

Research Seminar

**Investigation of emerging zoonotic
viruses in Europe and Africa**

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Centre de Biologie pour la Gestion des Populations - CBGP

Montferrier-sur-Lez, June 25th 2020

General plan

- Introduction on viral emergence
- Approaches to study zoonotic viruses
- Zoonotic transmission of retroviruses in Central Africa
- Molecular tools for characterizing emerging viruses
- Hantaviruses in Indian Ocean
- Tracking transmission of hantavirus in Madagascar

General plan

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Definition of viral emergence

- Emerging pathogen / virus / disease: overused/exploited term ??

NIAID, NIH

“ Emerging infectious diseases can be defined as **infectious diseases** that have **newly appeared** in a population **or** have **existed but** are **rapidly increasing** in **incidence** or **geographic range**, or that are caused by one of the NIAID Category A, B, or C priority pathogens.”

CDC

“ Emerging infectious diseases are those whose incidence in humans has increased in the past 2 decades or threaten to increase in the near future. These diseases, which respect **no national boundaries**, can challenge efforts to protect workers as **prevention and control** recommendations may **not** be **immediately available**. ”

- SARS-CoV-2 !!

Factors playing in viral emergence

- Viruses recently appeared
- Frequent origin from wildlife reservoirs and/or vectors

Viral

increase of virulence: mutation, recombination, reassortment..
RNA viruses (e.g. Chikungunya, influenza....)

Ecological

environmental, climate changes (e.g. global warming)..
(e.g. Crimean-Congo haemorrhagic fever..)

Human

land use, deforestation, urbanization, trade increase, globalization
(e.g. arboviruses, hantaviruses..)

Intrinsic (*more research → more finding*)

XXIst century: **“Emergence of knowledge”**

new viruses/variants discovered by cutting-edge technologies

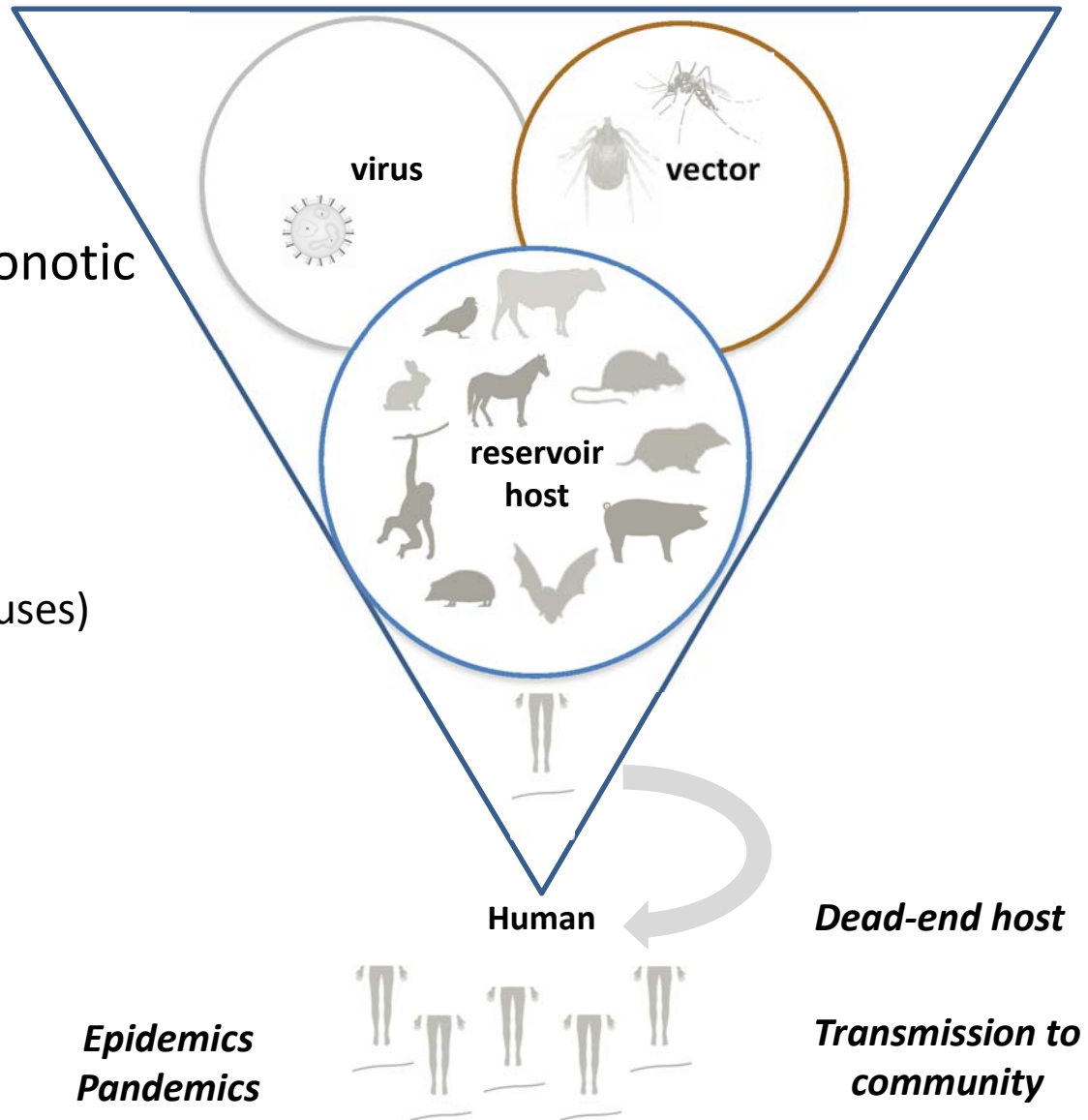
➤ Spread
new hosts
geographical areas

*Jones KE et al. Nature.2008.
Lloyd-Smith JO et al. Science. 2009.
Keesing F et al. Nature. 2010.
Holmes EC. Curr Opin Virol. 2013.*

Emerging zoonosis

Emerging viruses mostly zoonotic

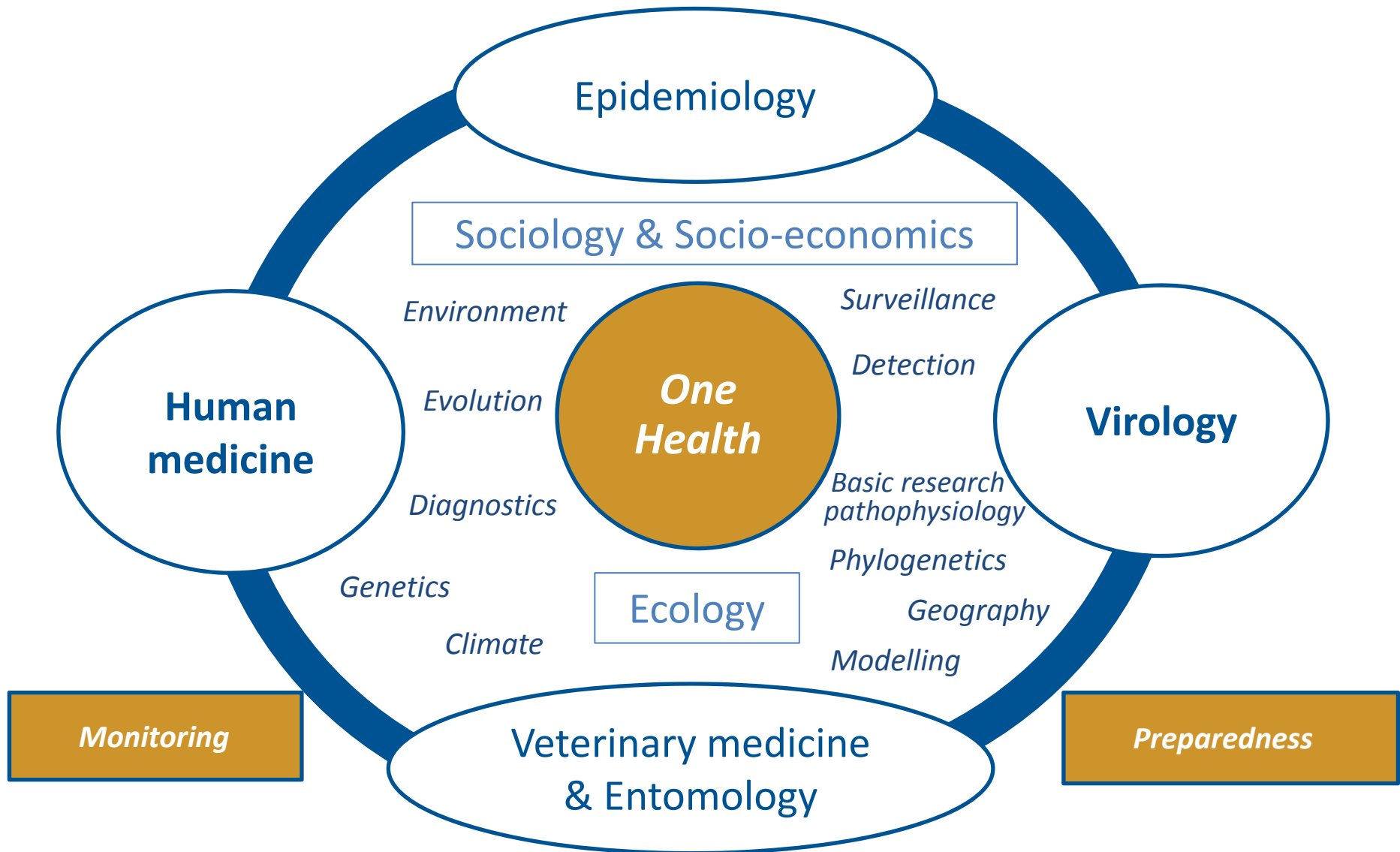
- Zoonosis *stricto sensu*
- Vector-borne
(arbovirus: arthropod-borne viruses)
-both (e.g. CCHFV, WNV, ...)



General plan

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Comprehension of viral emergence: achievable only with synergy of efforts



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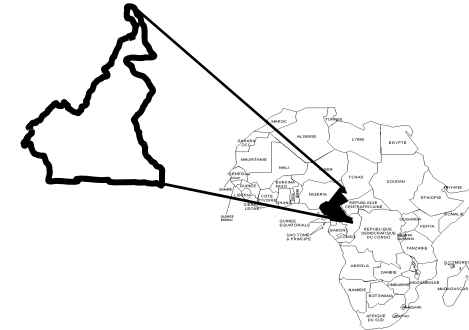
Retroviruses in Central Africa

Rural Cameroun

➤ region highly endemic for retroviruses

- HIV
- HTLV
- Foamy virus

Non-Human primates (NHP) origin of human retroviruses



- Epidemiological & Familial studies
- Molecular data & Phylogenetic descriptions

Arguments supporting inter-species transmission

High frequency of contacts of humans with potentially infected NHP

➤ Hunting



➤ Pets



➤ Bushmeat



EDITORIAL REVIEW

Cross-species transmission of simian retroviruses: how and why they could lead to the emergence of new diseases in the human population

Sabrina Locatelli and Martine Peeters

AIDS 2012, 26:659–673

Keywords: Africa, cross-species transmission, emerging disease, non-human primate, simian foamy virus, simian immunodeficiency virus, simian T-cell lymphotropic virus

REVIEW

10.1111/j.1469-0691.2012.03843.x

Simian retroviruses in African apes

M. Peeters and E. Delaporte

UMI 233, TransVIHMI, Institut de Recherche pour le Développement and University of Montpellier 1, Montpellier, France

Field work in Center and East regions of Cameroun



- *First mission:* Lomé, Messok
- *Second mission:* Lomé, Messok, Messaména, Somalomo, Akonolinga
- *Third mission:* Somalomo, Réserve du Dja, Akonolinga



On the road

....day



....and night



Investigation on Human T-lymphotropic virus 1 (HTLV-1)

- Oncogenic retrovirus

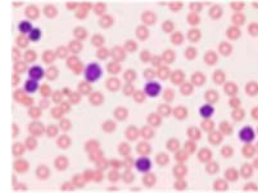
- Taxonomy

Family: *Retroviridae*

Subfamily: *Orthoretrovirinae*

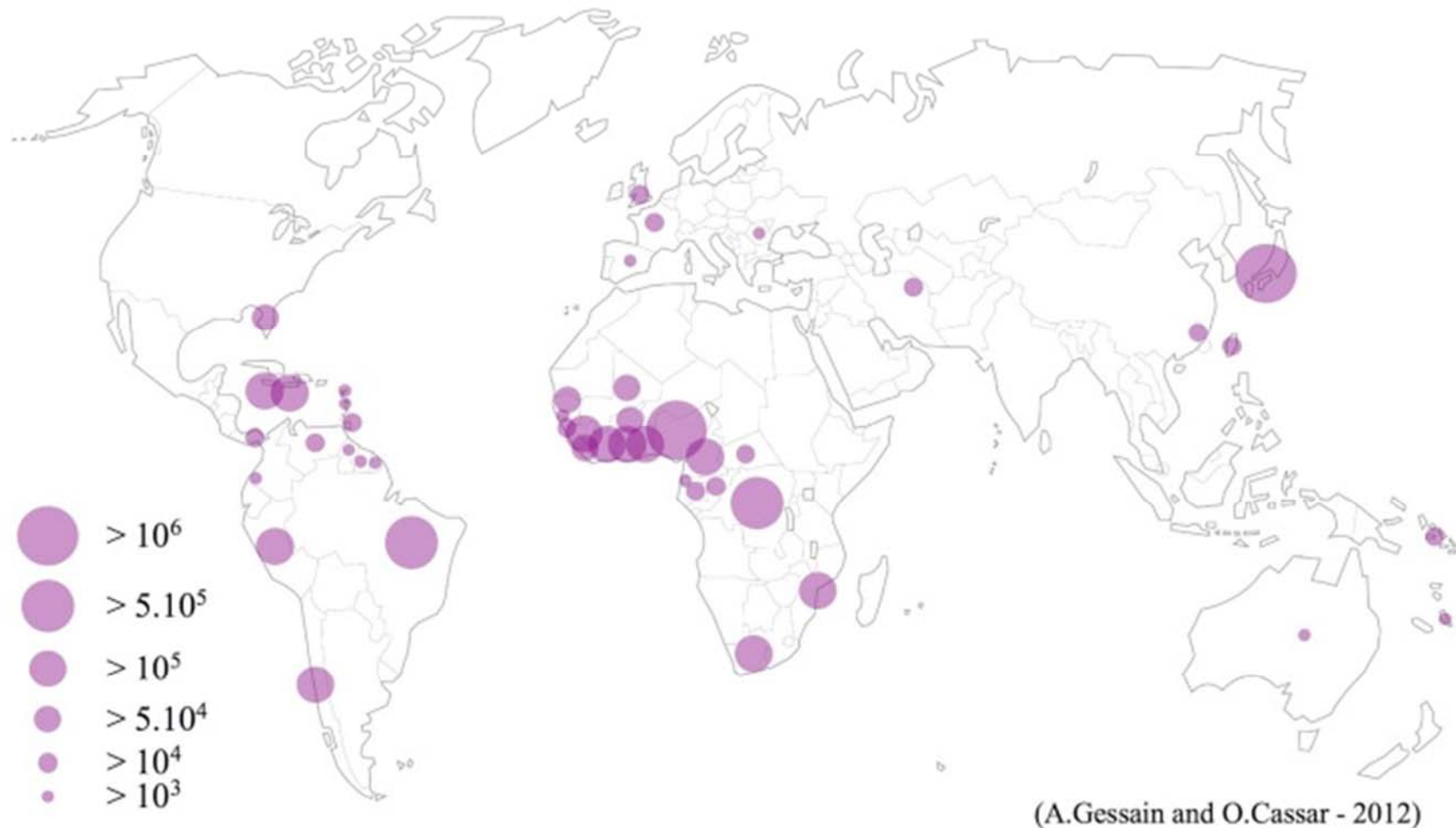
Genus: *Deltaretrovirus*

Species: *Primate T-lymphotropic virus 1*



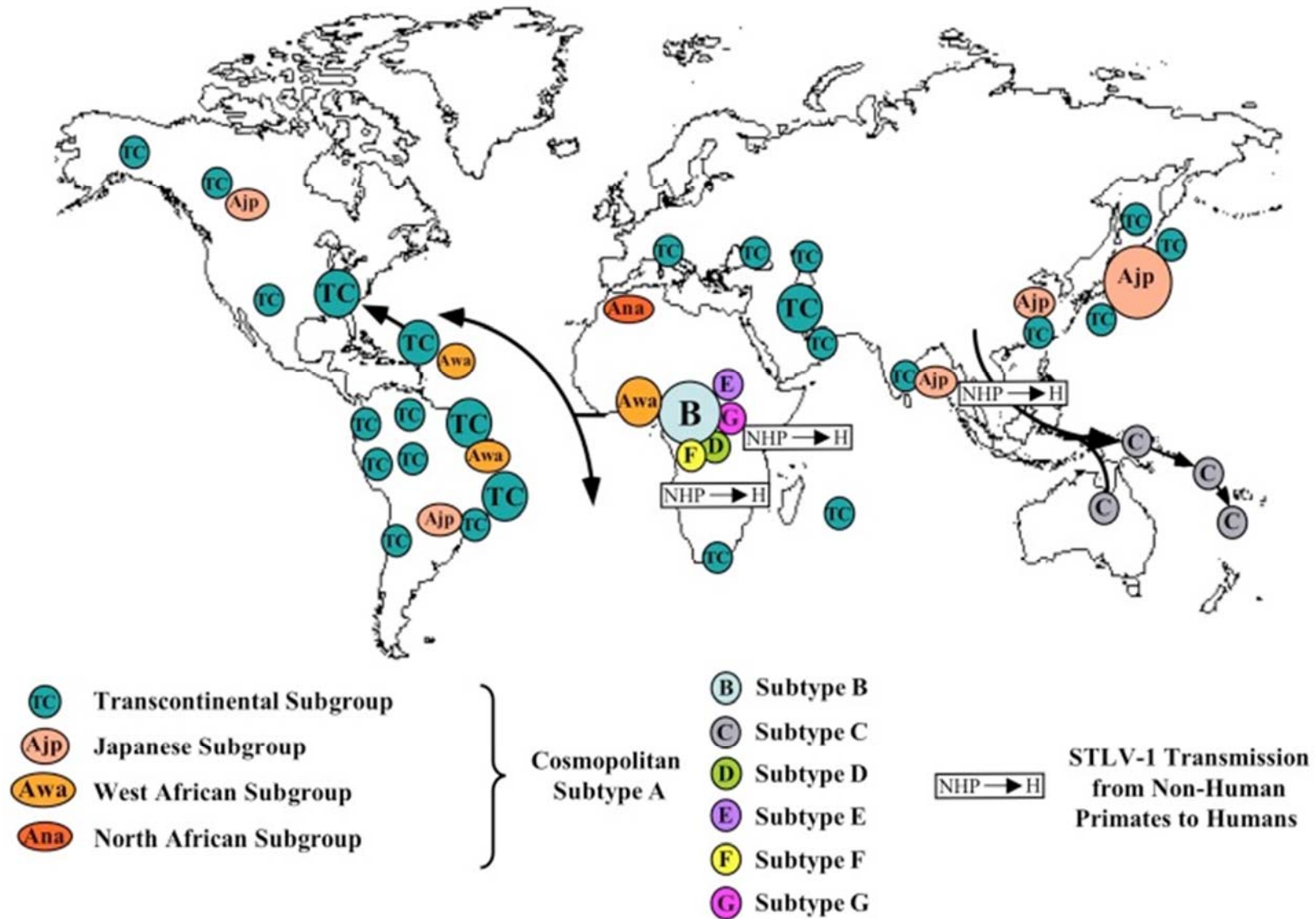
- Transmission by several routes (blood, sexual, mother-to-child, NHP..)
- Associated diseases (2-7% of infected individuals)
leukemia/lymphoma, myelopathy, myositis...
- Geographical distribution: endemic foci (**sub-Saharan Africa**, Japan, South-America..)

