Le Réseau International des Instituts Pasteur est un ensemble de 29 Instituts répartis sur cinq continents, regroupant 9 500 personnes.

Le Réseau International des Instituts Pasteur est né de la volonté de Louis Pasteur de former, dans l’établissement de Paris, des savants pour qu’ils appliquent et transmettent leur savoir dans les pays lointains.

Unis par la même vocation, la même culture scientifique et les mêmes valeurs, ces Instituts mettent leurs compétences et leurs moyens au service des besoins de santé de leur pays.

La mission du Réseau international des Instituts Pasteur est de contribuer à la lutte contre les maladies infectieuses touchant les populations des pays et régions où ils sont implantés par leurs activités de :

- Recherche
- Santé Publique
- Formation et Enseignement
- Services
- Production

Le Réseau contribue à une veille sanitaire et à une surveillance épidémiologique à l’échelle de la planète.
Best practices in rabies diagnosis and surveillance

- Introduction
- Importance of clinical surveillance
- Post mortem diagnosis
- Intra vitam diagnosis
- Detection of rabies antibodies
- Conclusion

Hervé Bourhy
UPRE Lysavirus dynamics and host adaptation
National Reference centre for rabies
WHO Collaborating centre for reference and research on rabies
Institut Pasteur, Paris
Factors in re-emergence (lyssaviruses)

- Public health infrastructure
  - Breakdown of public health measures
  - Inadequate communicable disease surveillance and diagnostic capacity
  - Lack of trained personnel

- Population movements
  - Increased long-distance travel

- Virus evolution and adaptation
  - Changes in virulence
  - Adaptation to new econiches

- Ecosystem disturbance
  - Environmental changes due to economical development
  - Animal translocation
  - Agriculture
  - Modification of the landscape, Deforestation

- New tools for characterization

(Adapted from McMichael, 2001)
Rabies in humans 1996-2006

Clinically diagnosed

? A lack of laboratory confirmation

Confirmed in the laboratory

(Source: OMS)
Rabies in humans
1996-2006
Clinically diagnosed
(Source: OMS)

Rabies in dogs
1996-2006
Confirmed in the laboratory

The burden of rabies is underestimated
Reservoirs of lyssavirus

(Murphy et al., Science, 2001)
Reservoirs in the Order Carnivora

In Asia and Africa: more than 99% of the human cases are due to canine rabies

(Adapted from Flynn et al., Syst Biol 2005)
Rabies
(lyssavirus, genotype 1)

Reservoir

Dead end infections

Domestic Animals

Vectors
### Different species/genotypes of lyssavirus

<table>
<thead>
<tr>
<th>Genotypes or Species</th>
<th>Geographic distribution</th>
<th>Animal species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Rabies (+ vaccine strains)</td>
<td>Worldwide, except: Australia, British Islands, Ireland, New Zealand, Japan, Scandinavia, Hawaii</td>
<td>Man, wild and domestic carnivores and herbivores, hematophagous bats and frugivorous bats</td>
</tr>
<tr>
<td>2. Lagos bat</td>
<td>Nigeria, Central African Republic, South Africa, Zimbabwe, Guinea, Senegal, Ethiopia, Egypt</td>
<td>Frugivorous bats, cats, dog</td>
</tr>
<tr>
<td>4. Duvenhage</td>
<td>South Africa, Zimbabwe</td>
<td>Man, insectivorous bats</td>
</tr>
<tr>
<td>5. European bat lyssavirus 1 (EBL1)</td>
<td>Europe</td>
<td>Man, stone marten, sheep, insectivorous bats</td>
</tr>
<tr>
<td>6. European bat lyssavirus 2 (EBL2)</td>
<td>Europe</td>
<td>Man, insectivorous bats</td>
</tr>
<tr>
<td>7. Australian bat lyssavirus (ABL)</td>
<td>Australia</td>
<td>Man, insectivorous bats, frugivorous bats</td>
</tr>
<tr>
<td>8., 9., 10.</td>
<td>Central Asian Republic, west caucase</td>
<td>Insectivorous bats</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>
Intersectorial collaboration in surveillance

Ministry of Agriculture

Ministry of Health

Informations required for a proper medical decision

Veterinary services

Antirabies clinics

Clinical surveillance

Laboratory diagnosis

Patients
Late phase of infection

> Laboratory diagnosis

Early phase of infection

Role des tissus nerveux et non nerveux
Infection Transmission Modification of behaviour

Observation period (Dogs and cats)

Laboratory diagnosis

Viral excretion

Observation period (Dogs and cats):
- Infection
- Transmission
- Modification of behaviour

- 2-5 Days
- 10 days
- (2 months)
- Death

2-5 Days

Viral excretion

Laboratory diagnosis
Laboratory diagnosis of rabies: Why?

