sputum, chest pain, and difficulty in breathing (incubation 24 h, without treatment = death in 2d)**

Biological diagnosis
In the Central Plague Laboratory

- **Bacteriology (Gold Standard)**
  Y. pestis isolation/acute phase specimen or post-mortem organs, Time 6-15d
- **Detection of F1 Ag (ELISA)**
  Acute phase specimen (threshold 2ng/ml)
  Specificity 100%, Sensitivity: 100% Time 6h
- **Serology anti-F1 IgG (ELISA)**
  (serum > 7 days after first clinical sign)
  Specificity 98%, Sensitivity 91%, Time 4h

Problems in endemic countries

Strategy

late clinical diagnosis

(high mortality)

late responses

biological confirmation long

>early detection, treatment

suspect cases,

chemoprophylaxis of contacts

>Urgent confirmation

>fleas control (insecticides)

>rodents control

Need for health workers: A rapid, easy test, used at primary health care level

RDT for F1 antigen detection: PLAGUE DIAGNOSIS

Rapid Diagnosis Test for F1 Ag detection

- **Development (2000):** IP Madagascar – IP Paris
- **Evaluation (2001):** IP Madagascar, IP Paris (lab. and field)
- **Validation, Diffusion (2001, 2002):** Madagascar
- **Diffusion (2003…):** Diagnostic use in other countries

S. Chanteau, L. Rahalison, L. Ralafiarisoa, J. Foulon, M. Ratsitorahina, L. Ratsifasoamanana, E. Carniel et F. Nato. Development and testing of a rapid diagnostic test for bubonic and pneumonic plague. Lancet, 361 (9353): 211-6, Jan 18, 2003.

Target: *Y. Pestis* F1 ANTIGEN

Capsular Glycoprotein - coded by plasmidic DNA (pFra)

***Y. pestis* specific**

Abundant (secreted at 37° C), heat stable

Different kinds samples (human, rodent)

Not influenced contaminants, treatment

Rapid Diagnostic test = dipstick

Principle : immunochromatography colloïdal gold particles (vertical flow)

Samples : bubo, sputum, serum, urine, post-mortem, spleen / liver (rats)

One-step, 15 mn, cut-off : 0.5 ng/ml F1

Internal validation, evaluation

- **Reference tests**

Microscopy Gram stain
Bacteriology
ELISA for F1 Antigen Detection
PCR

- **Samples:**

rodent and patients' samples
strains

Internal validation…

- **Validation of F1 Dips on control clinical samples**

  Specificity 100% (on 420 specimen from plague free and healthy individuals)

  Sensitivity 100% (on 166 sputa, sera, urine and bubo)

- **Validation of F1 Dips on rodent samples**

  Specificity 100% (on 78 healthy rats and mice specimen)

  Sensitivity 96-100% (on 64 dead rats and mice)

Internal validation

Validation of F1 Dips on other *Yersinia* cultures

Specificity 100% (on 134 strains of Y. enterocolitica, Y. pseudo-tuberculosis, non pathogenic Yersinia) (E. Carniel IPP)