HTLV-1 epidemiology



- Seroprevalence: 1- 25%, higher in women and age-related

HTLV-1 molecular epidemiology



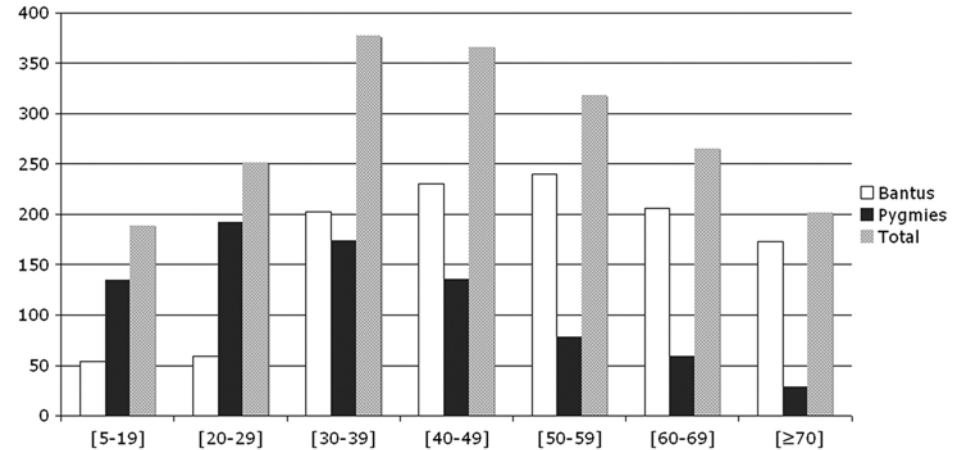
HTLV analysis of Bantus and Pygmies in rural Cameroun

I. Large survey

Study on ≈ 2000 persons

➤ General population

(Ethical Committee Cameroun)



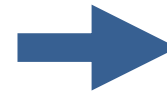
- Systematic screening by Western Blot
- Research of proviral DNA (battery of PCRs generic, specific, nested....)
Sequencing
 - HTLV-1 prevalence
 - Identification of a new WB HTLV atypical profile

HTLV analysis in rural Cameroun

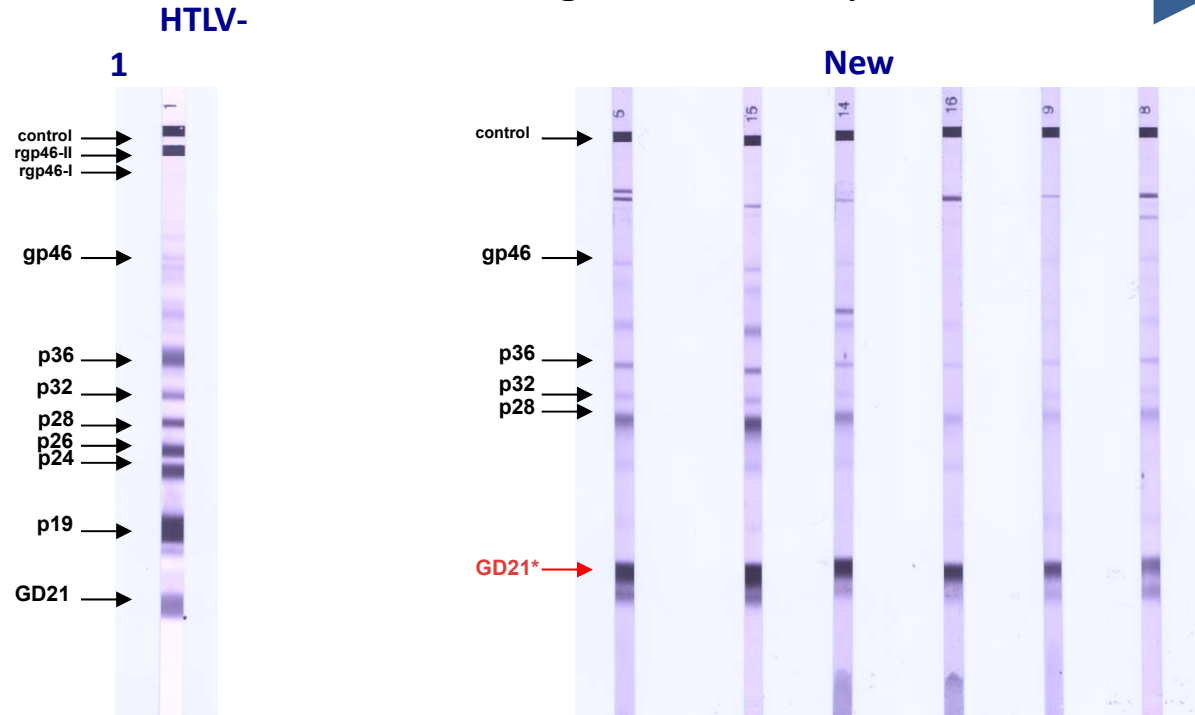
- **HTLV-1+**: prevalence **2.07%** (serology + molecular confirmation)

41/1985 HTLV-1: 2.07%. 26/815 **Pygmies**: 3,19%. 15/1170 Bantus: 1.28%

- Indeterminate serological reactivity: 56.9%



New: 68 cases (3.43%)

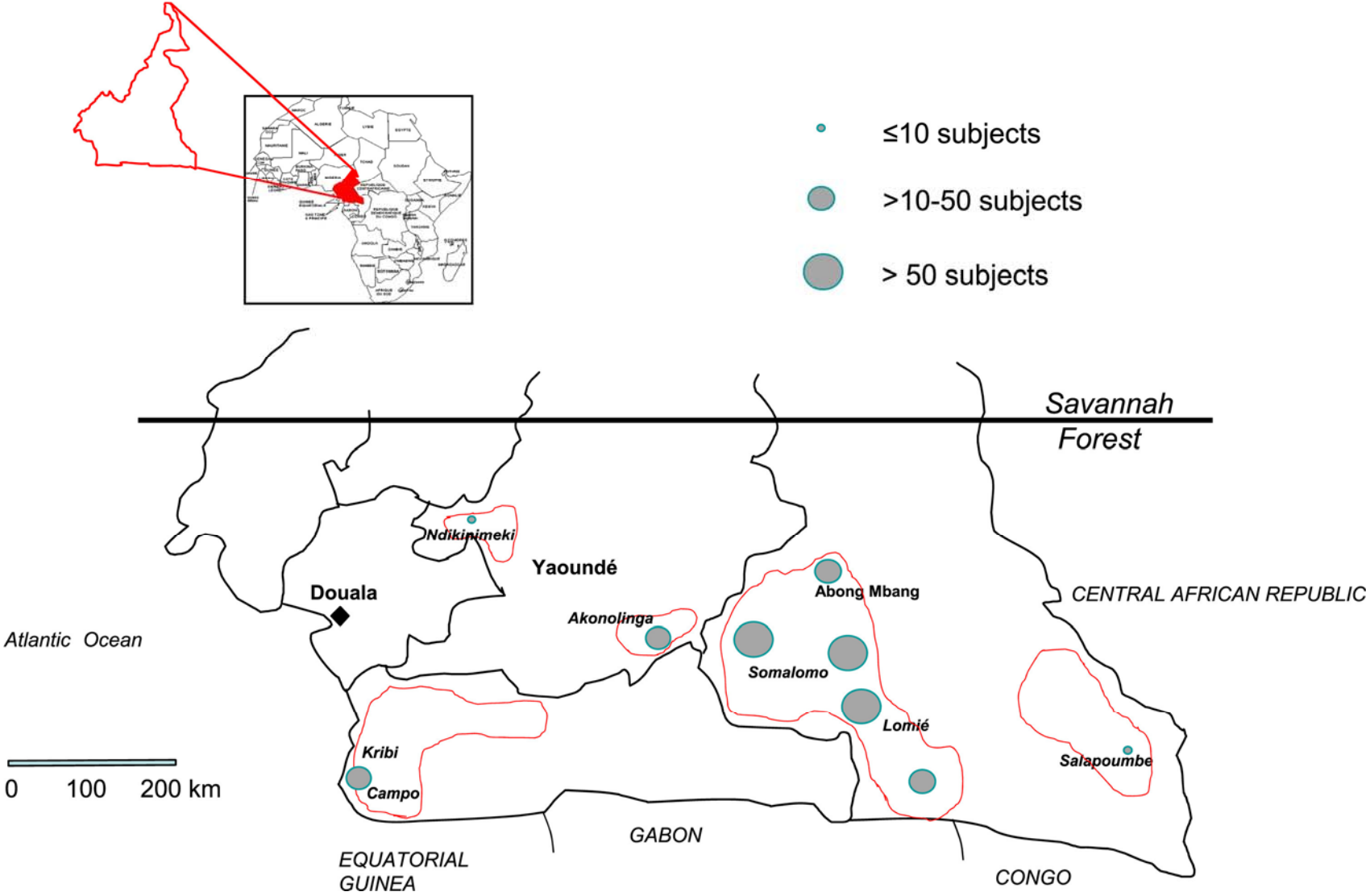


- Different epidemiological determinants (*age, sex, ethnics..*)

- cross-reactivity of the new indeterminate WB pattern with other infections (e.g. parasites)
- long-term follow-up of persons presenting the new pattern: significance

Field work in Center and East regions of Cameroun

HTLV-1: case - control study



HTLV-1: case - control study

II. Specific survey

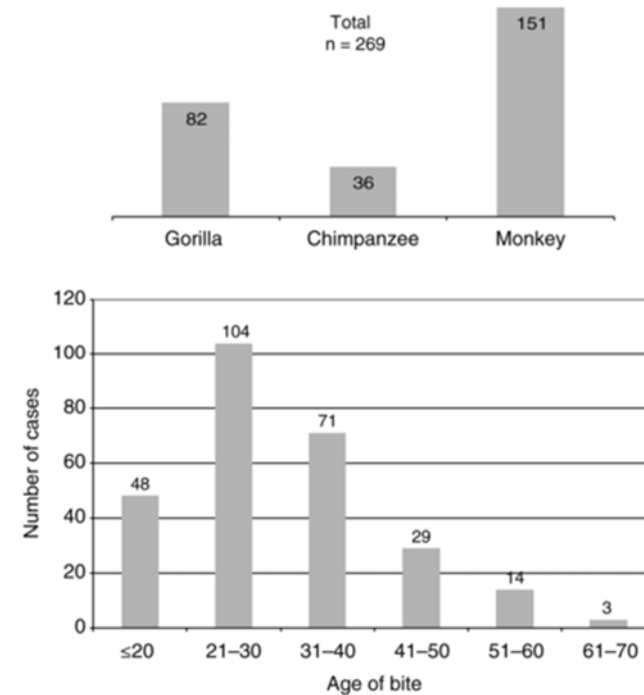
persons bitten by NHP

269 (254 men, 15 women)

average age: 43,5 years (2-87)

➤ matched with **non exposed controls**

- Definition **exposition *stricto sensu***:
i.e. bite or other contact
provoking blood-blood or saliva-blood exchange
- Situation: hunting, pet....



Gorilla gorilla gorilla



Pan troglodytes



Mandrillus sphinx



Cercopithecus Nictitans

Other monkeys

Serological Analysis by Western Blot

- Confirmatory test HTLV Blot 2.4 MP Diagnostics
→ on all the 538 plasma samples

Research of proviral DNA by PCR

- 3 generic PCR (tax, tax nested, pol nested) for all the PTLV
→ on **DNA** extracted from buffy-coats

8.5% bitten (23/269) vs 1.5% (4/269) control HTLV-1+

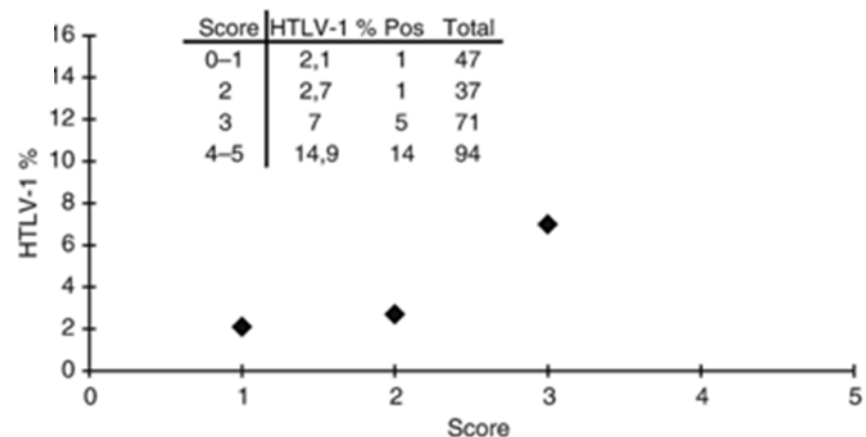
HTLV-1 transmitted by NHP bite?

- HTLV-1 prevalence (8.5%) among bitten individuals more important than in the control population (1.5%) ➤ Significant difference : $p < 0.001$

✓ Among the 23 HTLV-1+ bitten individuals:
17 bitten by a gorilla, 3 by a chimpanzee, 3 by a monkey

✓ Association between bite severity and prevalence of HTLV-1 infection.

(score 0-5 according to wound, bleeding, hospitalization, scar, handicap)



✓ Infection associated with ethnics (Pygmy, $p < 0.001$), bite by an ape ($p < 0.001$), severity of wound ($p = 0.033$), co-infection with Foamy virus ($p < 0.001$).

Simian Foamy Virus analysis in the same population

- Cross-transmission of Foamy virus is reported and clearly demonstrated in individuals in contact with NHP

RESEARCH

Simian Foamy Virus Transmission from Apes to Humans, Rural Cameroon

Sara Calattini,^{*} Edouard Betsem A. Betsem,[†] Alain Froment,[‡] Philippe Maucière,^{††§} Patricia Tortevoye,^{*} Christine Schmitt,^{*} Richard Njouom,[§] Ali Saib,[¶] and Antoine Gessain^{*}

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 13, No. 9, September 2007



Cross-Species Transmission of Simian Foamy Virus to Humans in Rural Gabon, Central Africa

Augustin Mouinga-Ondémé,[‡] Mélanie Caron,[‡] Dieudonné Nkoghé,[‡] Paul Telfer,[‡] Preston Marx,[‡] Ali Saib,[¶] Eric Leroy,[‡] Jean-Paul Gonzalez,[‡] Antoine Gessain,^{*} and Mirdad Kazanji^{‡*}

OPEN ACCESS Freely available online

PLOS PATHOGENS

Frequent and Recent Human Acquisition of Simian Foamy Viruses Through Apes' Bites in Central Africa

Edouard Betsem^{1,2,3*}, Réjane Rua^{1,2}, Patricia Tortevoye^{1,2}, Alain Froment⁴, Antoine Gessain^{1,2*}

¹ Unit of Epidemiology and Pathophysiology of Oncogenic Viruses, Department of Virology, Institut Pasteur, Paris, France, ² Centre National de la Recherche Scientifique (CNRS), URA 3015, Paris, France, ³ Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroun, ⁴ Institute of Research for Development, Musée de l'Homme, Paris, France



HTLV-1 transmitted by NHP bite?

- Individuals exposed to NHP potentially infected by HTLV-1
- Transmission may occur through the bite from NHP to humans (by blood-blood and/or saliva-blood exchange)

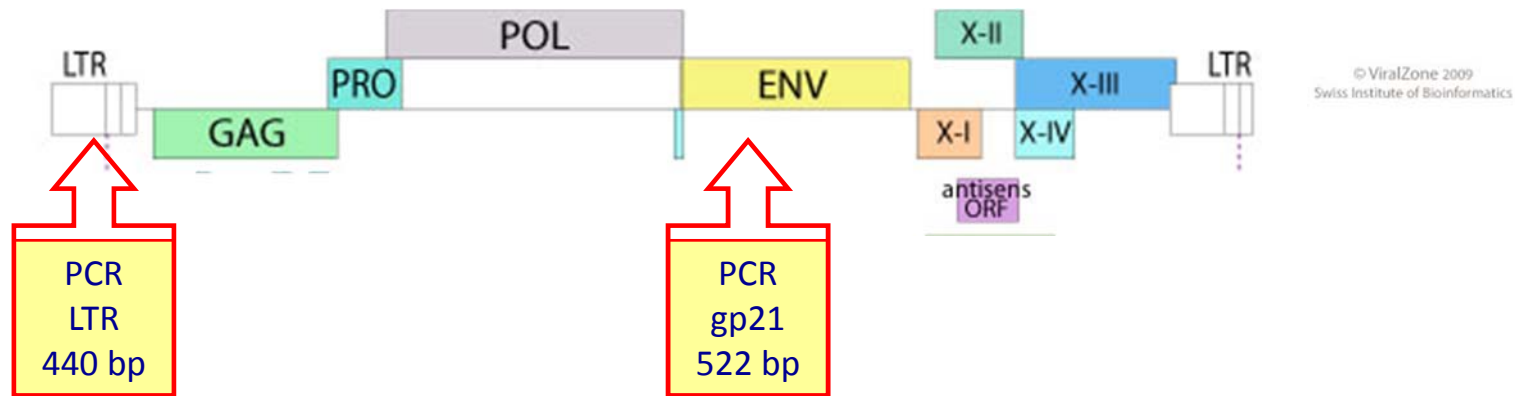


Can HTLV-1 share the same route of transmission as Foamy virus?