Accurate and prompt diagnosis of rabies essential for:
- Allowing reliable assessment of disease burden
- Monitoring trends of the disease and evaluate vaccination and control programs
- Proper medical decisions re: PET when implicated in a potential rabies exposure
  - Especially useful where cost of rabies PET is prohibitive
Laboratories operate at different levels

• **Peripheral** - Collect/process/transport specimens, simple tests

• **Intermediate** - Collect/process/transport, confirmatory tests, report results, join investigations

• **National** - Specialised tests, transport to International labs, report results, join investigations, training, production of reagents, others

• **International** - (Collaborating Centre, specialized laboratory) - Highly specialised tests, report results, join investigations, training, reagent production and as part of global networks
Diagnosis of rabies: problems

• Clinical diagnosis in humans is not always reliable
Varied clinical manifestation of rabies
Differenciation with other meningoencephalitis is sometimes difficult
Other etiologies are often being considered (ex: malaria)
Only spastic forms in humans are reported
A lack of history of exposure is not uncommon
Rabies can show long incubation periods

>>> Consider rabies in patients with risk factors and consistent or otherwise unexplained neurologic symptoms
>>> Confirm the clinical suspicion by laboratory diagnosis
Diagnosis of rabies: problems

• Collection and shipment of samples is sometimes difficult

Human brain samples are taken in exceptional circumstances
The shipment of the specimens (humans and animals) is difficult: submission of samples decreased significantly with distance from the laboratory
Association of increased risk for canine rabies and areas of low socio-economic status

• Inadequate laboratory facilities
Safety Measures

Level 2 (3) of biological safety

- Lab coats
- Masks
- Goggles
- Gloves
- Overshoes
- Safety hoods
- Vaccination of the staff
Post-mortem diagnosis

(In humans and animals)
The specimens
The techniques
Post-mortem specimens
Rapid collection of specimens
Easy ways of shipment
Trucut needle biopsy through superior orbital fissure

from Tong et al., The Lancet, 1999.
Biopsy by the occipital route

True cut biopsy needle or lumbar ponction needle

(cliché de B.M. Diop, CHU Fann, Dakar)
Targets of Post-mortem diagnosis

**TRANSCRIPTION**

- **GENOME (negative polarity)** 12 kb
- Leader
- **N P M G**
- Trailer

**TRANSLATION**

- Nucleo-protein
- Phospho-protein
- Matrix protein
- Glyco-protein
- Polymerase

**REPLICATION**

- Genome (-)
- Antigenome (+)
- Polymerase complex

**MORPHOGENESIS**

- Trailer
- Polymerase complex

**Targets of Post-mortem diagnosis**

- **RT-PCR**
- **FAT, ELISA**
- Fat, ELISA
- RFFIT, ELISA
- Isolation
FAT (Immunofluorescence)

The reference technique
Sensitivity and specificity > 99.9%
Results obtained rapidly
Need for trained personnel
Isolation on cell culture
(should replace MIT)

Specificity > 99%
Sensitivity 94-97%
ELISA for Rabies Ag detection: WELYSSA

(Xu et al., Biologicals, 2007)
WELYSSA

Threshold of detection = 0.8 ng/ml
1030 specimens from 19 animal species
7 genotypes of lyssavirus
Comparison with FAT and RTCIT
Specificity 0.999
Sensitivity 0.970
Easy, no need for trained personnel

(Xu et al., Biologicals, 2007)
Ante-mortem diagnosis

(In humans)
The techniques
The specimens
Principal clinical signs

Fever, anorexia, nausea,
Local pain at the site of bite

Agitation, depression

Incoordination, hydrophobia,
aerophobia, confusion,
hyperactivity

Paralysis (40%)

Coma
Modification of cardiac rhythm
Hypoventilation

Exposure
Incubation period
Duration 20-90 days

First symptoms
Prodromes
2-10 days

First neuronal signs
Acute neurol. phase
2-10 days

Death
0-14 days

(From Fisbein, D.B., 1991)
Targets of the Intra-vitam diagnosis

- RT-PCR
- FAT, ELISA
- Isolation
- MNT, RFFIT, ELISA
Similarity between the ORFs

N (79.8%)  L (77.9%)  M (76.3%)  G (58.4%)  P (47.4%)

(adapted from le Mercier et al., 1997)
## Results from a multicentric study
*(Cambodia, Senegal, Madagascar, France)*