Validation on *Y. pestis* cultures

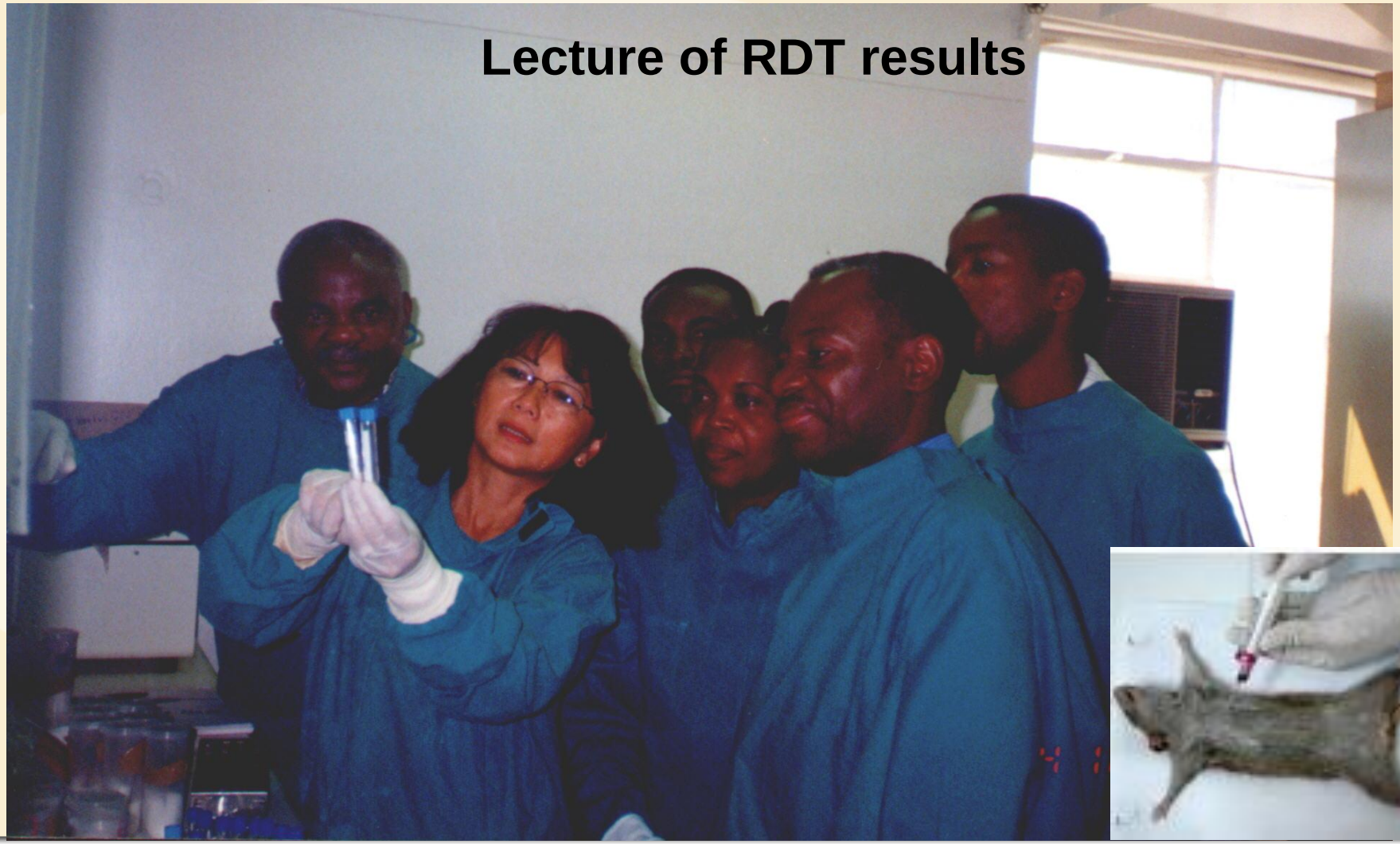
93-100% positive

(70 strains from Madagascar, African and Asian countries)

Implementation, use

1. Pilot Health Care Centres (6 hospitals, 20 primary health care level): Diagnostic Tool
2. Central Plague Laboratory
   Surveillance and Diagnostic Tool
3. General diffusion in Madagascar