- Among 51/269 Foamy virus+ individuals, 13 are HTLV-1+ (25%)

Molecular Analysis

Which are the strains harbored by the HTLV-1+ bitten individuals?



- Direct sequencing of the PCR amplicons
- BLASTN analysis and alignment with all known HTLV-1
- Viral strain characterization
 - Phylogenetic analyses with focus on species-specific peculiarities

Results

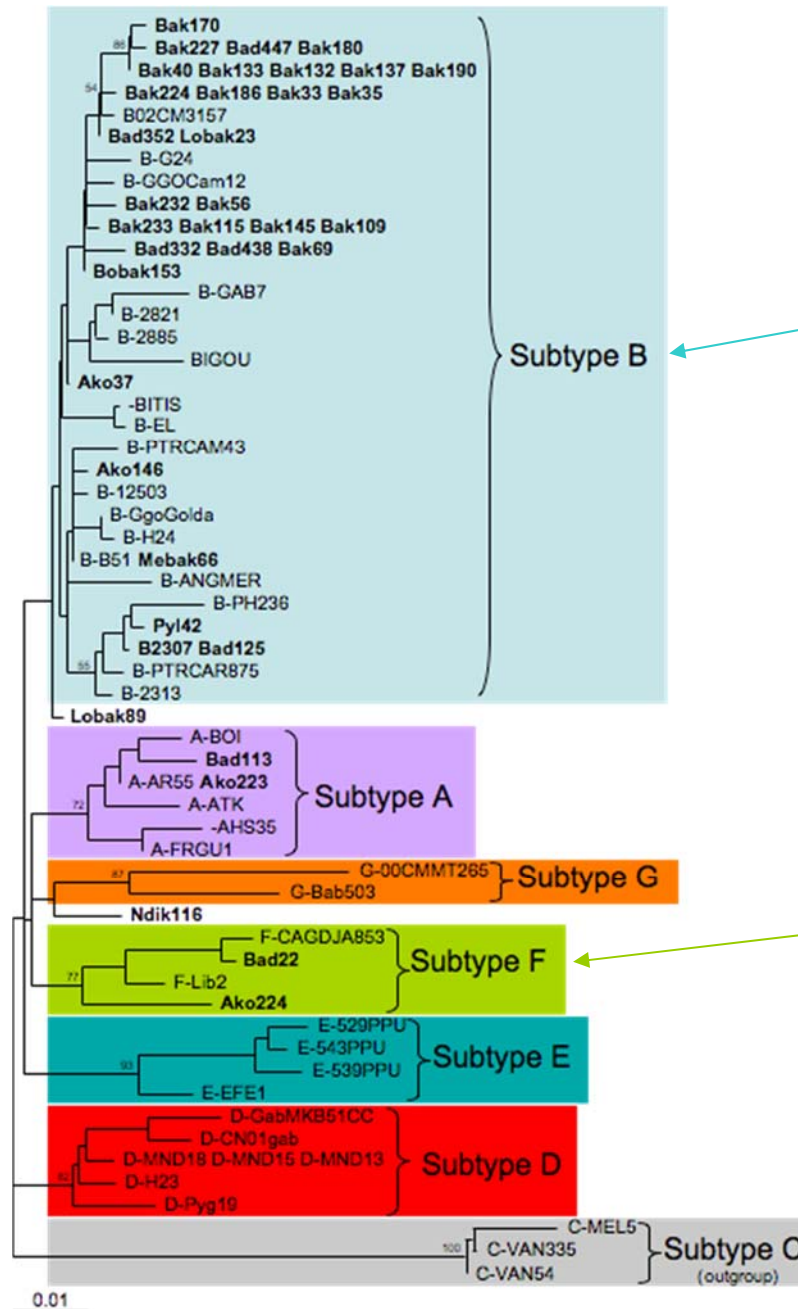
HTLV-1 molecular analysis

- Most of the obtained HTLV-1 sequences belong to the **subtype B**:
 - ✓ in the general population
 - ✓ among individuals bitten by a gorilla
 - ✓ in one individual bitten by a chimpanzee
- *Subtype B is the most frequently circulating in Central African individuals and gorillas. Thus it is difficult to certainly assess the non-human primate origin since their high homology*
- HTLV-1 obtained from two individuals bitten by a monkey belong to the **subtype F**

Phylogenetic analysis PTLV

- neighbor-joining
Env 519 bp

- Subtype B:
no significant difference between gorilla and human
- Subtype A:
non-exposed subjects
- Subtype F:
subjects exposed to monkey



➤ Human or NHP origin?

HTLV-1 F in two individuals bitten by a monkey: close to the strain isolated from *Cercocebus agilis*

➤ Cross-species transmission!

How to strengthen the link between NHP bite and acquisition of HTLV-1?

- ✓ Additional investigations to exclude that infection could occur through one of the other three assessed transmission routes
 - Vertical transmission from mother to child
 - Horizontal transmission by sexual contact
 - Transfusions of infected blood

- ✓ ? Demonstration that prevalence of HTLV-1 infection among the mothers of bitten individuals is lower than among the mothers of control population ?

Analysis of one individual HTLV-1f+ at 'Reserve du Dja'



- **Case: Bad 22**
HTLV-1+ bitten by a monkey
(*Cercocebus agilis*)
subtype F (not very frequent):
same cluster of the subtype from monkey
- ✓ mother HTLV-1 neg by WB and PCR
- ✓ no transfusion
- **argument for inter-species transmission**



Conclusions

- **HTLV-1** high prevalence in NHP hunters in South-Cameroon
- **Strong suggestion for cross-species transmission supported by:**
 - **epidemiological** data (high prevalence compared to general population & co-infection with Foamy virus)
 - **genetic** information (e.g. sequence homology between subtype F from an individual bitten from a monkey and subtype F from *Cercocebus agilis*)
 - **intra-familial** studies (plausible indication: i.e. vertical transmission excluded)

Clinical Infectious Diseases

CID 2015:60

MAJOR ARTICLE

A Severe Bite From a Nonhuman Primate is a
Major Risk Factor for HTLV-1 Infection in
Hunters From Central Africa

Claudia Filippone,^{1,2} Edouard Betsem,^{1,2,3} Patricia Tortevoye,^{1,2} Olivier Cassar,^{1,2} Sylviane Bassot,^{1,2} Alain Froment,⁴
Arnaud Fontanet,^{5,6} and Antoine Gessain^{1,2}

High level of sero-reactivity directed against Simian Immunodeficiency Virus

- Same population: case-control study

Matching according to age, sex, ethnics of subjects bitten by NHP vs subjects not bitten
492 individuals

bitten: 246 (170 Bantus: 12 F, 158 M ; 76 Pygmies: 2F, 74 M)

not bitten: 246 (170 Bantus: 12 F, 158 M; 76 Pygmies: 2F, 74 M)

- Serology (Elisa)
- Epidemiological analysis
- Molecular analysis (PCRs, generic, specific..)

Serology PLIA (Primate Lentiviruses Immuno-Assay)

Detection and differentiation of primate Lentivirus (SIV, HIV) by **multiplex ELISA**

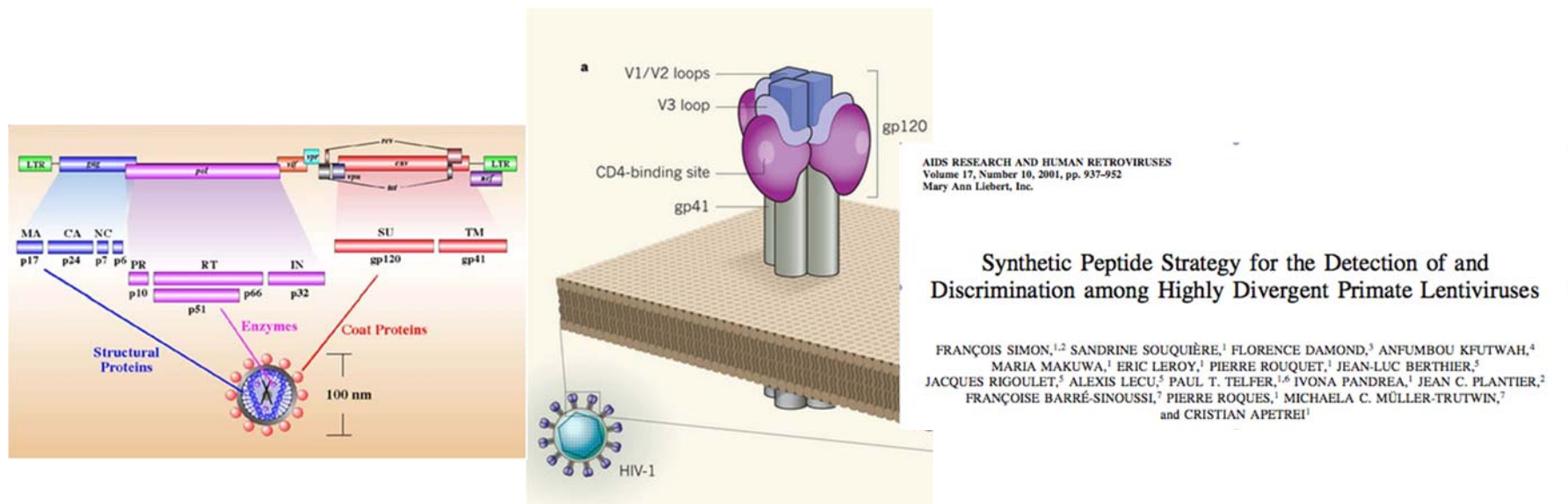
Lineages: HIV-1/SIVcpz/SIVgor, HIV-2/SIVsm, SIVagm, SIVmnd..

- **non specific screening**

10 peptides (**conserved epitopes**) of the membrane glycoprotein **gp41 TM**

- **identification**

13 peptides (**variable epitopes**) of the loop V3 of the **gp120 V3**



Paul R. Clapham et al, Nature, 2011)

Results Primate Lentivirus Serology

I. **PLIA negative** (DO < 0.15); (between 0.1 and 0.150)

Total: 339/492 (68.9%)

bitten: 176/246 (71.5%); not bitten: 163/246 (66.2%)

➤ *No additional analysis*

II. **PLIA positive** (DO > 0.150 for the peptides specific or crossing HIV-1)

Total: 26/492 (5.3%)

bitten: 10/246 (4.1%); not-bitten: 16/246 (6.5%)

molecular
confirmation
HIV-1 M

III: **PLIA indeterminate/atypical** (DO > 0.150 at least for one gp41/ gp120 peptides)

Total: 127/492 (25.8)

bitten: 60/246 (24.4%); not bitten: 67/246 (27.2%)

negative
molecular
analysis

Significance of SIV seroreactivity

No difference of prevalence in the groups (neg), (HIV-1+)(ind), for bitten and not bitten groups

- Atypical reactivity in Pygmies > Bantus
- Pygmies bitten by gorilla: higher intensity of signal

No virus detection: **Quid** Significance?

- immunological memory

bite from animal: one isolated contact

(serological response with no establishment of chronic infection)

- weak titer
- host genetic factors
- variability
- all population is exposed (*sensu lato*)

General plan

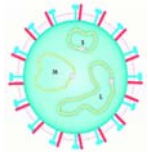
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Emerging zoonotic viruses

1. Investigation in the **animal reservoir/vector** of viruses potentially transmissible to humans
 2. Research of etiologic agents of diseases in **humans**
- Orientation of diagnostics according to:
host, area, disease....

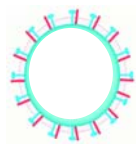
Diagnostic approaches: traditional vs new

Direct Methods: Virological diagnosis



Virus isolation

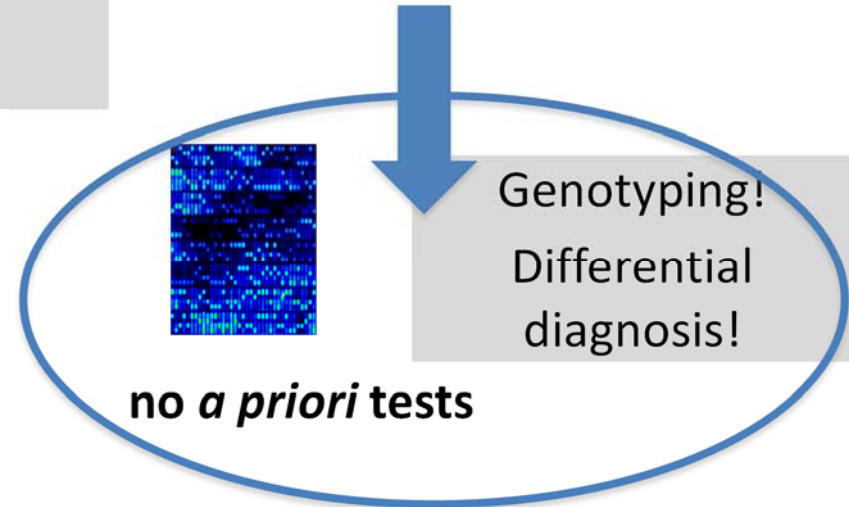
**BSL-3,
BSL-4**



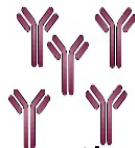
Antigen
detection



Genome
detection
RT-PCR



Indirect Methods: Serological diagnosis



Research of antibodies (IgM, IgG) - ELISA,
IF, WB, neutralization assay

Serosurveillance (IgG)

Traditional vs new molecular methods pro and cons

Viral genome detection

➤ **Research *ad hoc***

PCR, RT-PCR → *issue when considering viral high genetic variability*

high **sensitivity**

- RT-PCR (specific, degenerate/consensus)
- Nested RT-PCR
- Real-time RT-PCR (i.e. Taqman probes → high specificity)
- Multiplex RT-PCR

➤ **Research without *a priori***

- High Throughput Sequencing (HTS), Next Generation Sequencing (NGS)
- Resequencing Microarray Assay (RMA)
- Others: Nanopore sequencing...

→ *discovery of new genetic variants/species*

PathogenID[®] Microarray

Research Transversal Project IP

DEVA

Detection of Emerging Viral Agents



Affymetrix, Inc.

Categories of genetic elements	Number of genes
16S rRNA	51
Toxins/ pathogenicity genes	229
Antibiotic resistance genes	390
Conserved genes	72
MLST	7
Sequences with point mutations	167
Virus sequences	42
18S rRNA	4
Total	962

Genus	Species
Alphavirus	Eastern Equine Encephalitis virus
Alphavirus	Venezuelan equine encephalitis virus
Alphavirus	Western equine encephalomyelitis virus
Arenavirus	Guanarito virus
Arenavirus	Junin virus
Arenavirus	²
Arenavirus	Lymphocytic choriomeningitis virus
Arenavirus	Machupo virus
Coronavirus	SARS coronavirus
Ebolavirus	Reston ebolavirus (was Reston Ebola virus)
Ebolavirus	Zaire virus (was Zaire Ebola virus)
Ephemerovirus	Bovine ephemeral fever virus
Flavivirus	Dengue virus type 2
Flavivirus	Japanese encephalitis virus
Flavivirus	Kyasanur forest disease virus strain W371
Flavivirus	Tick-borne encephalitis virus
Flavivirus	Yellow fever virus
Hantavirus	Andes virus
Hantavirus	Dobrava virus
Hantavirus	Hantaan virus
Hantavirus	Puumala virus
Hantavirus	Seoul virus
Hantavirus	Sin Nombre virus
Henipavirus	Hendravirus
Henipavirus	Nipahvirus
Hepatovirus	Hepatitis A virus
Influenzavirus A	Influenza A virus
Influenzavirus B	Influenza B virus
Influenzavirus C	Influenza C virus
Lyssavirus	Rabies virus
Marburgvirus	Lake Victoria marburgvirus
Metapneumovirus	Human Metapneumovirus
Morbillivirus	Measles virus
Nairovirus	Crimean-Congo hemorrhagic fever virus
Norovirus	Norwalk virus
Orthobunyavirus	California encephalitis serogroup virus LEIV
Orthobunyavirus	La Crosse virus
Orthopoxvirus	Variola virus
Phlebovirus	Rift Valley fever virus
Pneumovirus	Human respiratory syncytial virus
Respirovirus	Human parainfluenza virus 1 strain Washington
Rubulavirus	Mumps virus
Vesiculovirus	Vesicular stomatitis Indiana virus

3rd generation

✓ « pan-viral »

