



# Virologie moléculaire

- Champs :
  - Détection des virus influenza et Newcastle
  - Caractérisation moléculaire
  - Etude de la fonction de gènes
- Dans projets CIRAD :
  - FAO LoA
  - MESURES D'URGENCE
  - GRIPAVI
  - RIVERS
  - PORTFASTFLU



# Objectifs Gripavi

- **Ecologie virale :**  
Flux de virus, de gènes (virus influenza)
- **Formation et transfert technologique**



# Outils & méthodes

- Outils
  - Diagnostic rapide et haut débit
  - Virologie classique et moléculaire
  - Bioinformatique et analyse phylogénétique
- Questions de recherche:
  - Caractérisation moléculaire des virus influenza et Newcastle africains (faune sauvage, domestique et environnement) par rapport aux virus européens : approche sur les flux



# Diagnostic

- Etat des lieux

20 février 2006

15 septembre 2006

10 septembre 2007

Programme

Mise en place  
des tests moléculaires

Automatisation  
Haut-débit

Isolement viral

Séquençage  
Haut débit

Analyse  
moléculaire

Ecologie virale

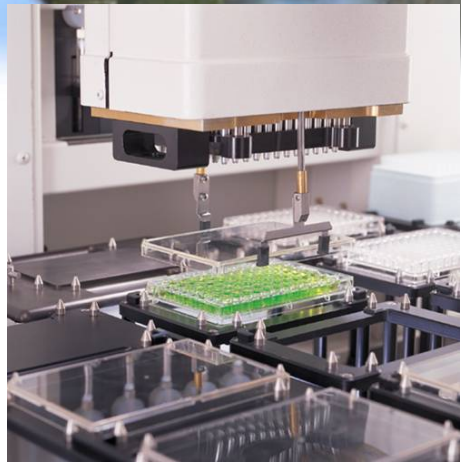


Influenza A, H5 et H7, Pathotype



# Avian Influenza

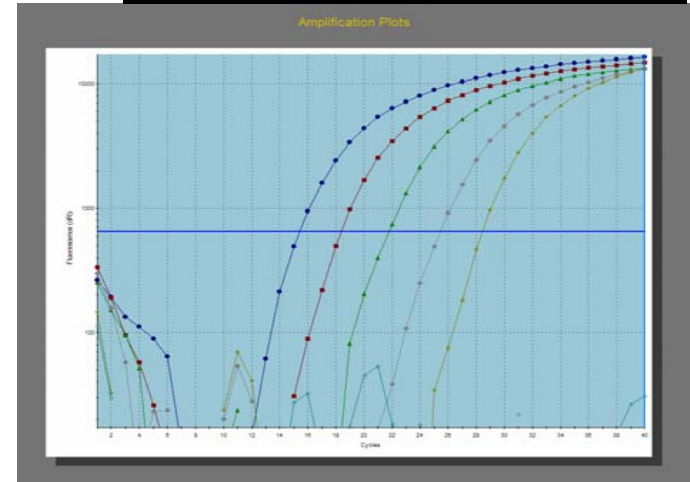
in Developing Countries  
**welcome**trust