Pilot Study: Staff Training



Institut Pasteur

Pilot site: use of Dips on primary level care

Sample collection, test on the bedside, treatment

Result, notification, report

Remaining sample to be sent to the CLP for comparison

Comparison Pilot sites vs Central Lab

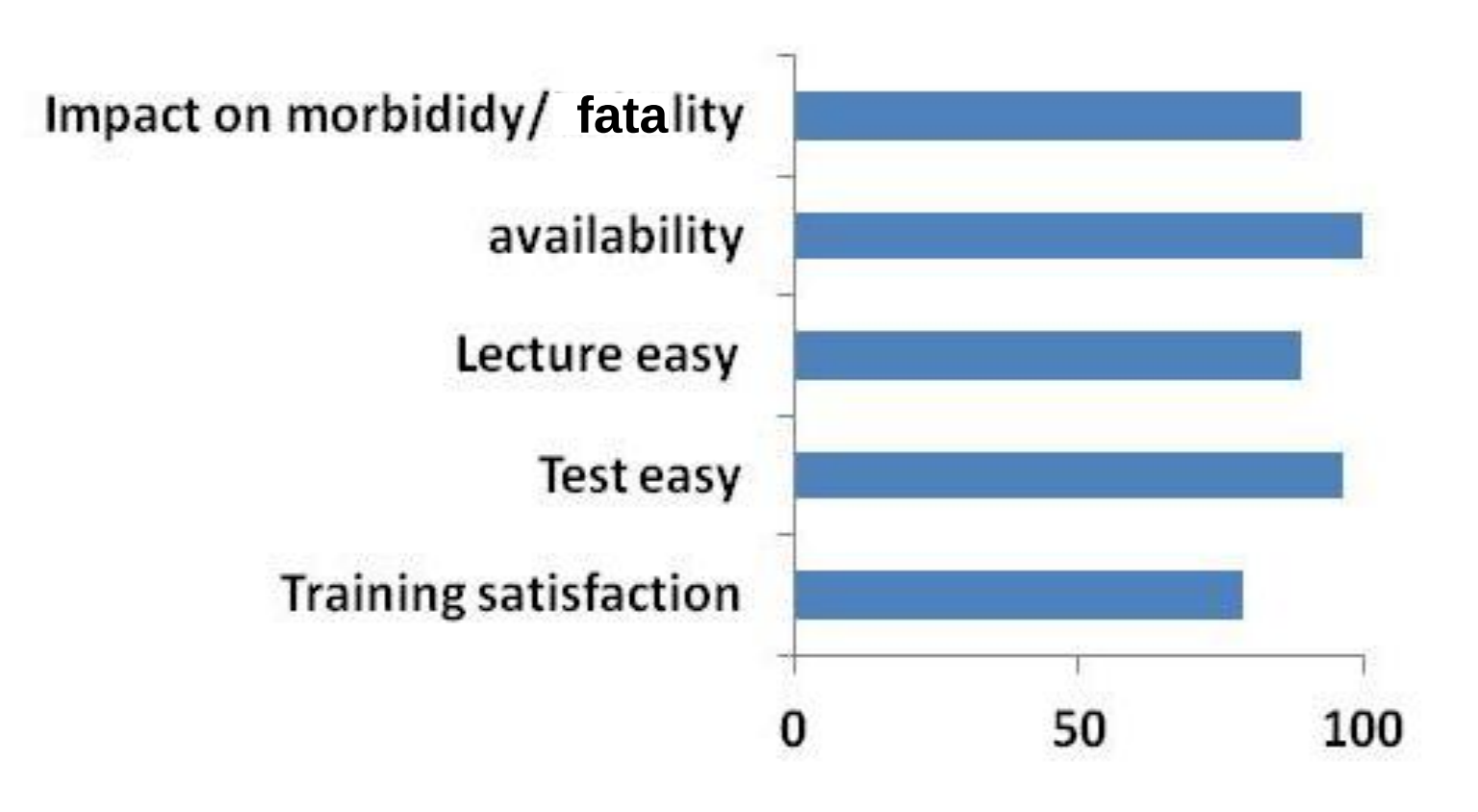
Central Lab.	Pilot Centres			
	Pos.	Neg.	Ind.	Total
Pos.	49	5	1	55
Neg.	4	66	3	73
Total	53	71	4	128

Agreement 90%
Disagreement 7% (weak pos.)
Indeterminate 3% (no control line)

Concordance F1 Dips / Bact. = 78.2%

Pilot study: users' satisfaction

Physician, Nurse, Technician, Health agent



Institut Pasteur

Diffusion of the RDT in Madagascar

Training 100 trainers from 42 District Health (SSD): Plague (clinical sign, differential diagnosis, use RDT, information...)