↓

• > 800 viral sequences

1st generation

✓ Bacteria

✓ Virus →

- 42 species
- 26 genera
- 11 families

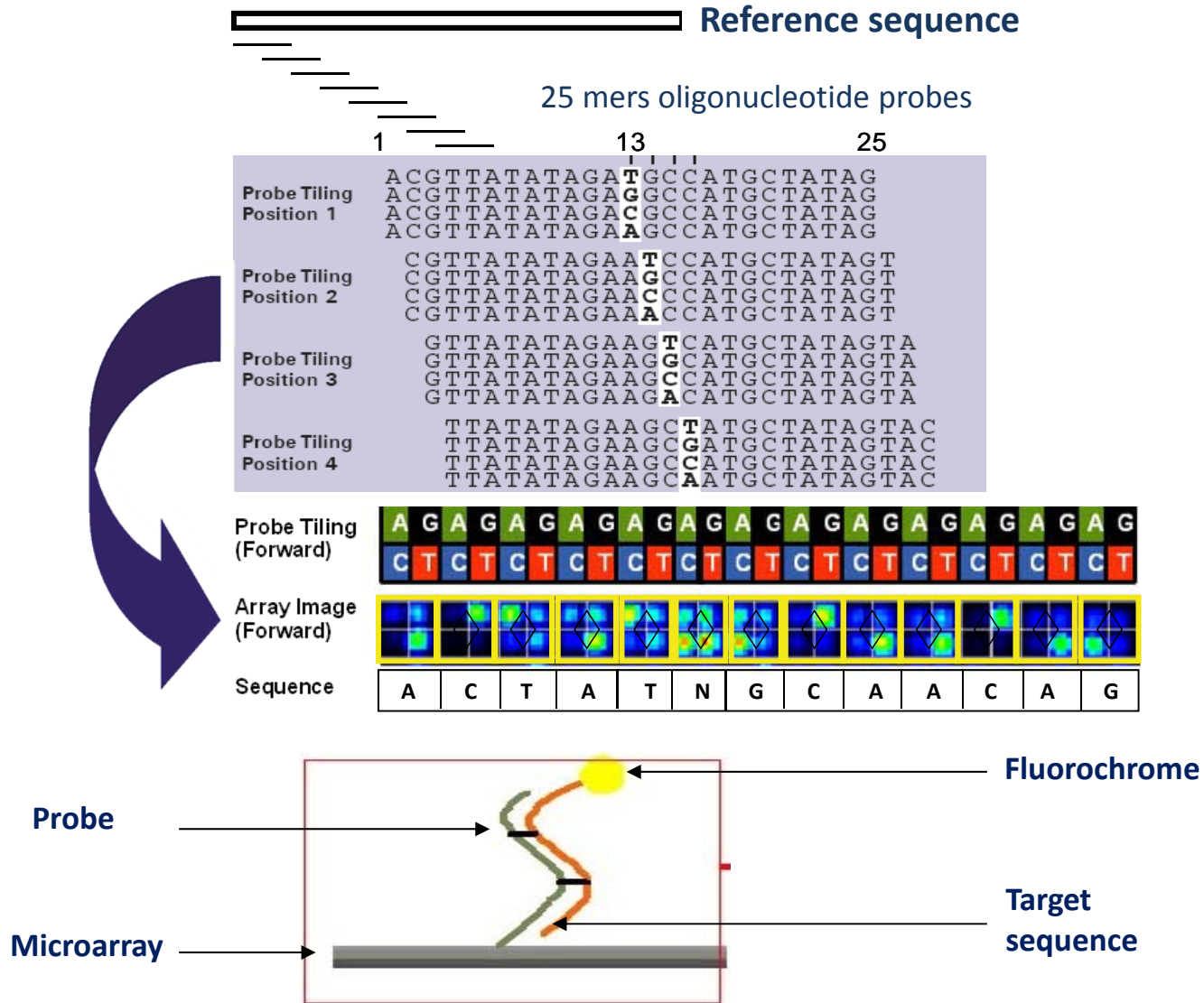
2nd generation

✓ Bacteria

✓ Virus →

- 55 species/variants
- 37 genera
- 14 families

Principle of resequencing microarray



- Resequencing approach

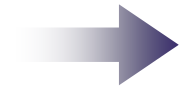
- Signal detection on the chip

Microarray assay overview: virus identification

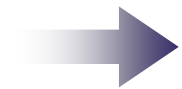
SAMPLE

- cell culture
- tissue, serum

RNA extraction

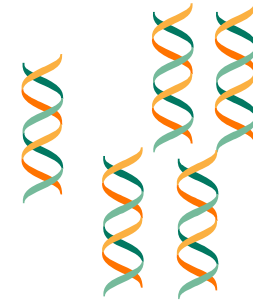
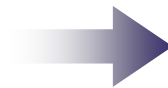


DNA extraction

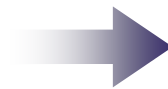


cDNA synthesis +

Φ29 WTA



Φ29 WGA

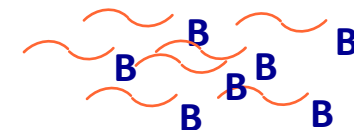


Fragmentation



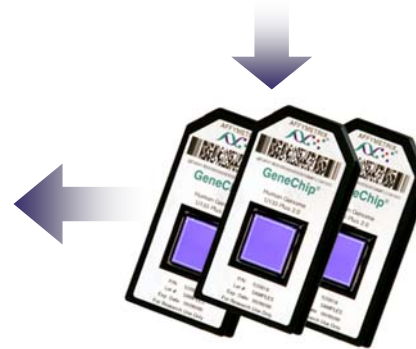
DNA fragments

Labelling

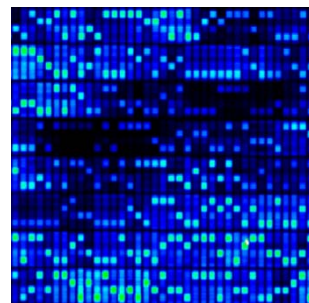


labeled DNA fragments

Hybridization, Washing, Detection



Scanning



```

>
ttatcNaaaNNacagctNNNgg
ttgaaagactggtttgtagag
tgccaatNNNNNNcaagcattt
tgactgtacaatgctaactctg
gcacttccaacatttgatgtNN
NtccaagatggtagNNNtaga
attaccatagacttcaatctgg
atgatatNNNaaggagcatctg
aatagNNNNNNNNgctcaac
tttgctaccctccatgtcgata
gatgtggaagatatNNNNNNN
Ncagatttcaccttggccac
ttagctgacaagactgacagac
tgtaaatgNNNcgt
    
```

Bioinformatic analysis and *Call rate* definition

Output: FASTA format sequence

for **BLASTN** analysis and call rate calculation

$$\text{Call rate} = \frac{\text{Number of read bases}}{\text{Total number of bases}} \times 100$$

```
>VIRDviNC-001474_polyprot_1402:IP_03_16_06_WGA_Dengue_typeII
NNggNNNcgcaatgtNcgccgaNNNcNcagNNNNaNgggacacaagaatcaNNcNNNNNNNNNNNNNNNNa
atgaagaaNNggNNNNaNNNgNNatgNaNggNgaacacaagaaactagccgaNNNNNNaNNNNNNNNNNNN
NNtNccNaaacaaggtggtgctgtgcaaagaccaacaccaagaggcacagtaatggatatcatatcga
gaagagaccaaNNacNcNNcNNNNNNNcccNcNNNgNNcNNNgNgNNgNNNNNgNNgNNNNNNNNcNNNN
NNNNNNNNcNNNNNNNNNNcNNNNNNNNNNNNcNNcNNNNgNNNNNNNNcNNNcNNNgNNgNNNNaN
NNNNNNNNNNNgNcNNNNgNNgNNNgNcNNNNgNNNNNNNNcNNNggNNNNccNNgggNgNcNNcNNNg
NNNgcgNcNNNNNNaNNNNNNN
```

=> Call rate: 47.6

Blast score and **call rate**: values indicating the **quality** of **hybridization** of the tested sequence on the tiled sequence

➤ identification of viral strains

Application of resequencing microarray to highly pathogenic viruses

- Viruses associated to hemorrhagic fevers or encephalitis (similar symptoms with other viruses, bacteria, parasites).
 - necessity for a rapid and differential diagnosis
- Several are associated to « bioterrorism » risk

Arenaviridae, Bunyaviridae, Flaviviridae, Filoviridae, Paramyxoviridae

530 bases (L segment of the polymerase)

ORIGINAL ARTICLE

Clin Microbiol Infect 2013; **19**: E118–E128
10.1111/1469-0691.12075

VIROLOGY

Molecular diagnostic and genetic characterization of highly pathogenic viruses: application during Crimean–Congo haemorrhagic fever virus outbreaks in Eastern Europe and the Middle East

C. Filippone^{1,2}, P. Marianneau³, S. Murri⁴, N. Mollard⁴, T. Avsic-Zupanc⁵, S. Chinikar⁶, P. Desprès⁷, V. Caro⁸, A. Gessain^{1,2}, N. Berthet^{1,2} and N. Tordo^{4,9}

Highly pathogenic viruses: sequences tiled on the chip

Family	Genus	Species/Subtypes	Tiled sequence (L) size (nt): location
ARENAVIRIDAE	Old World Arenavirus	Lassa virus	525: 4259-4783 (L)
	New World Arenavirus	Machupo virus	528: 2469-2996 (L)
		Guanarito virus	528: 4099-4626 (L)
		Junin virus	528: 2462-2989 (L)
BUNYAVIRIDAE	Nairovirus	Crimean Congo hemorrhagic fever virus	531: 2717-3247 (L)
	Hantavirus	Hantaan virus	510: 3131-3640 (L)
		Puumala virus	531: 4705-5235 (L)
		Seoul virus	552: 3055-3606 (L)
		Dobrava Belgrade virus	531: 3905-4435 (L)
		Sin Nombre virus	528: 4857-5384 (L)
	Phlebovirus	Rift Valley virus	549: 5026-5574 (L)
FLAVIVIRIDAE	Flavivirus	Kiasanur Forest disease virus	504: 8463-8966 (L)
		Yellow Fever virus	504: 8429-8932 (L)
FILOVIRIDAE	Ebolavirus	Reston Ebola virus	528: 13611-14138 *
		Zaire Ebola virus	528: 2146-2673 (L)
	Marburgvirus	Lake Victoria Marburg virus	528: 2266-2793 (L)
PARAMYXOVIRIDAE	Henipavirus	Nipah virus	528: 13743-14270 *
		Hendra virus	528: 13731-14258 *

Tiled viral sequence	Tested virus
Lassa virus	Lassa Josiah, Sierra Leone
	Ivory Coast
	Guinea
Junin virus	Junin
CCHF virus	CCHF Nigeria
	Mauritania
	Turkey
	China
Hantaan virus	Hantaan
	Sin Nombre
Seoul virus	Tchoupitoulas
Dobrava Belgrade virus	Dobrava
Rift Valley virus	Rift Valley
Kiasanur Forest disease virus	Kiasanur
Tick Borne encephalitis virus group	Omsk HF
Ebola virus	Reston
	Zaire (1995)
	Zaire (2001)
Marburg virus	Lake Victoria
	Popp
Nipah virus	Nipah
Hendra virus	Hendra

Validation of the microarray

- Detection of tilted viruses and their variants
- Detection of species related to the tiled viruses

Red: tiled viruses

Green: variants

Blue: related species

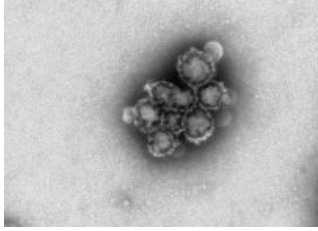
Simultaneous detection of different viruses

Different viral RNAs pooled in the same sample

- 12 pools (each containing 3 viruses) hybridized on 12 microarrays
 - Detection and characterization of the different virus/variant in a complex sample

- *Arenaviridae: Lassa Josiah virus*
- *Bunyaviridae: CCHFV Mauritania*
- *Filoviridae: Marburg Popp virus*

- *Bunyaviridae: Hantaan virus*
- *Flaviviridae: Omsk virus*
- *Filoviridae: Ebola Gabon virus*



Nairoviridae family

Orthonairovirus genus

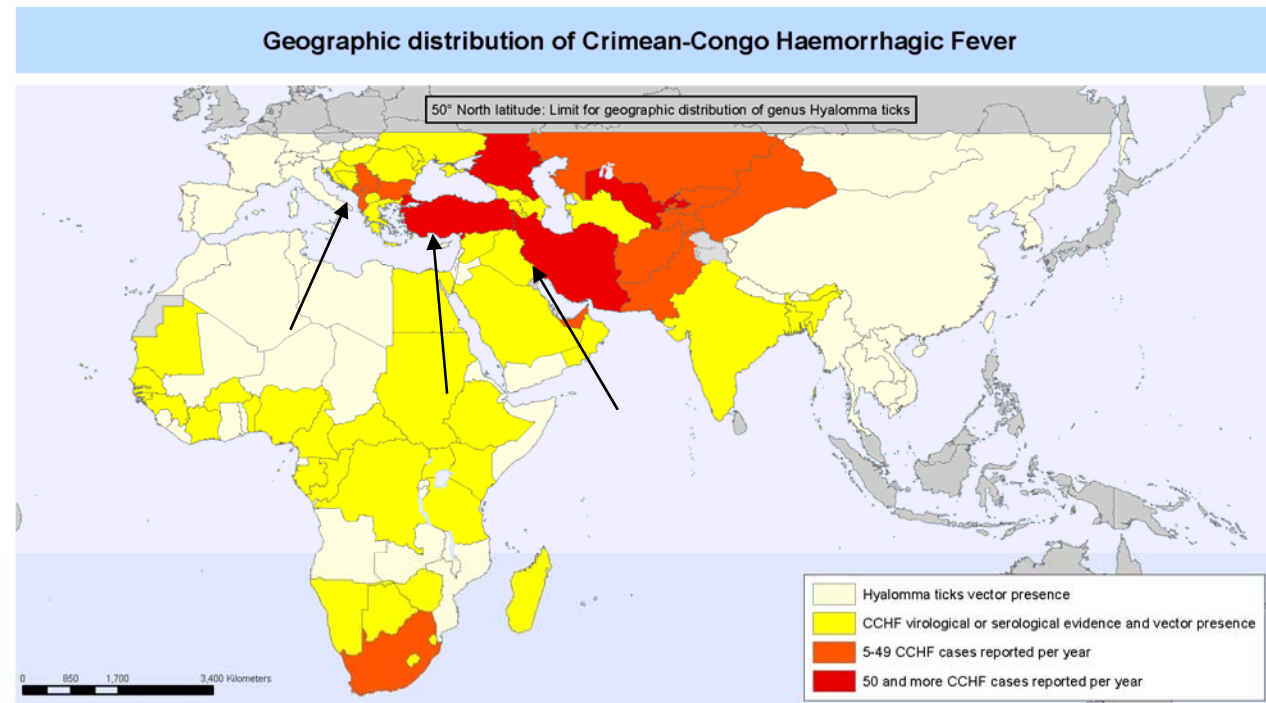
Application of the microarray during Crimean-Congo Hemorrhagic Fever outbreaks



transmitted by tick



animal reservoir



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization

 World Health Organization
© WHO 2008. All rights reserved

- 2 sera from Turkey
- 5 sera from Kosovo
- 5 sera from Iran

Sample	RNA (ffu)	cDNA (ffu)	φ29 (WTA) (ffu)	Call rate (%)	Divergence (%)
Nigeria* (supernatant)	5.9x10 ⁵	3.4x10 ⁵	1.0x10 ¹¹	99.6	0.2
Mauritania (supernatant)	8.1x10 ⁶	7.2x10 ⁵	2.8x10 ¹³	98.0	1.9
Turkey (supernatant)	6.2x10 ⁶	6.0x10 ⁵	1.4x10 ¹²	63.5	10.7
China (supernatant)	9.1x10 ⁵	8.8x10 ⁶	5.0x10 ⁹	31.9	13.7
Turkey 090137 (serum)	1.5x10 ⁰	7.1x10 ⁰	4.8x10 ⁷	45.6	10.15
Turkey 090139 (serum)	2.4x10 ²	4.8x10 ¹	9.9x10 ⁶	33.4	10
Kosovo 422 (serum)	1.2x10 ¹	9.7x10 ⁰	6.1x10 ⁶	38.9	n.s.
Kosovo 423 (serum)	2.3x10 ⁰	7.6x10 ⁰	3.5x10 ⁵	-	9.2
Kosovo 426 (serum)	1.1x10 ²	5.6x10 ¹	8.1x10 ⁵	n.d.	9.8
Kosovo 427 (serum)	1.8x10 ³	5.0x10 ³	1.9x10 ⁷	70.7	9.8
Kosovo 429 (serum)	2.6x10 ³	2.8x10 ³	2.8x10 ⁶	29.0	9.7
Iran 397 (serum)	7.1x10 ²	3.4x10 ³	2.5x10 ⁸	-	14.1
Iran 402 (serum)	3.2x10 ⁴	7.2x10 ³	3.9x10 ⁹	-	14.7
Iran 406 (serum)	1.8x10 ⁴	1.2x10 ⁴	3.6x10 ⁹	-	14.5
Iran 407 (serum)	3.9x10 ⁴	1.3x10 ⁴	5.0x10 ⁹	-	14.7
Iran 409 (serum)	4.7x10 ³	7.8x10 ³	8.9x10 ⁷	-	14.7

Evaluation of **sensitivity** and **specificity** CCFHV

- ✓ quantitative PCR
- ✓ microarray detection
- ✓ sequencing

Detection limit

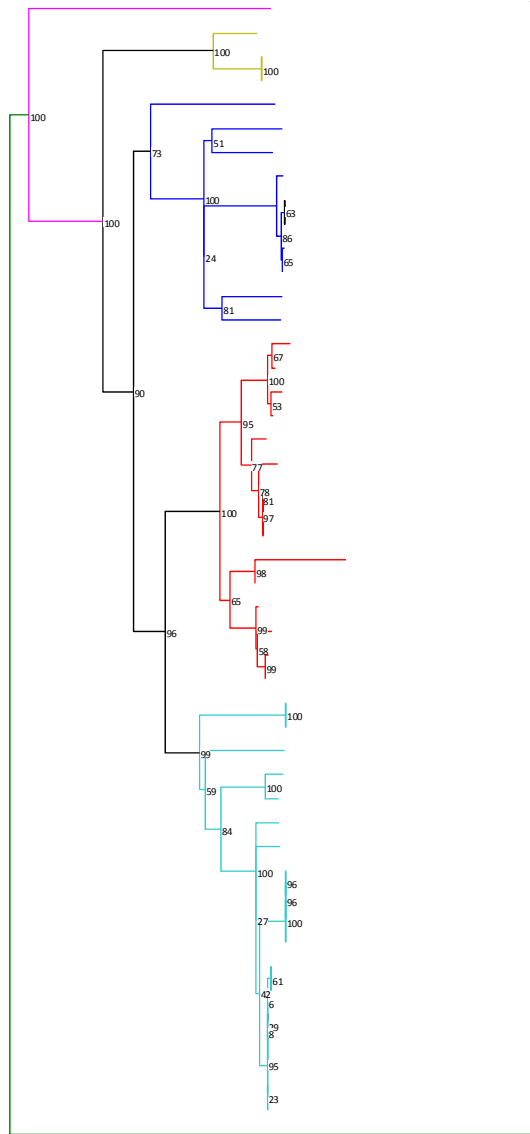
- ✓ 10⁶ ffu (10⁰-10¹)
- ✓ 14% divergence