**Automate**



**Real-time PCR**







View inside CIRAD BSL3



# Avian Influenza

Research in Developing Countries

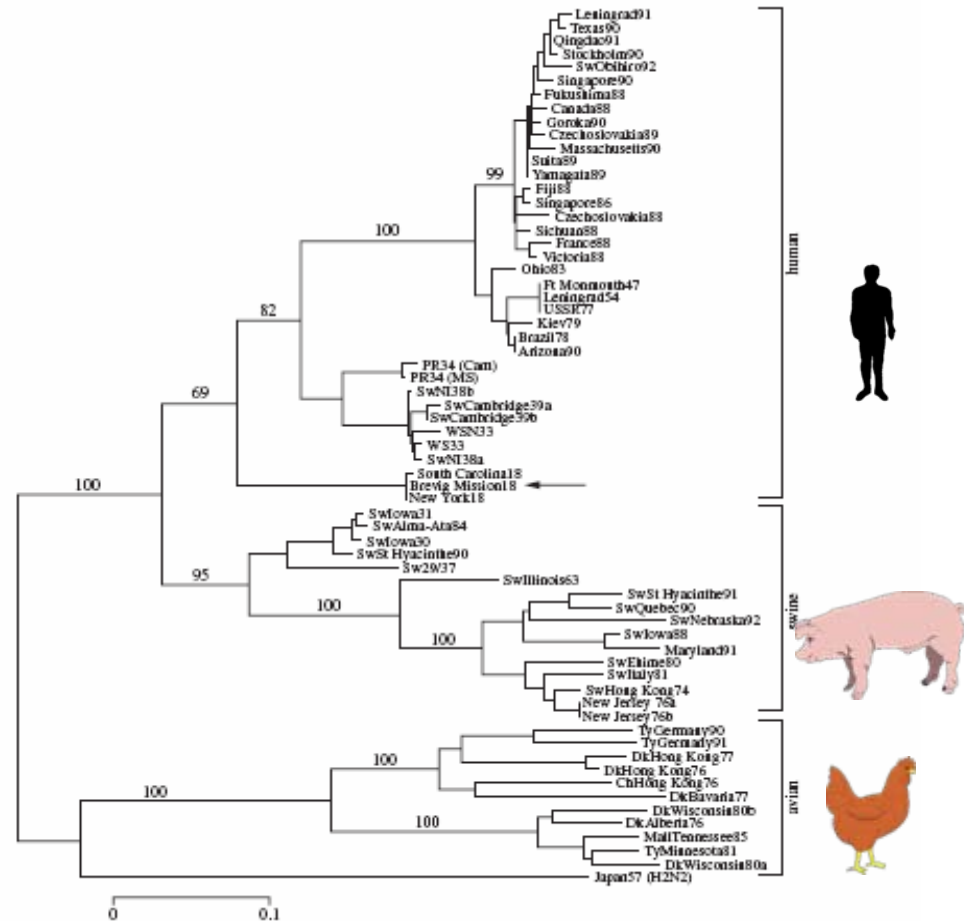
## Virologie

## Epidémiologie moléculaire

- Détection et typage des virus
- Analyse des séquences & phylogén

Arbre Phylogénétique basé sur le gène H

Taubenberger J.K, Reid A.H., Janczewski A., Fanning T., Integrating historical, clinical and molecular genetic in order to explain the origin and virulence of the 1918 sarnish influenza virus, *The royal society*, 2001.



# Detection of viral RNA

- ◆ Automated RNA extraction from samples
- ◆ Detection of type A influenza:
  - Matrix (M) gene by real-time RT-PCR
- ◆ Detection of H5 or H7 subtypes by real-time RT-PCR on AIV A positive samples
- ◆ RT-PCR on the cleavage site of HA for H5 or H7 positive samples
- ◆ Sequencing of the amplified cleavage site for determination of pathogenicity