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>Total</th>
<th>Per patient</th>
<th>Median range</th>
<th>Per sample</th>
<th>Per patient</th>
<th>Rate of positivity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Number of positive samples / total number of the considered sample; per patient: number of patients with at least one positive sample / number of total patients from whom the considered sample was collected.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saliva</td>
<td>84</td>
<td>1</td>
<td>0-6</td>
<td>70,2%</td>
<td>70,7%</td>
<td>57,5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(84)</td>
<td>(41)</td>
<td>(33)</td>
</tr>
<tr>
<td>Urine</td>
<td>63</td>
<td>1</td>
<td>0-5</td>
<td>11,11%</td>
<td>17,6%</td>
<td>8,3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(63)</td>
<td>(34)</td>
<td>(24)</td>
</tr>
<tr>
<td>Serum</td>
<td>46</td>
<td>1</td>
<td>1-3</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(46)</td>
<td>(43)</td>
<td>(33)</td>
</tr>
<tr>
<td>Skin biopsy</td>
<td>60</td>
<td>1.5</td>
<td>0-3</td>
<td>98,33%</td>
<td>96,7%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(60)</td>
<td>(31)</td>
<td>(33)</td>
</tr>
</tbody>
</table>
Skin biopsy from the nape of the neck

4 mm of diameter
Viral RNA in Saliva

Patient 1: D20 D21 D22 D23 D24
Result: 0 + + 0 +

Patient 2: D4 D5 D6 D7 D9 to D17
Result: + + + + 0

Excretion is not continuous
>> repeat collection of specimens

(Adapted from Crépin et al., 1998)
**Results of laboratory confirmation of rabies attempted in 55 patients during life**

<table>
<thead>
<tr>
<th>Time Frame</th>
<th>Number of Samples</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antigen in Skin Biopsies</strong></td>
<td>5/6 6/10 5/5 1/2 1/3</td>
<td>0.69</td>
</tr>
<tr>
<td><strong>Antibodies in Serum</strong></td>
<td>1/15 5/28 8/25 14/20 12/14</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Antibodies in CSF</strong></td>
<td>0/7 0/12 1/10 3/8 6/10</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>Antigen in Corneal Smears</strong></td>
<td>0/4 1/16 3/16 2/7 2/16</td>
<td>0.14</td>
</tr>
</tbody>
</table>

(Crepin et al., 1998)

**These results were obtained from the analysis of 22 (Anderson et al., 1984) and 17 (MMWR 1981 1996) cases of rabies in the USA and 16 cases from France (1970-May 1997) (unpublished results).**

*a Days after the onset of the symptoms*
## Specimens for rabies diagnostic

### Intra-vitam diagnosis

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Clinical phase</th>
<th>Temperature of shipment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-8 days</td>
<td>&gt;8 days</td>
</tr>
<tr>
<td>Saliva</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Urine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Skin biopsy</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Serum</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>CSF</td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

### Post-mortem diagnosis

- Brain biopsy (reference test) (+4 or -20°C)
- Skin biopsy (-20°C)
Conclusions

Clinical surveillance and laboratory diagnosis in animals
Clinical human suspicions should be laboratory confirmed:
Collection and shipment of specimen,
Standardization of laboratory techniques,
Expertise in rabies diagnosis: trained personnel,
National (regional) Reference Centre
Need for intersectorial collaboration

Clear and reliable picture of the epidemiological situation
International exchange of data
Similarity between the ORFs

N (79.8%)   L (77.9%)   M (76.3%)   G (58.4%)   P (47.4%)

(adapted from le Mercier et al., 1997)
Guidelines for determining when boosters should be administered.

Ș All people who work with live rabies virus in a diagnostic or research laboratory or in vaccine production should have *periodic antibody determinations to avoid unnecessary boosters.*

Ș People at continuous risk, e.g. rabies researchers, diagnostic laboratory workers (where virus is present continuously, often in high concentrations, and where specific exposures are likely to go unrecognized) should have *serological testing every 6 months.* A booster is recommended if the titre falls below 0.5 IU/ml.

Ș Responsible authorities should ensure that all people at risk are vaccinated and that serological status is monitored.

**ELISA**

- Cheap
- Rapid
- Detect all the rabies antibodies, not only the neutralizing fraction
## Platelia II Rabies

### Table: Platelia Results

<table>
<thead>
<tr>
<th>RFFIT</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>191</td>
<td>2</td>
<td>193</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>459</td>
<td>462</td>
</tr>
<tr>
<td>Total</td>
<td>194</td>
<td>461</td>
<td>655</td>
</tr>
</tbody>
</table>

- **655 patients**
- **193 vaccinated with PVRV**
- $\text{Se}=0.9896$
- $\text{Sp}=0.9935$
- $\text{Pv}+=0.9845$
- $\text{PV}-=0.9957$
- $\text{Conc}=0.9924$

5 discord. (all vaccinated) between 0.36 and 0.7 IU/ml

(Feyssaguet et al., Vaccine, 2007)
Perfect correlation with RFFIT in the range 0-4 UI/ml

(Feyssaguet et al., Vaccine, 2007)

N=655 sera

Linearity according to Bland and Altman, 1995
Thank you