*tiled on the array

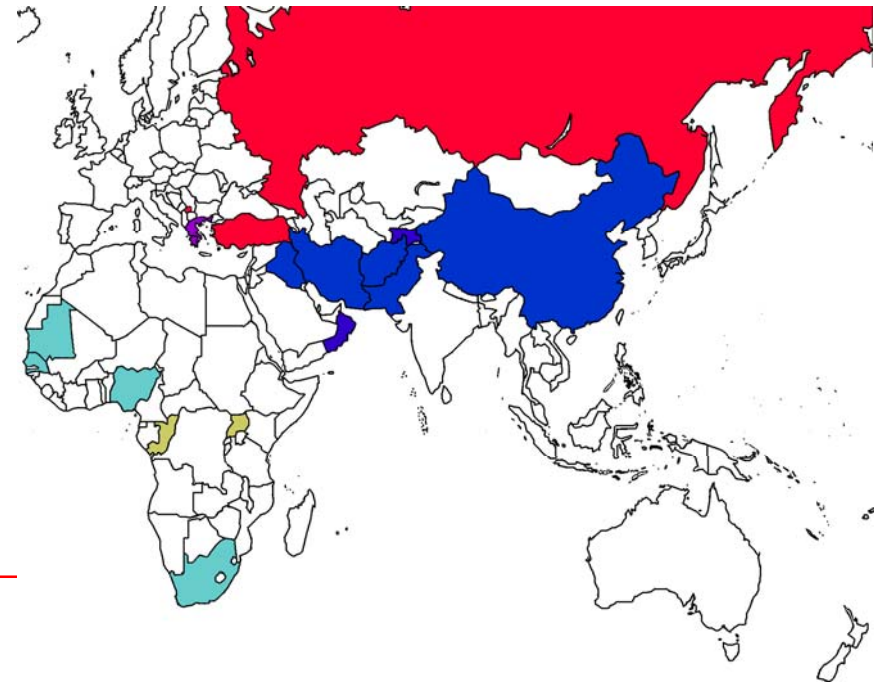
Phylogeographical analysis of CCHFV

Global (Gapsos t0%) Disc. unkn.
cchf

76 78 80 82 84 86 88 90 92 94 96 98 100



531_L.pol_DQ211612_AP92_Greece
 531_L.pol_DQ076412_S emunya_Uganda
 531_L.pol_DQ099335_Cong03010_Congo
 531_L.pol_DQ211624_UG3010_Congo
 531_L.pol_AY720893_TADJHU8966_Tajikistan
 531_L.pol_78191712_DQ211619_Oman
 531_L.pol_C68031_DQ211616_Cina
 Seq_Iran_397
 Seq_Iran_407
 Seq_Iran_409
 Seq_Iran_402
 Seq_Iran_406
 531_L.pol_AY422208_Matin_Pakistan
 531_L.pol_AY947890_Baghdad12_Iraq
 531_L.pol_AY675240_30908_Russia
 531_L.pol_DQ211617_Drosdov_Russia
 531_L.pol_DQ211618_Kashmanov_Russia
 531_L.pol_AY995166_VLV100_Russia
 Seq_Turkey_090137
 Seq_Kosovo_426
 Seq_Kosovo_423
 531_L.pol_EU044832_Kosova Hoti
 Seq_Kosovo_429
 Chip_Kosovo_427
 Seq_Kosovo_427
 Seq_Turkey_090139
 531_L.pol_GQ337055_Turkey_Kelkit06
 Seq_Turkey_0715_supernatant
 531_L.pol_DQ211623_Turkey2003108
 529_L.pol_DQ076414_SPU128817_South Africa
 531_L.pol_DQ076417_SPU481_South Africa
 531_L.pol_DQ211621_SPU10387_South Africa
 531_L.pol_DQ211622_SPU41585_South Africa
 531_L.pol_DQ211620_SPU9785_South Africa
 531_L.pol_DQ211614_ArD15786_Seneegal
 531_L.pol_DQ211613_ArD8194_Seneegal
 531_L.pol_DQ211615_ArD39554_Mauritania
 Chip_Mauritania_supernatant
 Seq_Mauritania_39554_supernatant
 Seq_Mauritania_supernatant
 531_L.pol_ref_005301_IbAr10200
 531_L.pol_tile_d_AY389361_IbAr10200
 531_L.pol_AY389508_IbAr10200
 Chip_Nigeria_supernatant
 531_L.pol_AY422209_IbAr10200
 531_L.pol_AY947891_IbAr10200
 Seq_Nigeria_06185_supernatant
 Kupe virus



* ←

➤ Sequence output of microarray
Kosovo isolate

* ←

* ←

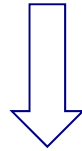
* *

Sequences obtained following hybridization on the microarray

Development of a 3rd generation Resequencing Microarray

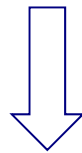


- Chip



- > 800 viral sequences

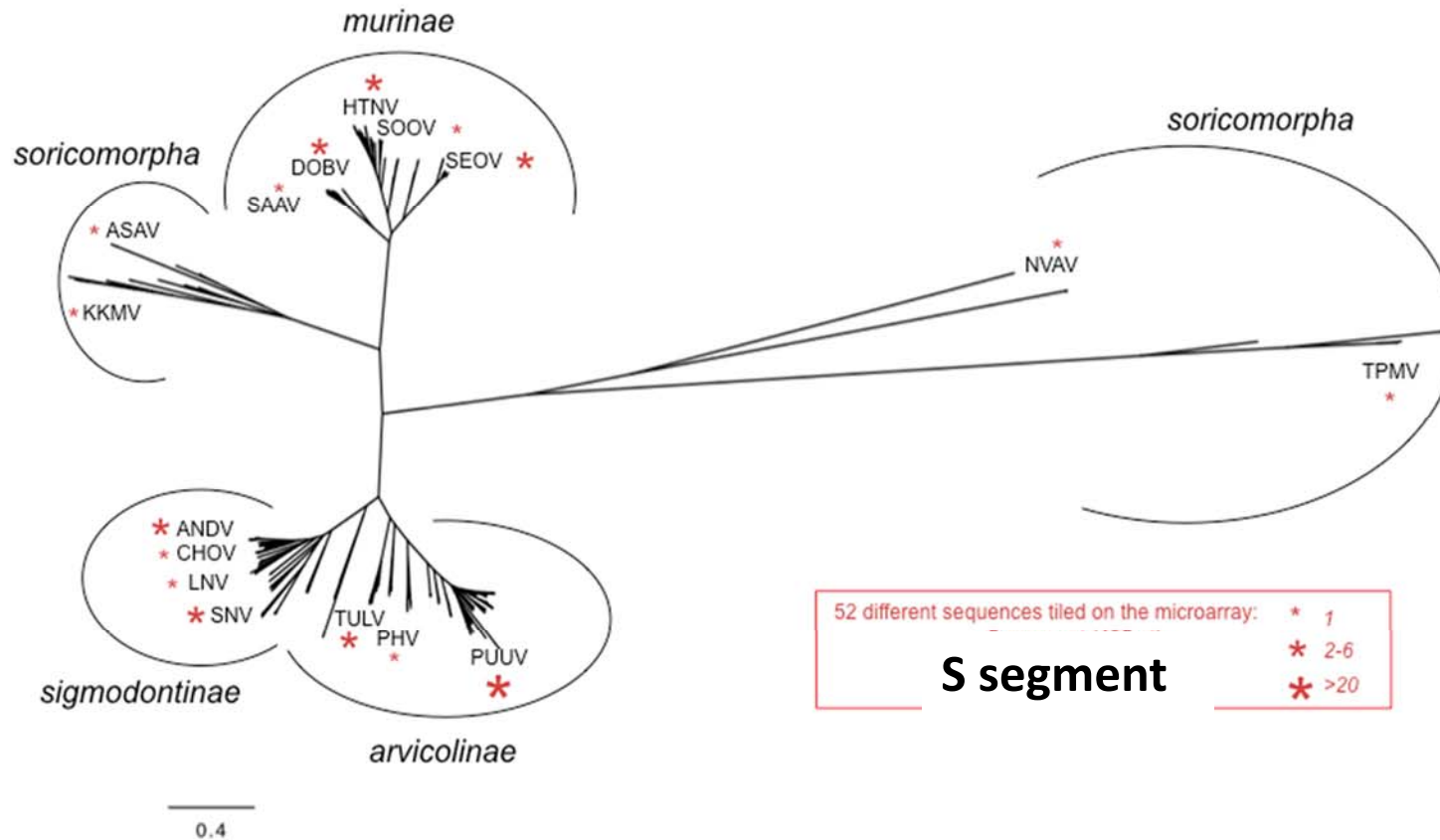
- each chosen sequence is tiled as a set of overlapping 25 mers probes



- up to 3×10^6 probes

pan-viral

3rd generation Resequencing Microarray: enrichment on Hantavirus



focus on hantavirus ?

- Responsible of zoonosis worldwide, including Europe

Model of zoonosis

Largely spread
new RNA virus family
new species discovered

Zoonotic
Circulation in small mammals
(rodents, insectivores, bats)

Hantaviruses

Responsible of severe diseases
Hemorrhagic Fever with Renal Syndrome
Nephropathia Epidemica
Hantavirus Cardio-Pulmonary Disease

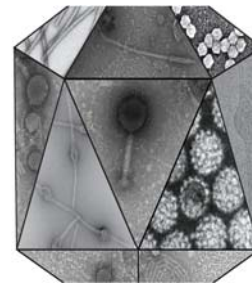
Investigations to monitor spread,
risk for humans and establish public
health measures

Hantavirus Taxonomy

- New and continuously evolving...
- Old Taxonomy: *Bunyaviridae* family
 - Hantaviruses the only ones not to be transmitted by arthropods

Chip design!

- 2017-2018
- Order: *Bunyavirales*
 Family: *Hantaviridae*



ICTV Report

Virus Taxonomy:
 The Classification and Nomenclature of Viruses

Source: <https://talk.ictvonline.org/taxonomy/>

proposed composition of *Hantaviridae* family

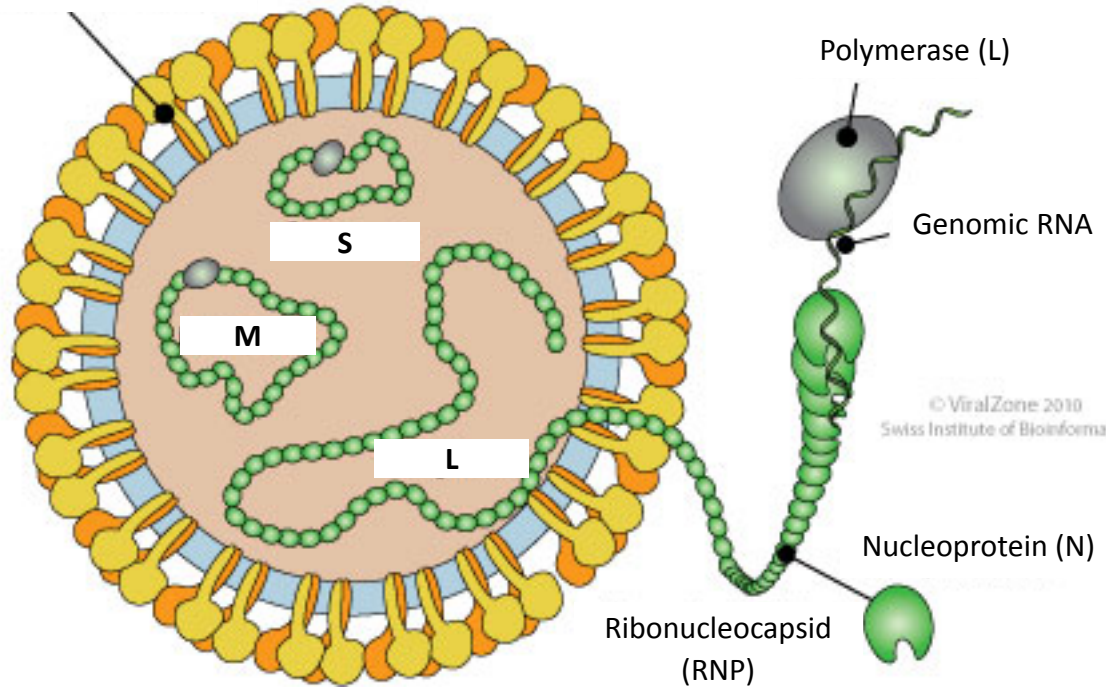
Family	Subfamily	Genus	Species	Host	
<i>Hantaviridae</i>	<i>Actantavirinae</i>	<i>Actinovirus</i>	#3	Ray-finned fish	
	<i>Agantavirinae</i>	<i>Agnathovirus</i>	#1	Jawless fish	
	<i>Mammantavirinae</i>		<i>Loanvirus</i>	#1	Mammalian (bat)
			<i>Mobatvirus</i>	#3	Mammalian (bat or mole)
		*	<i>Orthohantavirus</i>	#36	Mammalian (rodent)
		<i>Thottimvirus</i>	#2	Mammalian (shrew)	
	<i>Repantavirinae</i>	<i>Reptillovirus</i>	#1	Reptile	

Virus features

- Enveloped, spherical virus (80-120 nm)

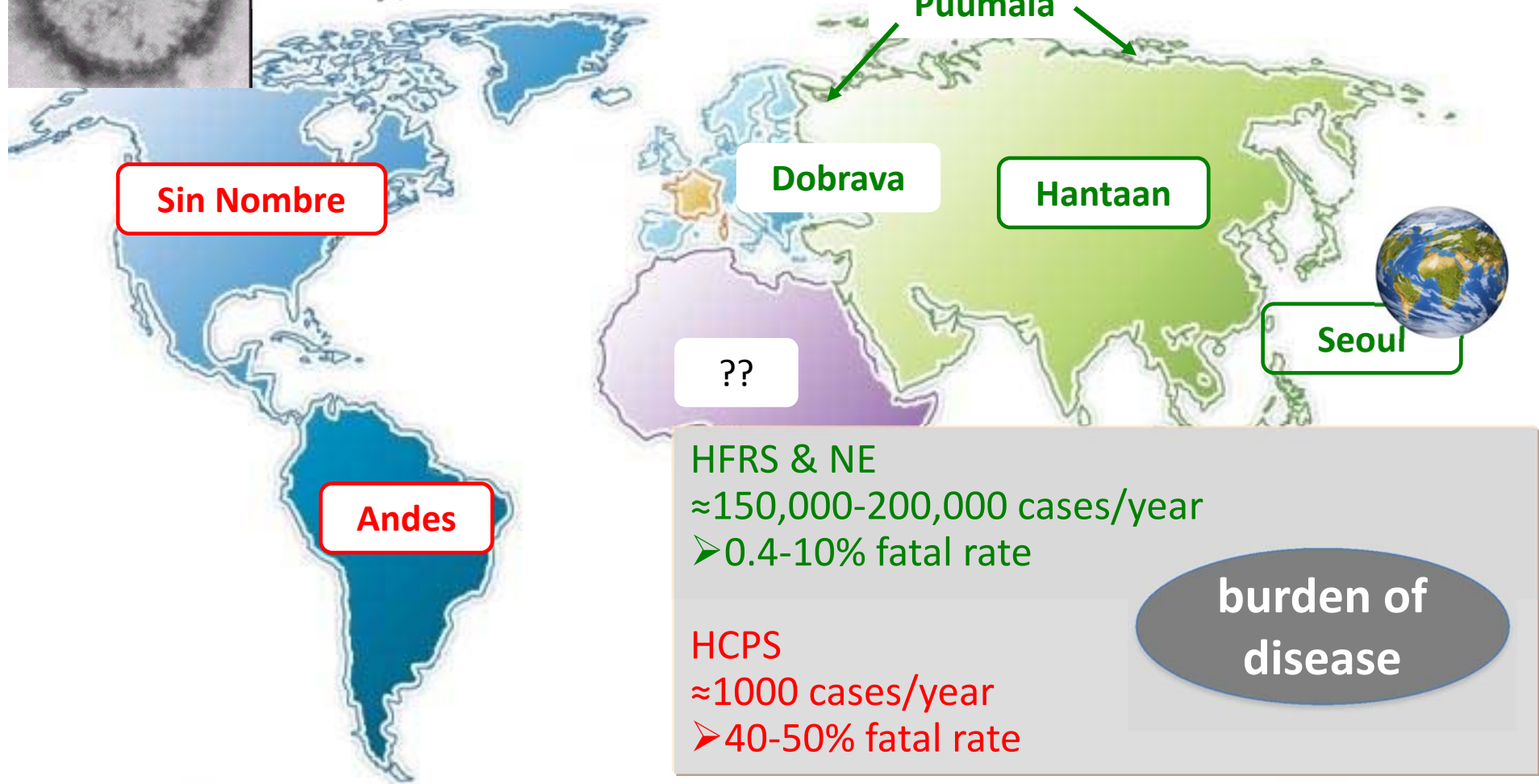
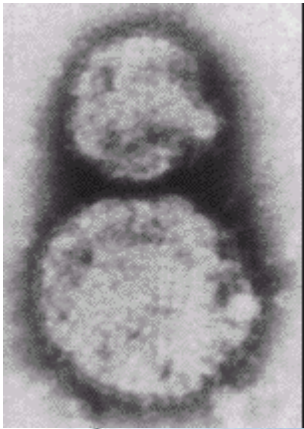
negative-stranded
tri-segmented RNA
genome