# Detection of type A influenza: real-time RT-PCR specific of M gene

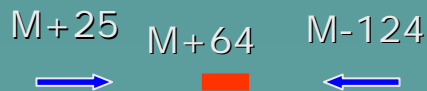
M+25

H5N2	(1)	-----GATATTGAAAATGAGCCTTCTAACCGAG
H1N1	(1)	-----GGTAGATATTAAAGATGAGTCTTCTAACCGAA
H3N2	(1)	-----AGCGAAAGCAGGTAGATATTGAAAGATGAGTCTTCTAACCGAG
H1N1	(1)	-----AGCGAAAGCAGGTAGATATTGAAAGATGAGTCTTCTAACCGAG
H1N1	(1)	-----ATGAGCCTTCTAACCGAG
H3N2	(1)	-----GCAAAAGCAGGTAGATATTGAAAGATGAGCCTTCTAACCGAG
H1N2	(1)	-----TAGATATTGAAAGATGAGCCTTCTAACCGAG
H9N2	(1)	GGGGAATTCCAAAAGCAGGTAGATATTGAAAGATGAGTCTTCTAACCGAG
H5N1	(1)	-----AGCGAAAGCAGGTAGATATTGAAAGATGAGTCTTCTAACCGAG
H5N1	(1)	-----AGCGAAAGCAGGTAGATATTGAAAATGAGTCTTCTAACCGAG
H5N1	(1)	-----CAAAGCAGGTAGATCTTGAAGATGAGTCTTCTAACCGAG
H5N1	(1)	-----GGAACTCATGAGTCTTCTAACCGAG
H5N1	(1)	-----AAGATGAGTCTTCTAACCGAG
H5N1	(1)	-----GATGAGTCTTCTAACCGAG
Consensus	(1)	GGTAGATATTGAAAGATGAGTCTTCTAACCGAG
M-AIVsens	(30)	51 GTCGAAACGTACGTTCTCTCTATCGTCCCGTCAGGCCCCCTCAAAGCCGA 100
IVAMM2E	(34)	GTCGAAACGTACGTTCTCTCTATCGTCCCGTCAGGCCCCCTCAAAGCCGA
AB036778	(44)	GTCGAAACGTACGTTCTCTCTATCATCCCGTCAGGCCCCCTCAAAGCCGA
NC_002016	(44)	GTCGAAACGTACGTTCTCTCTATCATCCCGTCAGGCCCCCTCAAAGCCGA
AY210255	(19)	GTCGAAACGTACGTTCTCTCTATCGTCCCGTCAGGCCCCCTCAAAGCCGA
CY000794	(43)	GTCGAAACGTACTGTTCTCTCTATCGTTCCATCAGGCCCCCTCAAAGCCGA
CY006188	(32)	GTCGAAACGTACTGTTCTCTCTATCGTTCCATCAGGCCCCCTCAAAGCCGA
NC_004907	(51)	GTCGAAACGTACGTTCTCTCTATCATCCCGTCAGGCCCCCTCAAAGCCGA
H5N2	(44)	GTCGAAACGTACGTTCTCTCTATCATCCCGTCAGGCCCCCTCAAAGCCGA
NC_007363	(44)	GTCGAAACGTACGTTCTCTCTATCGTCCCGTCAGGCCCCCTCAAAGCCGA
AY627887	(42)	GTCGAAACGTACGTTCTCTCTATCATCCCGTCAGGCCCCCTCAAAGCCGA
AM040045	(26)	GTCGAAACGTACGTTCTCTCTATCATCCCGTCAGGCCCCCTCAAAGCCGA
AB239319	(23)	GTCGAAACGTACGTTCTCTCTATCATCCCGTCAGGCCCCCTCAAAGCCGA
AB239326	(20)	GTCGAAACGTACGTTCTCTCTATCATCCCGTCAGGCCCCCTCAAAGCCGA
DQ100569	(1)	-----TATCATCCCGTCAGGCCCCCTCAAAGCCGA
Consensus	(51)	GTCGAAACGTACGTTCTCTCTATCATCCCGTCAGGCCCCCTCAAAGCCGA
M-AIVsens	(80)	101 GATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG 150
IVAMM2E	(84)	GATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG
AB036778	(94)	GATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG
NC_002016	(94)	GATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG
AY210255	(69)	GATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG
CY000794	(93)	AATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG
CY006188	(82)	AATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG
NC_004907	(101)	GATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG
H5N2	(94)	GATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG
NC_007363	(94)	GATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG
AY627887	(92)	GATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG
AM040045	(76)	GATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG
AB239319	(73)	GATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG
AB239326	(70)	GATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG
DQ100569	(31)	GATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG
Consensus	(101)	GATCGCCAGAACTTGAAGATGTCCTTGCAGGGAAGAACACAGATCTTTG

Taqman Technology

Primers Spackman: M+25/M-124  
Probe M+64 FAM-BHQ1

Q-RT-PCR OIE protocole



M gene

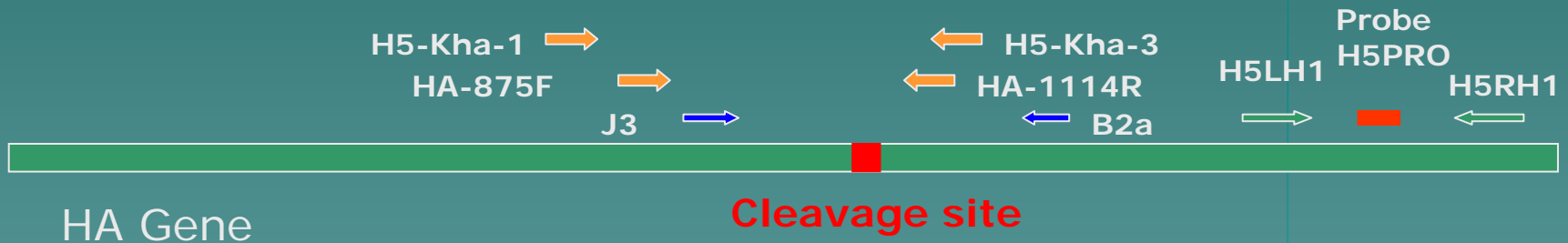


M-124

# Detection of H5 subtypes

## – Real-time RT-PCR

- ☞ Primers H5LH1 / H5RH1 (VLA, Weybridge, UK)
- ☞ Taqman probe H5PRO –5'(FAM ) /.../ (BHQ1)-3'