Diffusion:

kits in 300 plague endemic foci centres in Madagascar (SSD, CHD, CSB)

~ 3500 kits and ~ 300 supplies cases

Diffusion of the RDT in Madagascar

42 Districts in plague area (100 staffs)

Composition of a kit

- Syringe · Needle (18G)
- Eppendorf PBS
- Cary Blair tube . Swab
- Calibrated plastic tubes for assay
- Alcohol wipe
- Disinfectant wipe
- Elastoplast

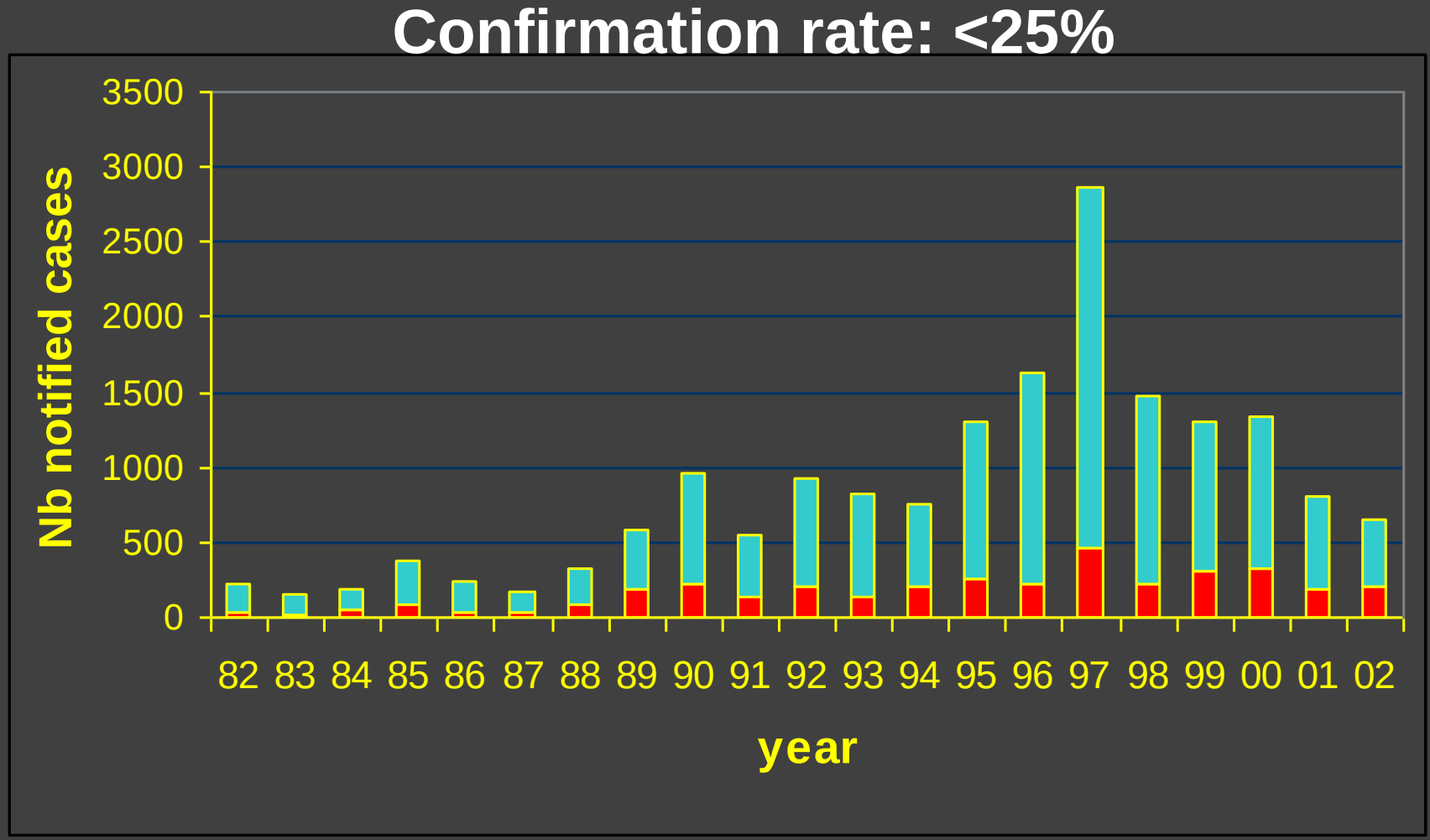
Institut Pasteur

Diffusion: kit preparation

Plague kits and supplies cases

Dispatching to plague area

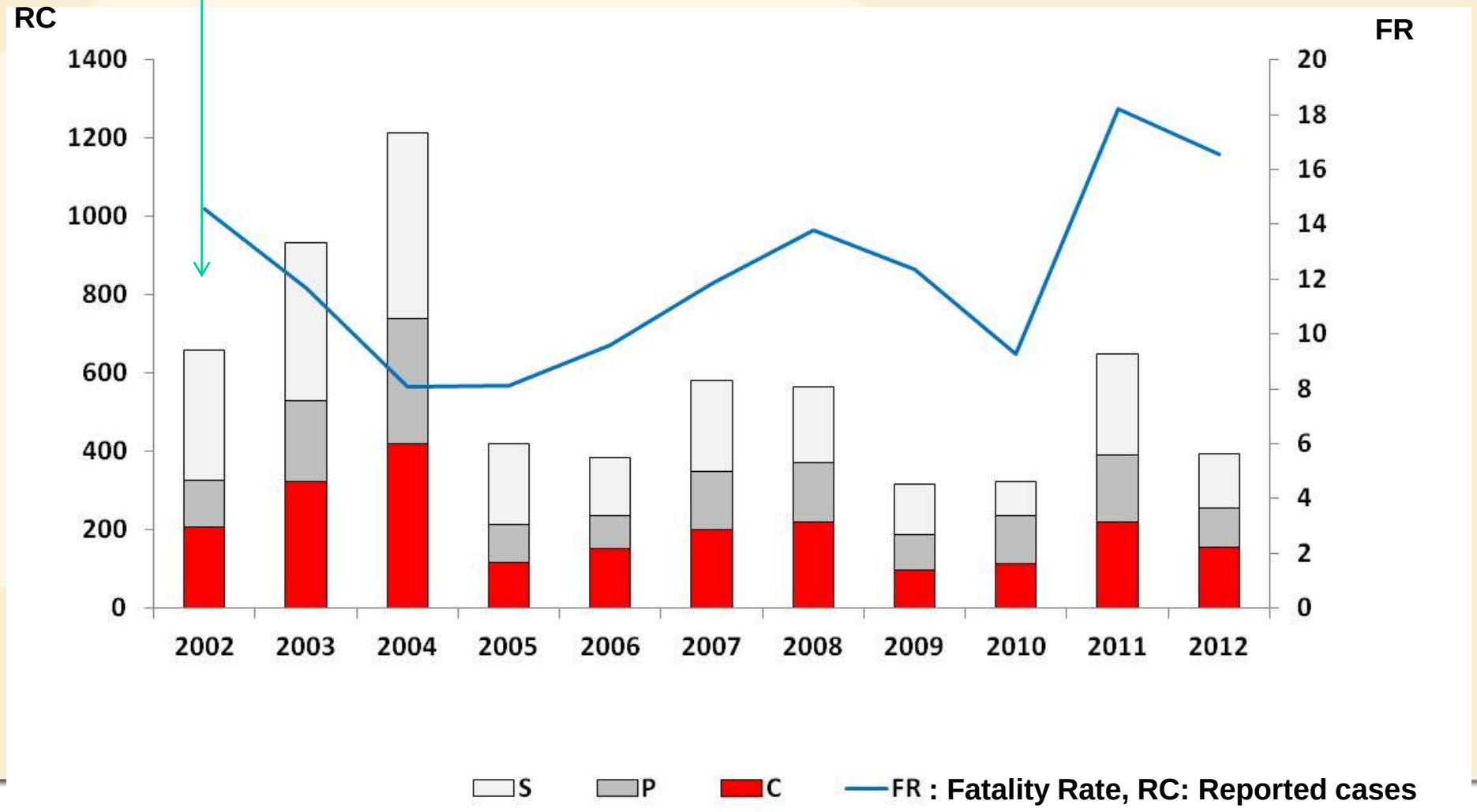
Plague situation before RDT use



Institut Pasteur

Situation from 2002 to 2012

Use RDT: Confirmation rate ++



Conclusion

Excellent internal value (Sp, Se~ 100%)

bubonic / pneumonic plague, epizootic

Validated in programme conditions

Substancial contribution in Central Lab