Glycoprotein (Gn and Gc)



Adapted from: *ViralZone, SIB Swiss Institute of Bioinformatics*

Geography and pathogenicity of hantaviruses



Resequencing of hantaviruses from animal reservoirs in Europe



Myodes glareolus



Microtus arvalis



Apodemus flavicolis



Apodemus agrarius



Rattus norvegicus



Lemmus sibiricus

Specimen:

- Lab strain cell supernatant
- Rodent lung tissue

Rodent reservoirs

RNA extraction



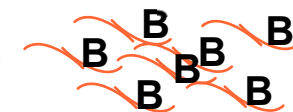
cDNA synthesis + Φ 29 WGA



Fragmentation



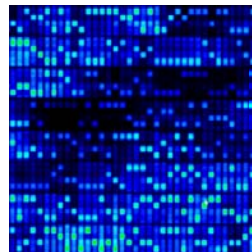
Labelling



Hybridization Washing



Scanning



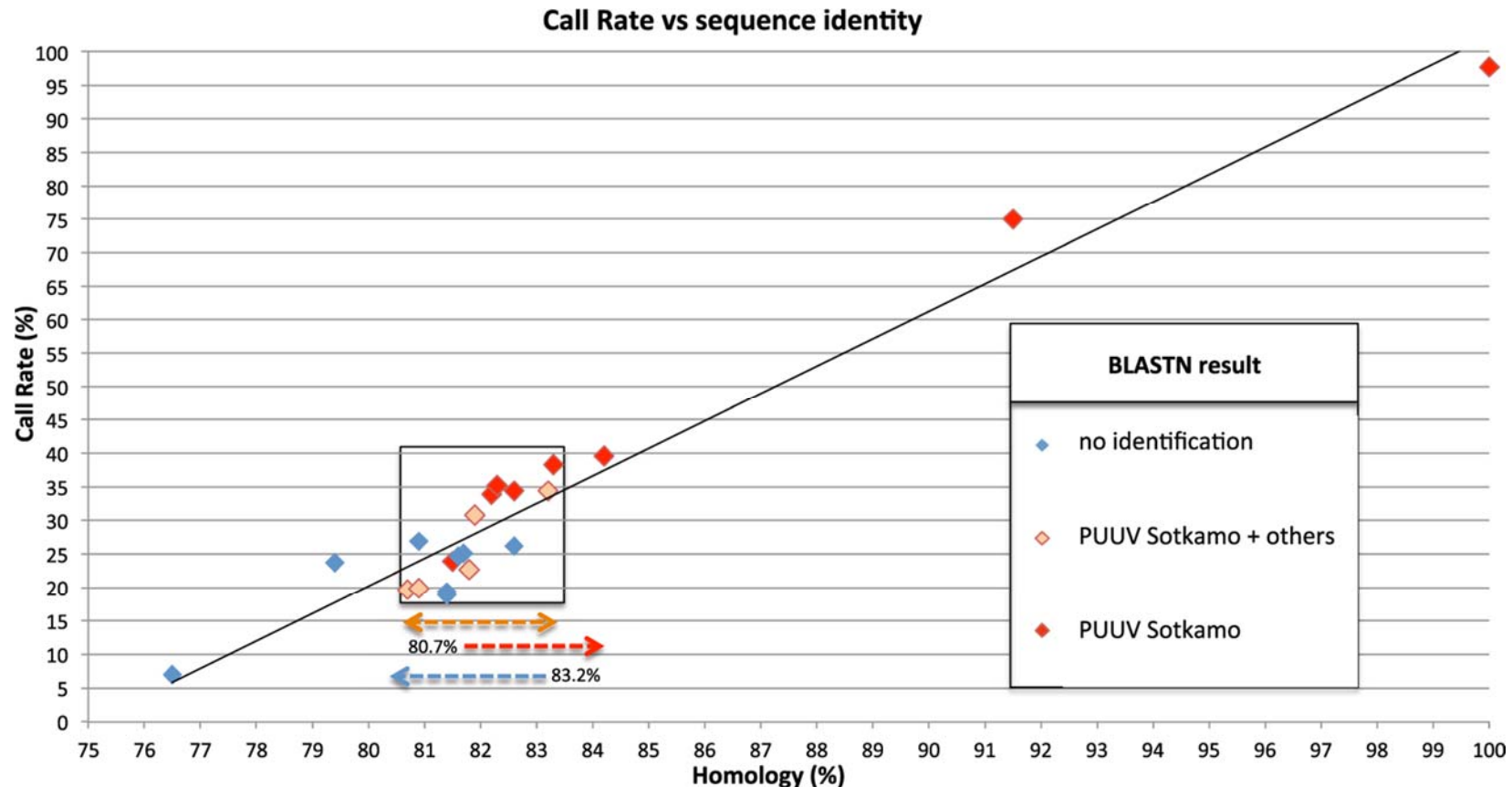
Resequencing analysis

```
>cacacttctattatcctgannnn
tnnnnnnnnntgtcaacacgtggg
agncaaaactgtgaaagaaaacaag
ggactcgtatccgtttcnaagntgn
cactncctncnaggacatcaatggt
atcaggagaccanagcatcnatag
ttccatgcccnaactgctcaatcaac
tatgaaggctgaagaattaaccctt
gggcgatttagaacatagtatgtg
gtctatttccancnc
```

Output sequence (FASTA)

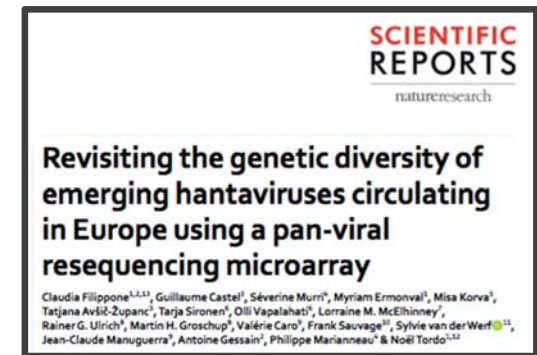
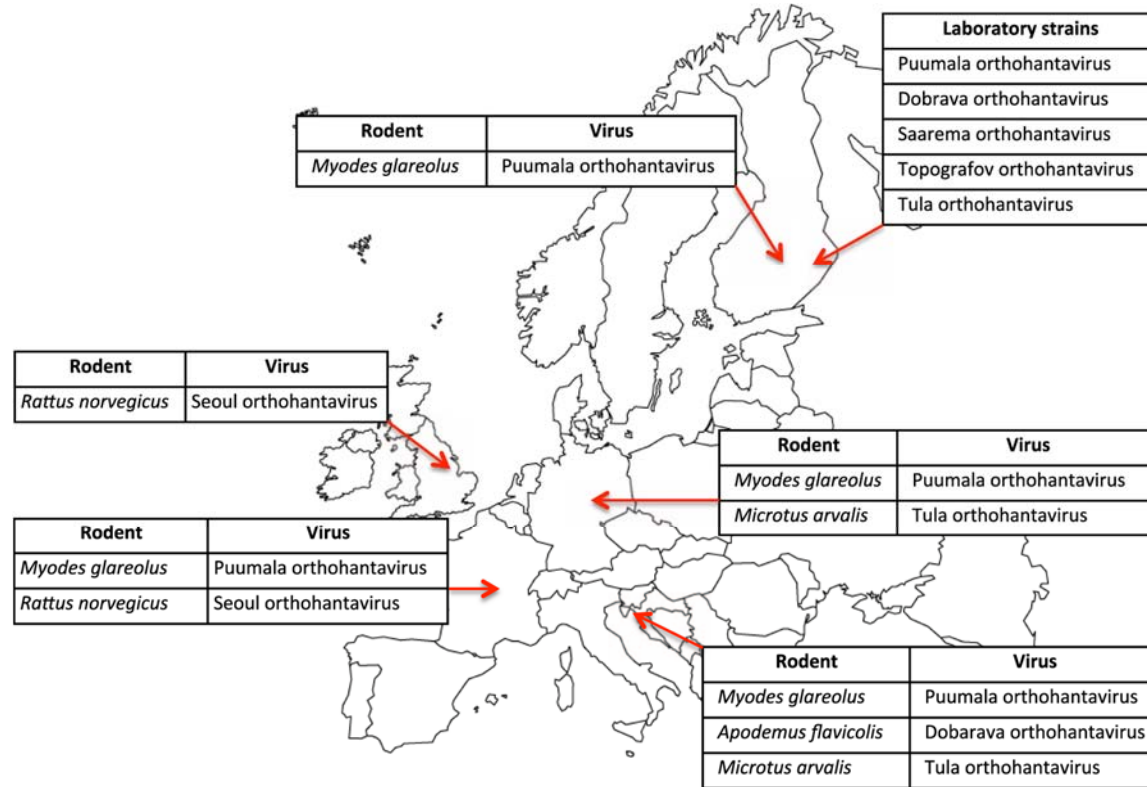
Hantavirus isolate typing (BLASTN)

Performance of PathogenID v3.0 microarray for Hantavirus detection



Reference prototype **PUUV** compared with all sequences tiled on the microarray. Percentage of positions identified (*call rate* value) proportional to percentage of homology of the reference sequence vs the tiled ones. Up to 20% of divergence was tolerated for efficient detection by the microarray.

Analysis of hantaviruses circulating in Europe



➔ Genetic characterization at the geographical variant level

Collaboration with G Castel, CBGP

Example of Seoul hantavirus: emerging virus in Europe


- United Kingdom, France, Belgium, Ireland, Portugal, Russia....
- Carried by Norway brown rat (*Rattus norvegicus*), or black rat (*Rattus rattus*)
- Associated with (mild) HFRS, severe SEOV-associated HFRS in France
- Laboratory-acquired human infections
→ Laboratory rats SEOV+
- Infected rats (e.g. viral RNA in lung, kidney; urine..)
→ human exposure
- Still several cases unnoticed/misdiagnosed → **Surveillance needed**

- Taori, SK et al. *The Lancet*. 2013.
- Reynes JM et al. *Emerg Infect Dis*. 2017.
- Kerins JL, VMD. *2017 Weekly / February 2, 2018*.
- Clement J et al. *Viruses*. 2019 Jul 17;11(7).

Hantaviruses at XXI century

- **Seoul virus**

TWEET THIS

 Hantavirus infection reports are popping up throughout the Western hemisphere.

FEB 5, 2018 @ 07:00 AM 3,932

Here's The Virus That People Are Catching From Their Pet Rats



CDC Home Based Rat Breeding Facility Triggers Seoul Virus Outbreak



➤ Several reported cases in France, UK, USA, Canada..

- Taori, SK et al. *The Lancet*. 2013.
- Reynes JM et al. *Emerg Infect Dis*. 2017.
- Kerins JL, VMD. *2017 Weekly / February 2, 2018*.
- Clement J et al. *Viruses*. 2019 Jul 17;11(7).

Detection of Seoul virus UK strain by microarray

Sequence on the chip

222_Puumalavirus_RNA_for_nucleocapsid_protein_CG14444_AJ277075
 223_Puumalavirus_CG13891_RNA_nucleocapsid_protein_gene_U22423
 224_Puumalavirus_Bavaria_CG9/04_AY954722_nucleocapsid_protein
 225_Puumalavirus_Sotkamo_NC_005224
 226_Puumalavirus_Puu/Kazan_Z84204
 227_Puumalavirus_Umea/hu_AY526219
 228_Puumalavirus_PUU/Ernstbrunn/Cg641/1995_AJ888752
 229_Puumalavirus_BerkeL_L36943
 230_Puumalavirus_Couvin/59Cg/97_AJ277034
 231_Puumalavirus_Pallasjarvi/63Cg/98_AJ314598
 232_Puumalavirus_Munga/Mg16/05_GQ339487
 233_Puumalavirus_PUU/Mignovillard/CgY02/2005_AM695638
 234_Puumalavirus_strainFrance/Perpignan1999_nucleocapsid_protein_gene_partial
 235_Puumala_Fusong90006_EF488806
 236_Puumala_CH-214_Franche_Comt»_
 237_Puumala_AR-21_Charleville_MeziAres
 238_Puumala_167-2Charleville-Mezieres
 239_Puumala_167-4Charleville-Mezieres
 240_Puumala_178-2Charleville-Mezieres
 241_Puumala_180-78Charleville-Mezieres
 242_Puumala_RU-11Ruminy-Troyes
 243_Puumala_OR-52_Orleans
 244_Pulmonary_syndrome_hantavirus_ConvictCreek107_L33683
 245_DobravaBelgrade_L41916
 246_Dobrava_Esl/81Aa/01_AY533120
 247_Saarema_virus_AJ616854
 248_Kenkemavirus_MSB148794_GQ306148
 249_Hantaan_M14626_reference
 250_Hantaan_KI_GU140098
 251_Hantaan_Z10_EF533944
 252_Hantaan_AA2499_AF427320
 253_Hantaan_CGHu1_EU092218
 254_Hantaan_Q32_ABO27097
255_Hantavirus_Z37_AF187082
 256_Hantavirus_Nc167_ABO27523
257_Hantavirus_L99_AF488708
258_Seoul_8039_NC005236
 259_Asamavirus_N10_EU929072
 260_Novavirus_MSB95703_FJ539178
 261_ProspectHill_M34011
 262_Soochong_SC-1_AY675349_mRNA
 263_Tula_Sennickerode_Sen05/204_EU439950
 264_Tula_Kosice144/Ma/95_Y13979
 265_Tula/Moravia/5293Ma/94_Z48574
 266_Tula/MG23/Omsk/AF442621
 267_Thottapalayam_AY526097
 268_Andes_Chile-9717869_AF291702
 269_Andes_NK104619_EU241691
 270_SinNombre_NMR11_L37904
 271_NewYorkhantavirus_nucleocapsid_U09488
 272_Choclovirus_segmentS_DQ285046
 273_LagunaNegra_AF005727

RAPID COMMUNICATIONS

The continued emergence of hantaviruses: isolation of a Seoul virus implicated in human disease, United Kingdom, October 2012

L J Jameson (lisa.jameson@hpa.org.uk)^{1,2}, C H Logue¹, B Atkinson¹, N Baker³, S E Galbraith², M W Carroll¹, T Brooks⁴, R Hewson¹
 1. Virology and Pathogenesis, Microbiology Services, Health Protection Agency, Porton Down, Wiltshire, United Kingdom
 2. Department of Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom
 3. National Collection of Pathogenic Viruses, Microbiology Services, Health Protection Agency, Porton Down, Wiltshire, United Kingdom
 4. Rare and Imported Pathogens Laboratory, Microbiology Services, Health Protection Agency, Porton Down, Wiltshire, United Kingdom

seq258:S_UK_03-10-2013_(VirIDv3r520827) Start=12...

RID ABMY5H1W01R (Expires on 12-10 21:29 pm)

Query ID lcl|35875

Description seq258:S_UK_03-10-2013_(VirIDv3r520827) Start=12 End=412

Molecule type nucleic acid

Query Length 401

Other reports: [Search Summary](#) [Taxonomy reports](#) [Distance tree of results](#)

Descriptions

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected:0

[Alignments](#) [Download](#) [GenBank](#) [Graphics](#) [Distance tree](#)

	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Seoul virus nucleocapsid protein gene, complete cds	86.0	136	33%	2e-13	74%	KC626089.1
<input type="checkbox"/> Seoul virus strain Humber segment S, complete sequence	86.0	136	33%	2e-13	74%	JX879769.1
<input type="checkbox"/> Seoul virus N gene for nucleocapsid protein, partial cds, strain: KS80	82.4	132	33%	3e-12	73%	AB697614.1
<input type="checkbox"/> Seoul virus N gene for nucleocapsid protein, partial cds, strain: KS74	82.4	132	33%	3e-12	73%	AB697613.1
<input type="checkbox"/> Seoul virus isolate DPRK08 segment S, complete sequence	82.4	127	33%	3e-12	73%	JX853575.1

General plan

- Introduction on viral emergence
- Approaches to study zoonotic viruses
- Zoonotic transmission of retroviruses in Central Africa
- Molecular tools for characterizing emerging viruses
- **Hantaviruses in Indian Ocean**
- Tracking transmission of hantavirus in Madagascar

Hantavirus in *Rattus rattus* in Mayotte

Journal of General Virology (2016), 97, 1060–1066

DOI 10.1099/jgv.0.000440

Short
Communication

Discovery of hantavirus circulating among *Rattus rattus* in French Mayotte island, Indian Ocean

Claudia Filippone,^{1†} Guillaume Castel,^{2†} Séverine Murri,³
Frédéric Beaulieux,⁴ Myriam Ermonval,¹ Corinne Jallet,¹ Emma L. Wise,⁵
Richard J. Ellis,⁵ Denise A. Marston,⁵ Lorraine M. McElhinney,^{5,6}
Anthony R. Fooks,^{5,6} Amélie Desvars,^{7‡} Lénaïg Halos,⁸
Gwenaél Vourc'h,⁷ Philippe Marianneau³ and Noël Tordo¹

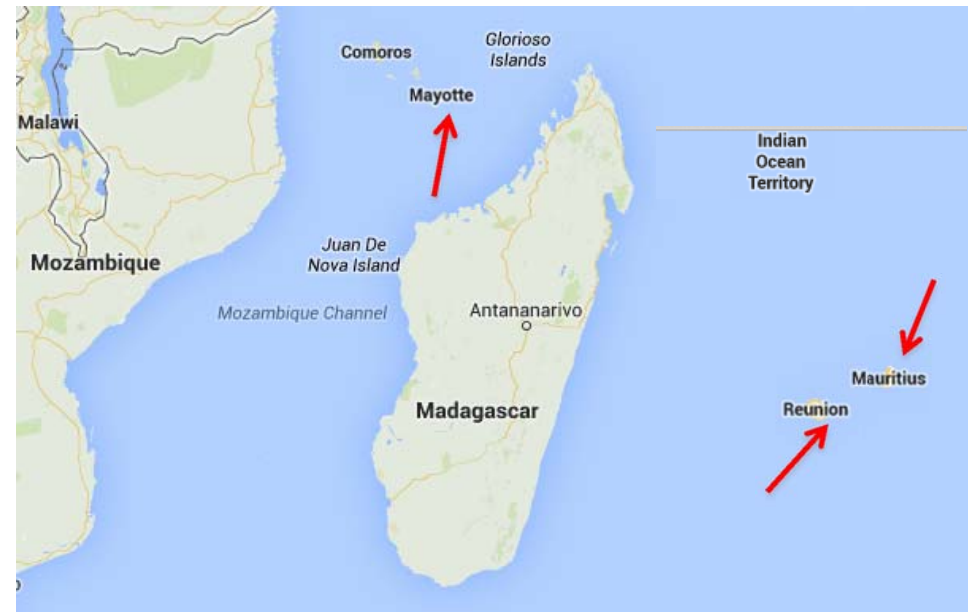
Collaboration with CBGP (Guillaume Castel)