## – Conventional RT-PCR: primers H5 from VLA, Weybridge, UK

- ☞ HA-875F / HA-1114R
- ☞ J3 / B2a
- ☞ H5-Kha1 / H5-Kha3

Different sensitivity and specificity

Complementary RT-PCR



Sequencing of the amplification product = pathotyping

# Detection of H7 subtypes

## – Real-time RT-PCR

- ☞ Primers LH6H7 / RH4H7 (VLA, Weybridge, UK)
- ☞ Taqman probe H7pro11 –5'(FAM ) /.../ (BHQ1)-3'



## – Conventional RT-PCR: primers H7 from VLA, Weybridge, UK

- ☞ Primer GK 7.3 / GK 7.4

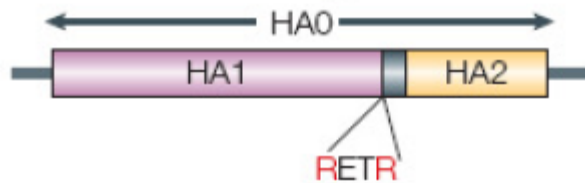
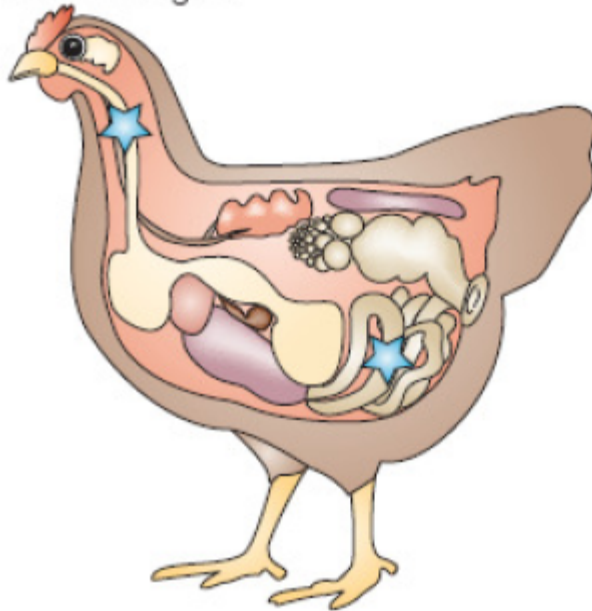


Sequencing of the amplification product = pathotyping

# Hemagglutinin and HPAI

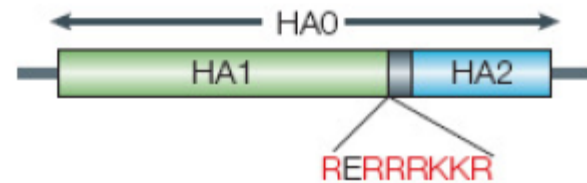
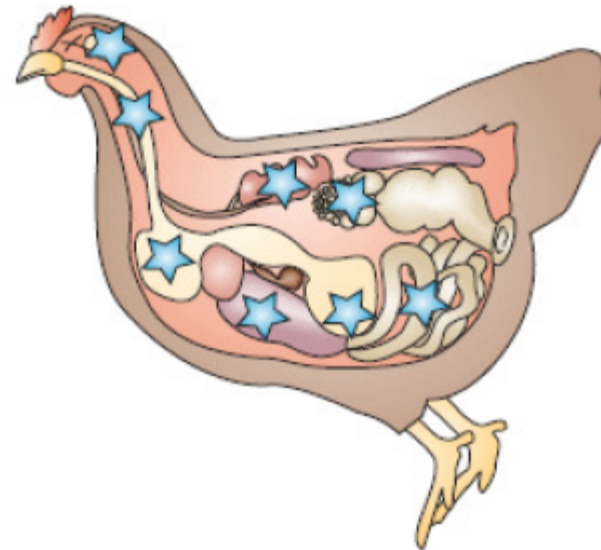
## LPAI

Proteases localized in respiratory and intestinal organs



## HPAI

Ubiquitous proteases



# Isolation in embryonated chicken eggs

- ◆ Samples processed in BSL3
  - Tracheal or cloacal swabs in transport medium
  - Conservation at low temperature : +4°C for few days or -80°C, dry ice, liquid nitrogen
  