Nevertheless, 5% false negative (low F1)

✞ treat every suspect patients

Conclusion

Detection at primary level (human, rat)→ Alerte rapid→Prevention of human cases

Use RDT F1: Interregional meeting on plague on april 2006, WHO took into account the latest scientific and technical advances : case definition

Plague case definition

SUSPECTED CASE: compatible clinical presentation; and consistent epidemiological features

PRESUMPTIVE CASE: (definition of suspected case +)
Putative new or re-emerging focus: at least two of the tests positive (microscopy, F1 antigen, single anti-F1 serology, PCR); Known endemic focus: at least one

CONFIRMED CASE: (the definition of suspected case +)
***Y. pestis* isolated, or**
fourfold rise in anti-F1 antibody titre in paired serum samples
F1 Ag + (*in endemic areas when no other confirmatory test can be performed*)

RDT CANNOT REPLACE THE BACTERIOLOGY

IMPORTANCE OF STRAIN FOR THE SURVEILLANCE OF THE SENSITIVITY ON ATB USE FOR THE TREATMENT AND CHIOMIOPROPHYLAXIE

RDT for antibodies anti-F1detection
PLAGUE SURVEILLANCE

Rapid Diagnosis Test for Ab anti-F1

Development (2004): IP Madagascar – IP Paris

Evaluation (2005-2006): IP Madagascar, IP Paris, Ip Algeria, IP NhaTrang (lab. and field)

Evaluation cont'd (2007….) Madagascar, Iran, DRC, Peru, other countries

Rajerison M, Dartevelle S, Ralafiarisoa LA, Bitam I, Tuyet DT, Andrianaivoarimanana V, Nato F, Rahalison L. Development and Evaluation of Two Simple, Rapid Immunochromatographic Tests for the Detection of Yersinia pestis Antibodies in Humans and Reservoirs. PLoS Negl Trop Dis. 2009;3(4):e421.

Rapid Diagnosis Test for Ab anti-F1

Internal value:

Humans Se=84.6%, Spe=98%.