Context:

Surveillance of outbreaks in Indian Ocean « Chikani » project

- Chikungunya virus (CHIKV) epidemics (2005-2006)
- Research of animal reservoirs among wild and domestic vertebrates

Vourc'h , 2014

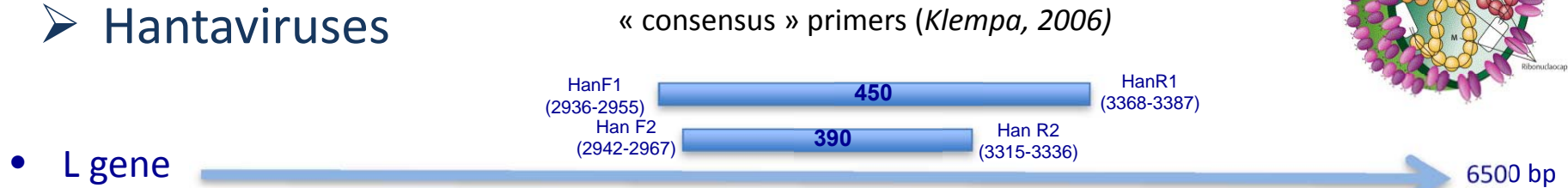
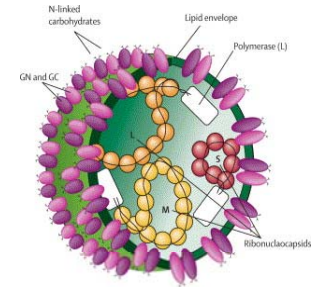


Our study

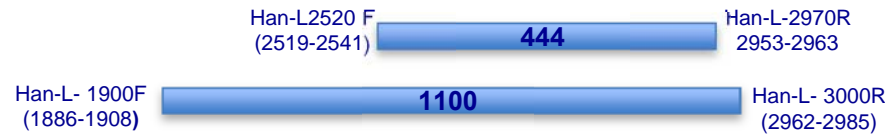
Virus hunting in 642 small mammals from La Réunion, Mayotte (2006-2007)

Search of Rodent- and Insectivore- borne viruses

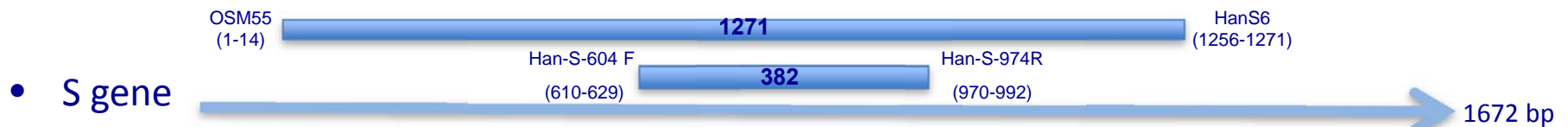
➤ Hantaviruses



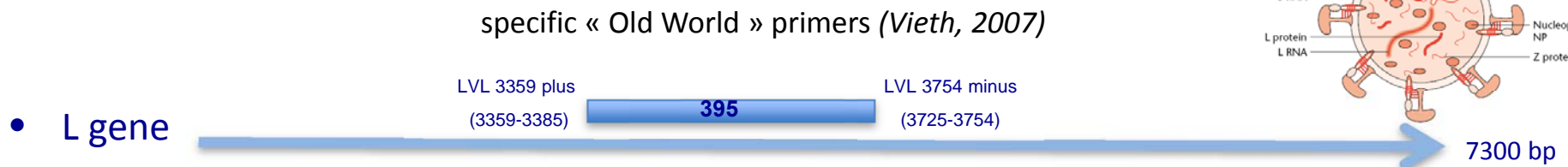
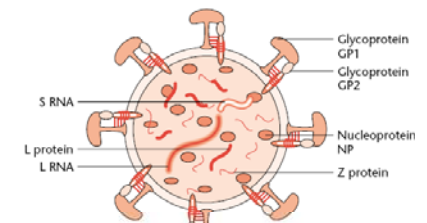
specific « insectivores » primers (Kang, 2009)



specific « insectivores » primers (Kang, 2009)



➤ Arenaviruses



Sera from rodents and insectivores tested for the presence of hantavirus and arenavirus



Species	La Réunion 2006	La Réunion 2007	Mayotte 2007
<i>Rattus rattus</i>	84	109	160
<i>Rattus norvegicus</i>	22	22	-
<i>Mus musculus</i>	46	21	-
<i>Suncus murinus</i>	22	111	-
<i>Tenrec ecaudatus</i>	-	45	-



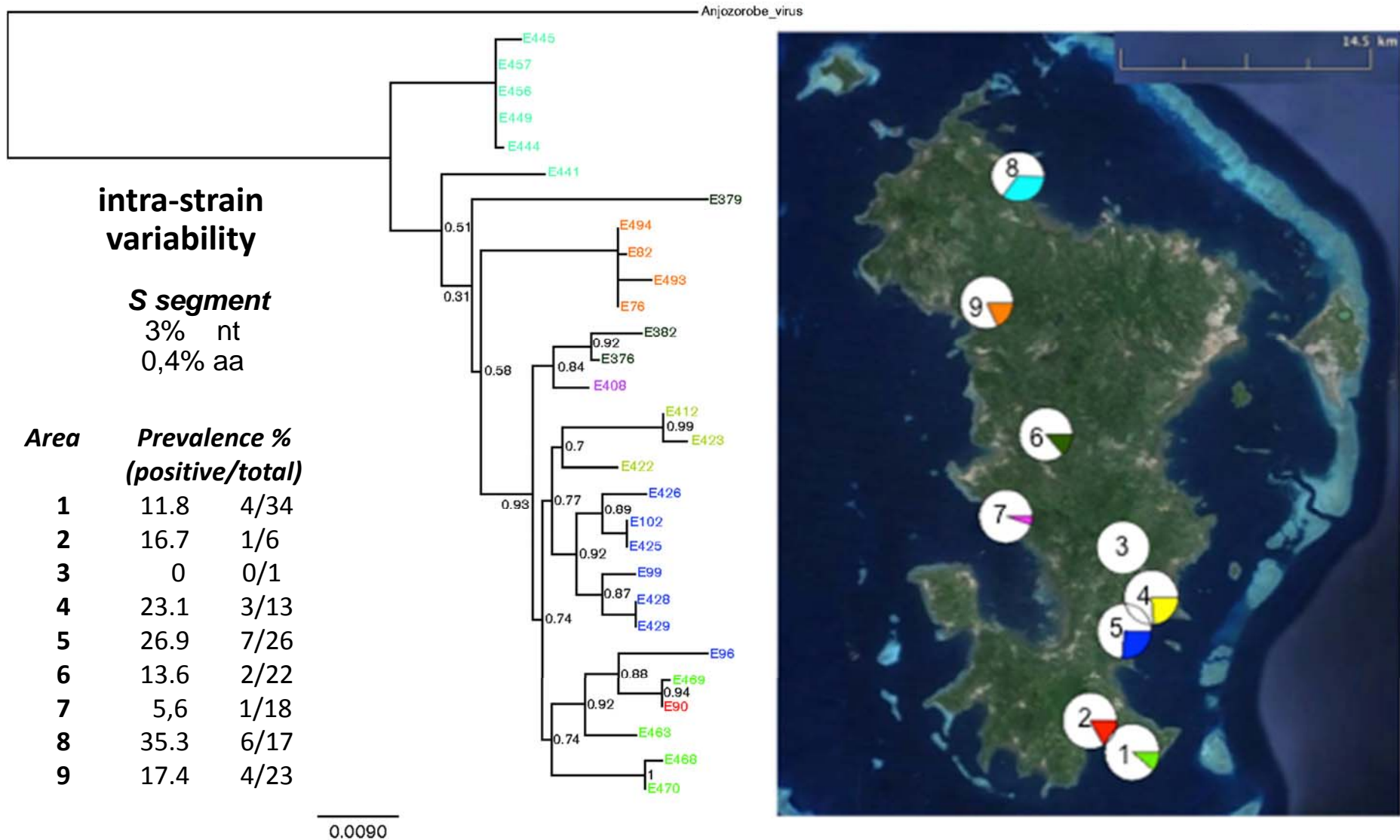
- no arenavirus detected
- **Hantavirus (L)+ in 29 (18%) of *Rattus rattus* in Mayotte** (serum, liver, spleen)

Molecular epidemiology of Mayotte hantavirus

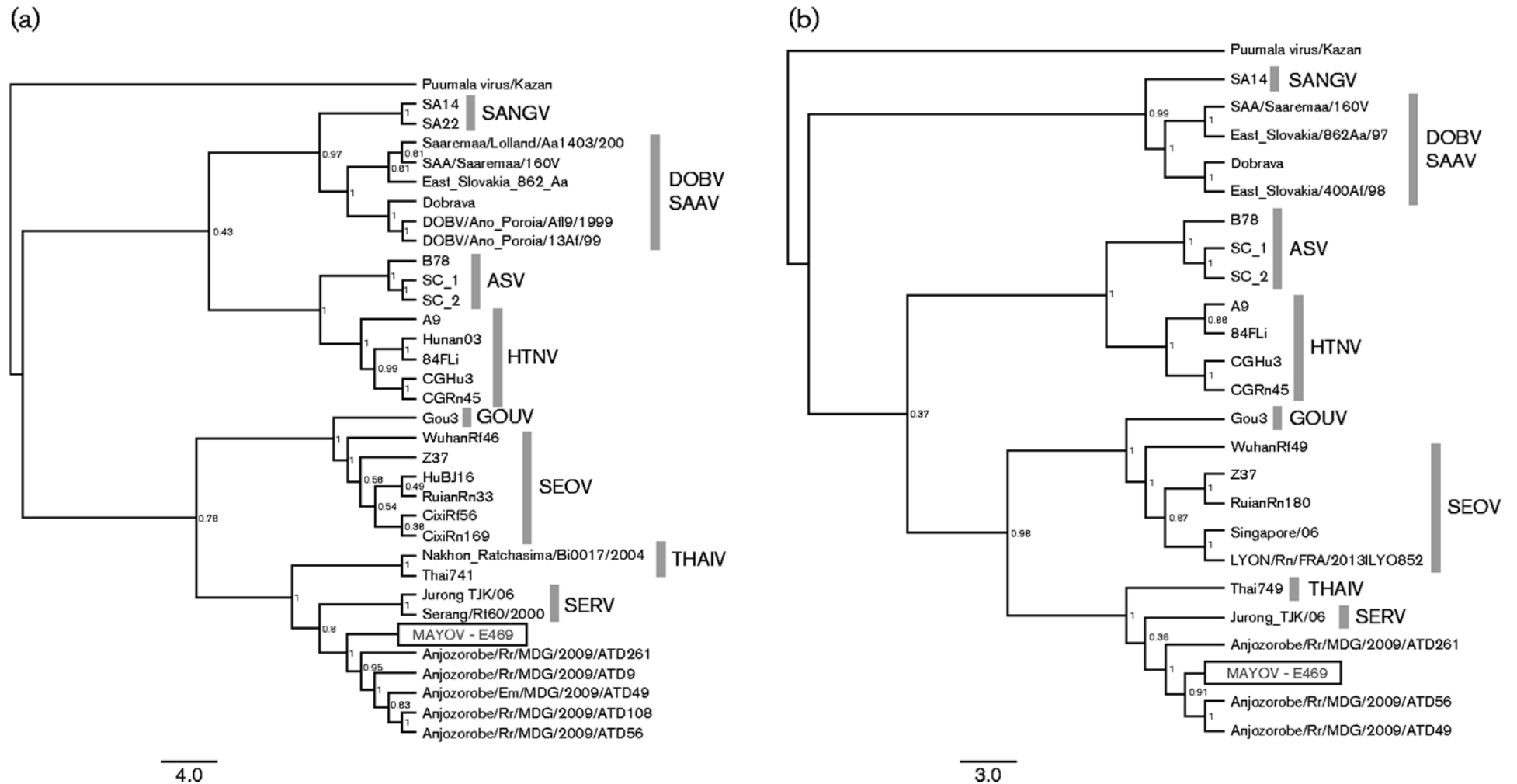
Spleen and liver tissues from the 29 hantavirus+ individuals

- set-up of a battery of RT-PCRs for S segment
Sanger sequencing
- Next Generation Sequencing (NGS)

Molecular epidemiology of Mayotte hantavirus



Mayotte hantavirus among *Murinae* hantaviruses



maximum-likelihood (ML) with PhyML v3.0

Hantavirus circulation in Indian Ocean

South-East Asia

Virology Journal



Research

Open Access

Genetic analysis of Thailand hantavirus in *Bandicota indica* trapped in Thailand

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Journal of General Virology (2009), 90, 205–209

DOI 10.1099/vir.0.006155-0

Short Communication

A newly recognized hantavirus in the Asian house rat (*Rattus tanezumi*) in Indonesia

Angelina Plyusnina,¹ Ima-Nurisa Ibrahim² and Alexander Plyusnin¹

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Johansson et al. *Virology Journal* 2010, 7:15
<http://www.virologyjournal.com/content/7/1/15>



VIROLOGY JOURNAL

RESEARCH

Open Access

Molecular characterization of two hantavirus strains from different rattus species in Singapore

Patrik Johansson¹, Grace Yap², Hwee-Teng Low¹, Chern-Chiang Siew¹, Relus Kek², Lee-Ching Ng^{2*}, Göran Bucht²

African Indian Ocean region - Madagascar

Arch. Inst. Pasteur Madagascar, 1985, 52, 1, 181-186

LA FIEVRE HEMORRAGIQUE AVEC SYNDROME RENAL
A MADAGASCAR.
PREMIERE ENQUETE SERO-EPIDEMIOLOGIQUE
SUR LES POPULATIONS DE RATS.

ROLLIN P.E. (★), MATHIOT Ch. (★★), NAWROCKA E. (★★★),
RAVAOALIMALALA V.E. (★★★★), COULANGES P. (★★),
SUREAU P. (★), McCORMICK J.B. (★★★★★)

VECTOR-BORNE AND ZOO NOTIC DISEASES
Volume 14, Number 3, 2014
© Mary Ann Liebert, Inc.
DOI: 10.1089/vbz.2013.1359

Anjzorobe Hantavirus, a New Genetic Variant of Thailand Virus Detected in Rodents from Madagascar

Jean-Marc Reynes¹, Nadia Kaloina Razafindralambo², Vincent Lacoste³, Marie-Marie Olive², Tony Andrianaivo Barivelo^{4,5}, Voahangy Soarimalala⁴, Jean-Michel Heraud² and Anne Lavergne³

How hantavirus has arrived in Mayotte?



- Recent invasion of *Rattus rattus* in the island?
- Spill-over from/to other species?

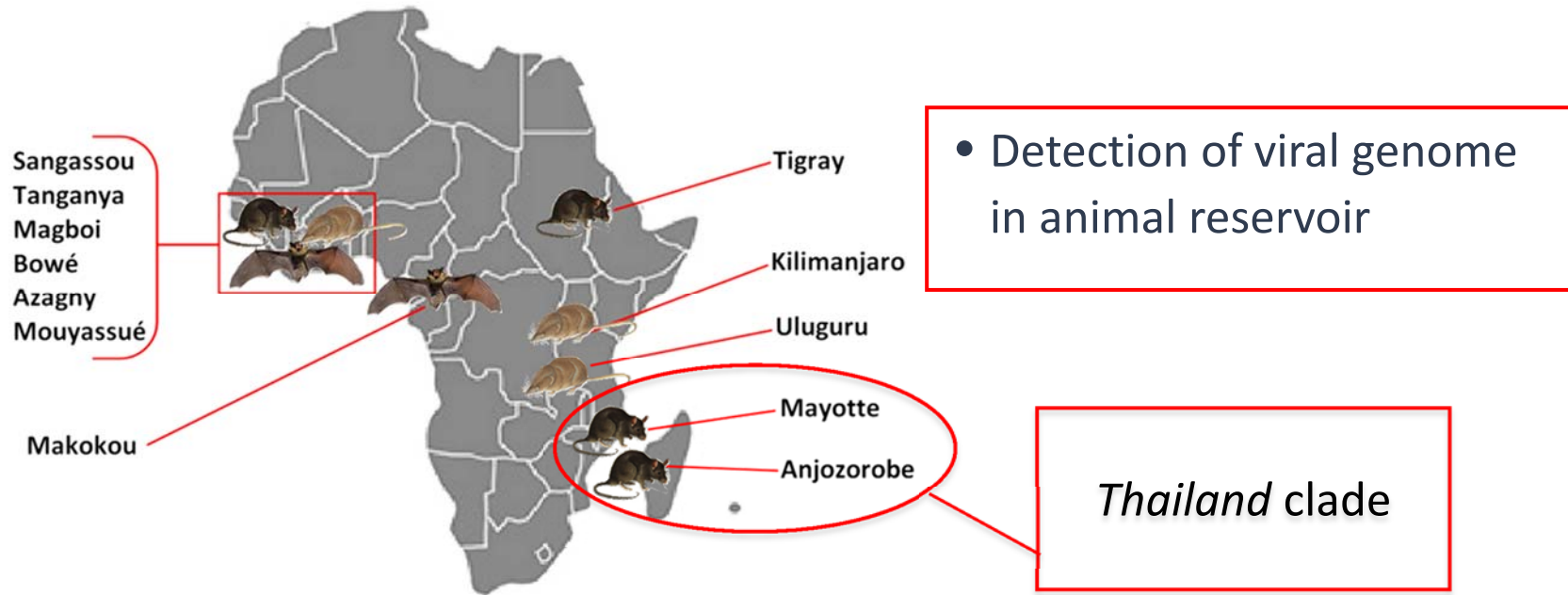
Perspectives for studies in the area

- Screening in Indian Ocean (e.g. Mayotte, Madagascar...)
 - **Surveillance** in the **rodent** population and other potential reservoirs
 - **Investigation in humans**
 - general population
 - patients with undiagnosed renal syndromes