- ◆ Inoculation of sample in allantoic cavity of embryo of 9 to 11 days
  
- ◆ Confirmation of isolation after 2 to 7 days of incubation at 37°C
  - Detection of haemagglutinating activity of allantoic fluid
  - Confirmation of the presence of Influenza A virus by RT-PCR







# Caractérisation moléculaire

- Pathotypage :
  - Site de clivage AIV et NDV
- Comparaison de séquences : analyse phylogénétique



# Caractérisation moléculaire

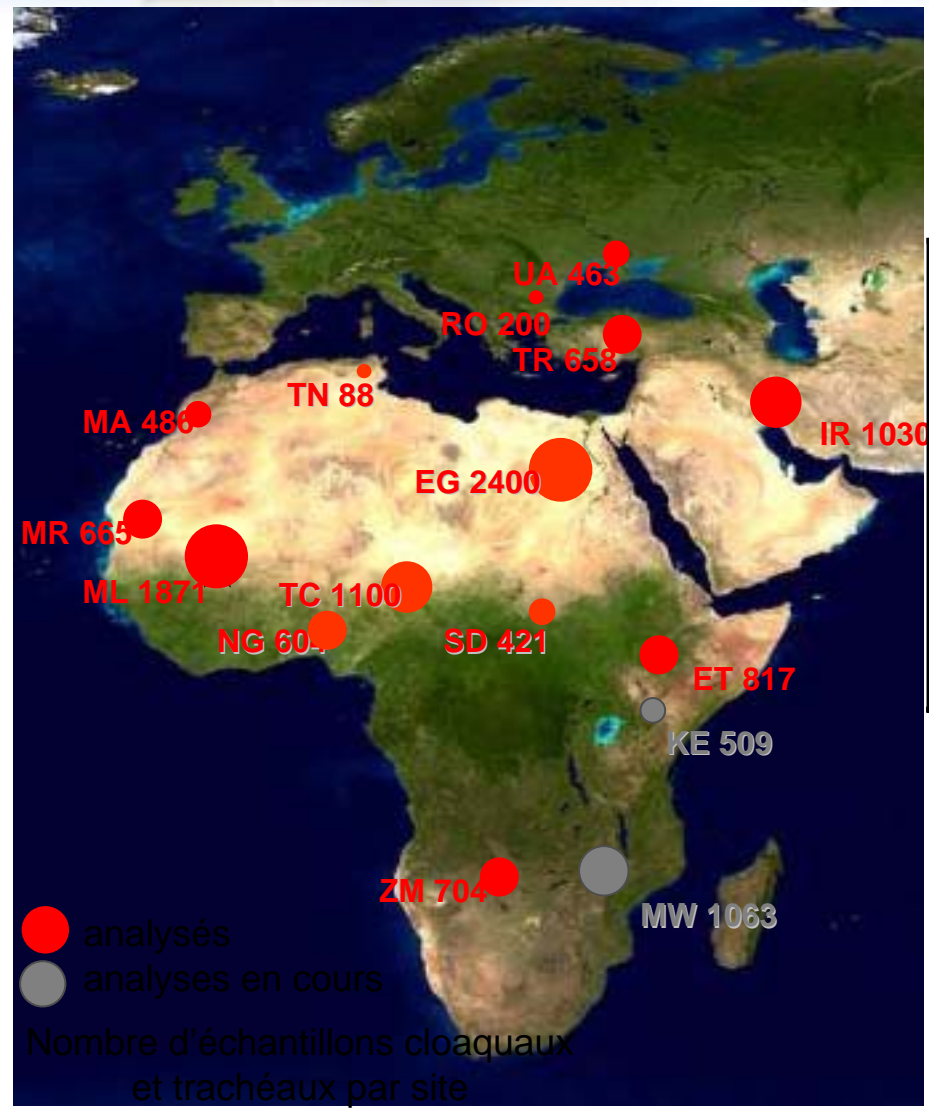
- Pathotypage :
  - Site de clivage AIV et NDV
- Comparaison de séquences : analyse phylogénétique
- Détection des déterminants de virulence et de spécificité d'hôte



# Avian Influenza

## Research in Developing Countries

### Résultats d'enquêtes 2006 & 2007



	N testés	N AIV +	%
N	10,755	236	2.2
prélèvement trachéal N	5,174	106	2.0
cloacal N	4,832	120	2.5
fecal N	849	10	1.2
N	6,516	233	3.6

oiseaux

3.5 % pendant la première campagne