Rodents , other small mammals:

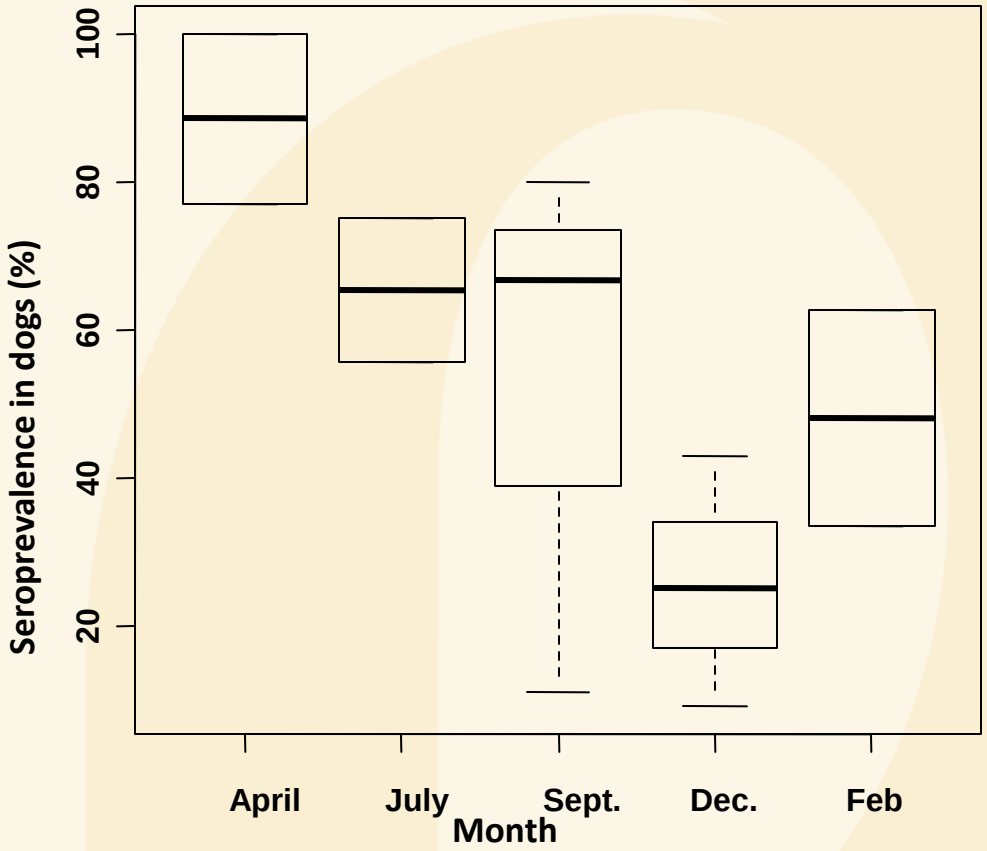
Se=87.8% , Spe = 90.3%

Dog: Se=93% , Spe=98%

Seroprevalence in dogs varies seasonally

Human cases of plague (Oct-Mar)

Effect of month:
χ2 =19.56 df=4
p=0.006



Seasonal pattern in dog seroprevalence is consistent with a loss of antibodies during the low season

Institut Pasteur

Use of dog on plague surveillance

Surveillance : low/high season

Presence of seroconverted dog (negative-positive) : a clue of plague circulation in the area

Presence of rodent carrier of plague bacilli

Alert, measure preventive,

Few Nb dog in a village vs rodent:
save a significant cost on assay

RDT Implementation in Peru:
Use in pilote sites
Production RDT for OPS

Collaboration between IP Madagascar-Paris and Peru

AGREEMENT N° E – 2009-OPD/INS

COLLABORATION AGREEMENT BETWEEN THE NATIONAL INSTITUTE OF HEALTH AND THE INSTITUT PASTEUR DE MADAGASCAR.

The present document consists of the Agreement established between

CONVENIO Nº E-2009-OPD/INS

CONVENIO DE COOPERACIÓN ENTRE EL INSTITUTO NACIONAL DE SALUD Y EL INSTITUTO PASTEUR DE MADAGASCAR

Conste por el presente documento, el Convenio que celebran de una parte:

General:

To create strategic alliances between the 2 Institutes, for the exchange of scientific and technological knowledge related to plague epidemiological surveillance and control, including the utilization of rapid tests for the diagnosis of plague in the field.