General plan

- Introduction on viral emergence
- Approaches to study zoonotic viruses
- Zoonotic transmission of retroviruses in Central Africa
- Molecular tools for characterizing emerging viruses
- Hantaviruses in Indian Ocean
- **Tracking transmission of hantavirus in Madagascar**

Hantavirus in Africa and Indian Ocean



Adapted from: *Witkowski et al, 2014 ; Manigold and Vial, 2014.*

- Molecular evidence in rodents, shrews and bats
- Discovery in continental Africa and Indian Ocean area
 - area with several patients with **unknown fevers**
 - serious issue for Public Health

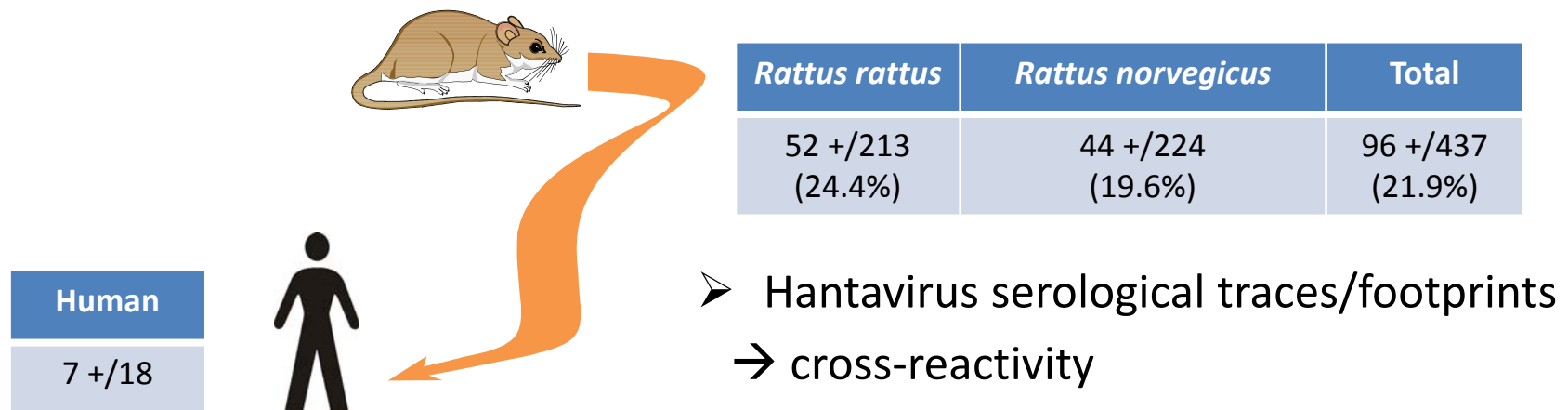
Need of screening in humans

Pioneer study at Institut Pasteur de Madagascar

- **≈ 30 years before the discovery of Anjzorobe virus**
 - Serological survey in Antananarivo + 4 sites
 - among rats (*Rattus rattus*, *Rattus norvegicus*)
 - among humans manipulating rats

Methodology: immunofluorescence by using infected cells with *Hantaan* and *Puumala* viruses

Rollin PE, Mathiot C, Nawrocka E, Ravaoalimalala VE, Coulanges P, Sureau P, McCormick JB. Hemorrhagic fever with renal syndrome in Madagascar. First seroepidemiologic survey of rat populations. Arch Inst Pasteur Madagascar. 1986;52(1):181-6.



Circulation of hantavirus in Madagascar

National survey

ZORA

(Zoonosis, Rodents, Arbovirus)

Project IPM & Wellcome Trust

November 2011 - May 2013

28 zones around health centers of the country

- **Animal study**

Population of study: 1242 small mammals

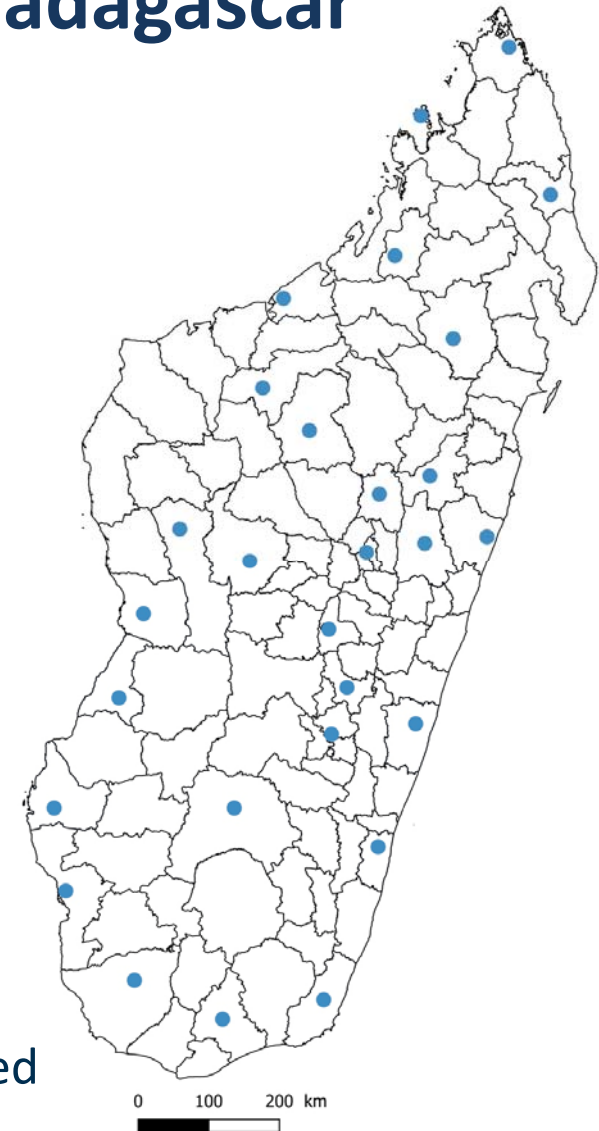
- 7 species (rodents, insectivores)

Rattus rattus, *Rattus norvegicus*, *Mus musculus*, *Suncus murinus*,
Setifer setosus, *Tenrec ecaudatus*, *Hemicentetes semispinosus*

- **Human study**

Population of study: 1680 individuals randomly selected

- average age: 37 years [18-99]
- collected informations: contact with rodents, activity...



Circulation among the animal reservoir

- **Molecular detection of hantavirus**

Research of viral genome by RT-PCR

- important prevalence among *Rattus rattus*

12.4% (111/897)

- 1.6% (2/125) among *Mus musculus*

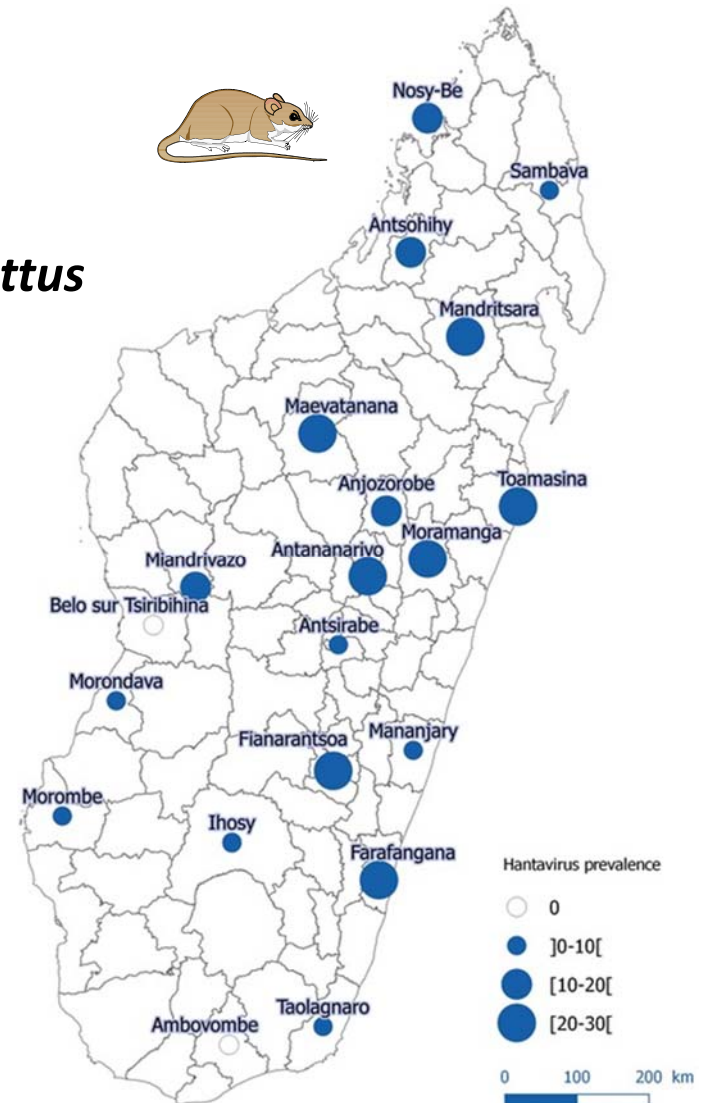
- **Geographical distribution**

- in all the country

0-29%

- **Risk factors for infection**

- habitat, high precipitation weight..



Molecular detection of Malagasy hantavirus

- Development of a specific TaqMan real-time RT-PCR
 - validation (sensitivity / specificity) compared with Klempa RT-PCR

Table 2. Limit of detection of Thailand orthohantavirus (Anjzorobe virus) assay by using the two platforms of real time RT-PCR.

System	LightCycler 480 Instrument II (Roche)	Applied Biosystems 7500 (Applied-Biosystems)	Pan-Hantavirus Nested RT-PCR ¹
RT-PCR Reagents	SensiFAST Probe One-Step kit (Bioline)	SuperScript III Platinum One-Step Kit (Invitrogen)	
THAIV RNA Concentration (FFU/mL) ²	Ct	Ct	
3.45 × 10 ⁵	21.1	18.7	Positive
3.45 × 10 ⁴	24.6	22.8	Positive
3.45 × 10 ³	27.8	26.9	Positive
3.45 × 10 ²	31.5	30.8	Positive
3.45 × 10 ¹	34.3	32.9	Positive
3.45	36.4	36.2	Positive
3.45 × 10 ⁻¹	Negative	Negative	Negative
3.45 × 10 ⁻²	Negative	Negative	Negative

¹ Conventional RT-PCR according Klempa et al. [10]. ² Thailand orthohantavirus reference virus (THAIV strain 749) RNA was extracted from a serial ten-fold dilution of a viral stock at 3.45 × 10⁶ FFU/mL.

*Coll CNR Hantavirus
France*

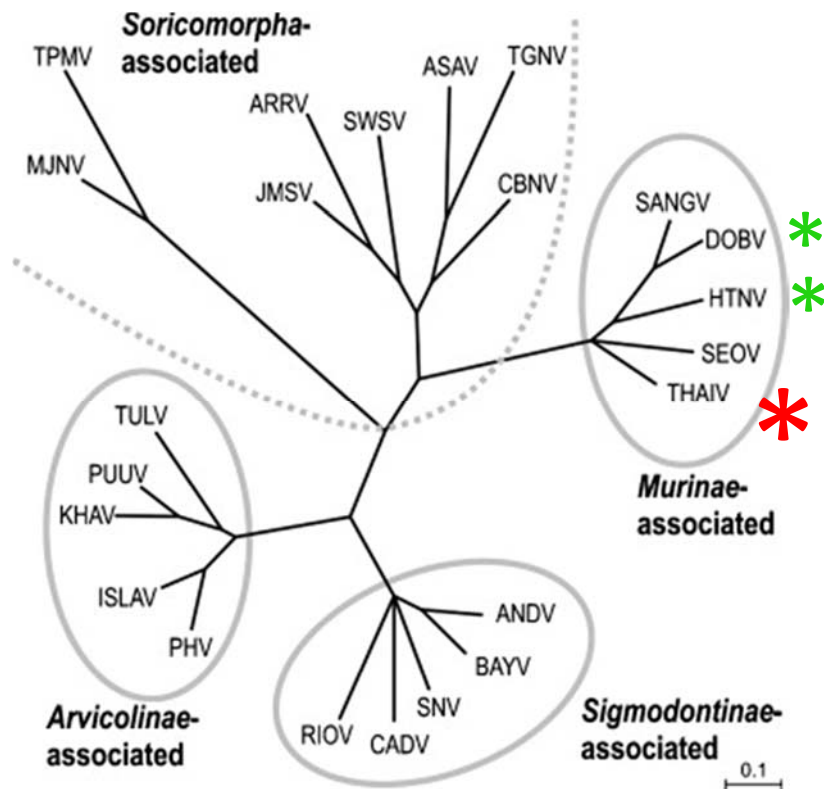
Table 4. Methods comparison between rRT-PCR using Applied Biosystems 7500 and reference method nested RT-PCR by Klempa et al. [10].

Applied Biosystems 7500 (Our Assay)	Pan-Hantavirus Nested RT-PCR (Klempa)	
	Positive	Negative
Positive	113	1
Negative	0	341

➤ *....For additional investigations in animal and humans*

Serological investigation in human

- Sub-family *Murinae*



Charrel et al, Antiviral Research. 2011



Anjzorobe hantavirus, genetic variant of prototype species *Thalande*

REAGENA
Reagena Dobrava-Hantaan IgG EIA

- IgG test based on recombinant N antigen (DOBV, HTNV)

Serological investigation in human

- **Seroprevalence study**

Methodology: ELISA for searching IgG against nuclecapsid antigen/protein

- hantavirus associated to *Murinae* sub-family (*Hantaan..*)

Commercial Kit

- specific to *Anjzorobe* virus from Madagascar

home-made by using recombinants antigens produced in baculovirus

(*Projet Transversale Recherche 'Hantarev' IPP & IPM, CNR Hantavirus*)

Hantavirus in human in Madagascar

- **First human survey at the national scale**
- IgG+ individuals all over the country

Prevalence: 2.7% (46/1680)

➤ absence of association with age, sex, geographical origin

➤ **Signs of past infection in human**

➤ To be extended to specific areas

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 26, No. 3, March 2020

DISPATCHES

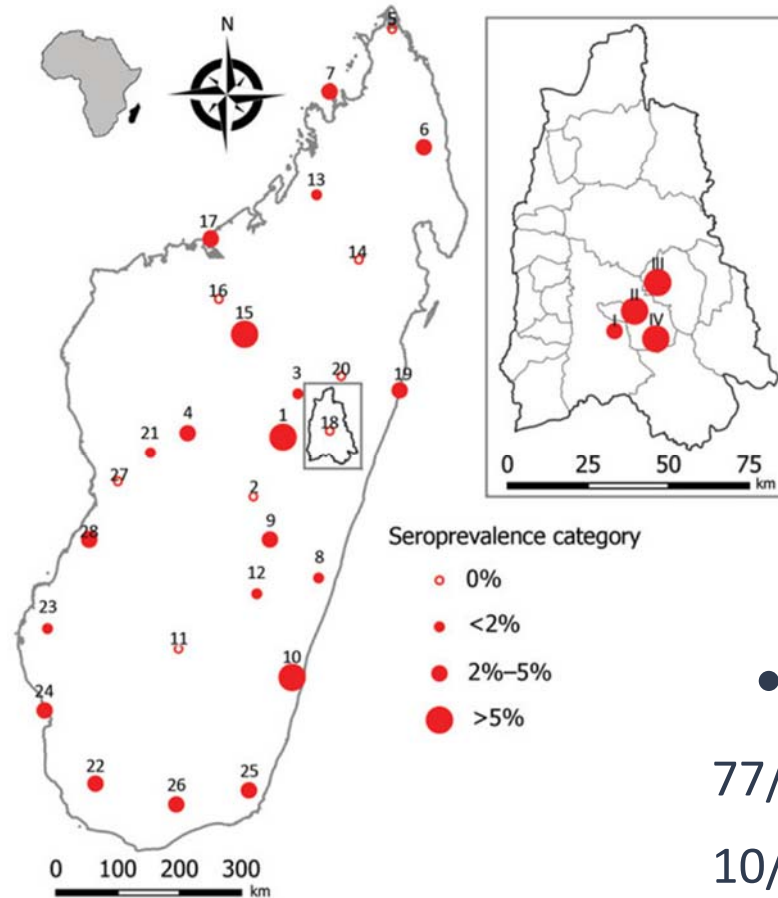
Human Exposure to Hantaviruses Associated with Rodents of the *Murinae* Subfamily, Madagascar

Harinirina Aina Rabemananjara,¹ Vololoniaina Raharinosy,¹ Ravo Michèle Razafimahefa,
Jean Pierre Ravalohery, Jean Théophile Rafisandratanisoa, Soa Fy Andriamandimby,
Minoarisoa Rajerison, Soanandrasana Raheliniirina, Aina Harimanana, Judickaëlle Irinantenaina,
Marie-Marie Olive, Christophe Rogier, Noël Tordo, Rainer G. Ulrich, Jean-Marc Reynes,
Stéphane Petres, Jean-Michel Heraud,² Sandra Teller,² Claudia Filippone²

National scale vs forest district

- ZORA

2.7% (46/1680)



- PRIZM

Prediction

Risk

Zoonosis

Moramanga

- Moramanga district

77/237 animal: 32.5%

10/139 human: 7.2%

Etiological link with nephropathy or (hemorrhagic) fever?

- ***Anjzorobe* virus → Thailand clade associated to HFRS**
- **Evaluation of the risk of hantavirus infection in human**
 - Is hantavirus circulating in Madagascar at the origin of human disease?



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- Unité d'Epidémiologie et Physiopathologie des Virus Oncogènes: A Gessain and coll
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CIBU: JC Manuguerra and coll ; Proteopole: S Petres

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