Use RDT: Pilot study in Peru

August 2010 (Investigation of PP)

Training of Lab Technician, Biologist, MD

Health Centers : INS , GERESA La Libertad, LRR Lambayeque

Supplies: RDT, video for instruction use in spanish version (WHO-PAHO-INS)

Pilot site: RDT training

Use of rapid test on human samples
Importance of sample in plague dianosis

Samples collection according to clinical form (kits for Peru Health Center, instruction use sheet in spanish)

Sample testing process on rodent
Lecture of RDT results
Practices (field condition for rodent surveillance)

Training on sample collection

Institut Pasteur

Training in field condition

Institut Pasteur

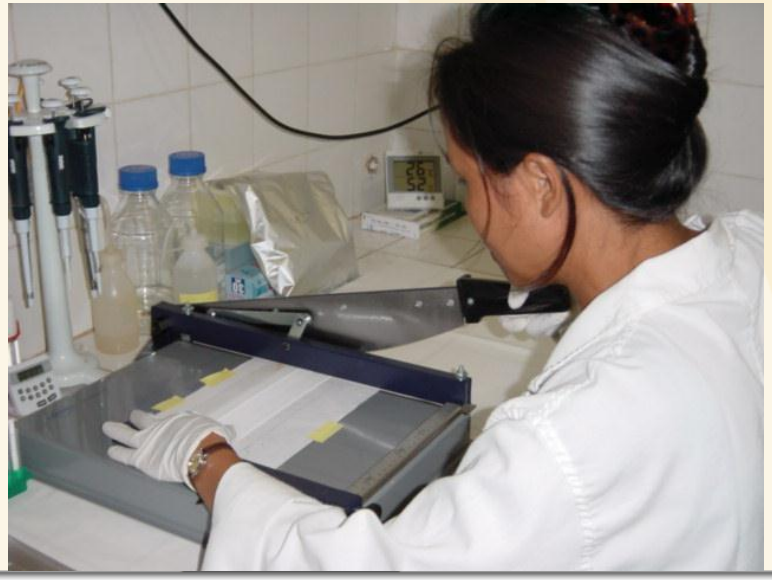
Production: technology transfer

Mab used for RDT production

Patent not exclusive (Conservation Matériels

Scientifique IP Paris)

Technical assistance (IPM –Paris PF5)



Conclusion, perspectives

Improve the technical capacities of the National lab reference INS Peru

- **Serology, RDT, Bacteriology(IPM)**
- **Molecular biology (IP Paris)**

Include the use of RDT (Ag F1; Ab) in the National system surveillance in Peru: training of staff from plague foci (pilot) in 2010

Next step:

- **Training of Peru lab staff (in Paris, Madagascar)**
- **To strengthen plague epidemiological system: share experience Peru vs Madagascar (meeting in Madagascar)**

Laboratory support for outbreak response:

- ✓ **Essential functions and Roles of laboratoires: rapid detection= alerte rapid**

- ✓ **During the investigation: to confirm the identification of disease.**

Acknowledgment:

Financial support
World Bank, IP, OMS, MoH,
IP, Wellcome-Trust,
OMS, PAHO, INS , IP

Staffs involved in these studies

Institut Pasteur

Merci de votre aimable attention

Recommandations

Importance du réseau de laboratoire: maintient / amélioration de la qualité (technique, personnel, ….) pour pouvoir confirmer à temps une épidémie

Définition des cas selon critères OMS

Quelles sont les tendances spatiales et temporelles dans les nombres des rats et des puces ?

Quels facteurs influencent les tendances spatio-temporelles?
Climat, végétation,
Déplacements (situation habituelle, forte pression=feux dans les champs de canne à sucre)

Est-ce que les rats des différents endroits dans la zone endémique ont une sensibilité différente à *Y. pestis*?

Quels facteurs influencent la sensibilité ?

Adaptater les mesures de lutte

Peste une maladie multifactorielle: (socio-economique, politique, comportement humain, ……..)