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

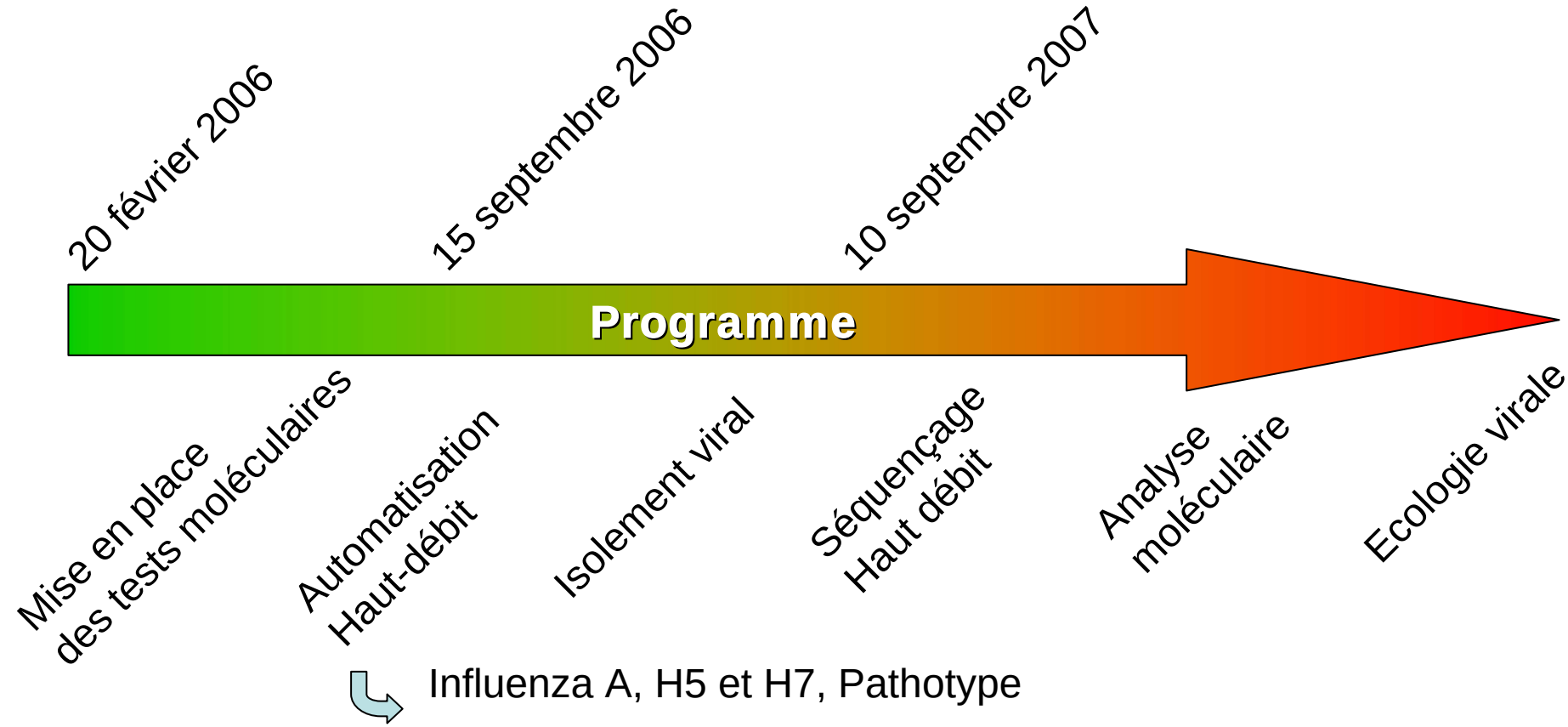
Outils & méthodes

- 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



Avian Influenza

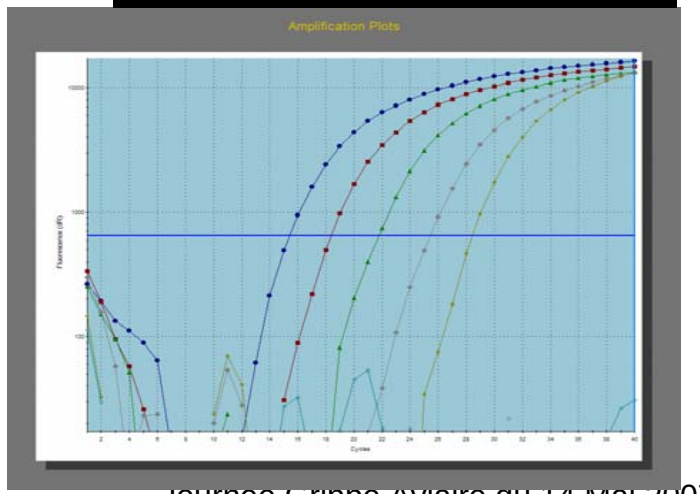
n Developina Countries

wellcome trust

la Région Languedoc Roussillon

Automate

Real-time PCR



View inside CIRAD BSL3

Avian Influenza
Research in Developing Countries

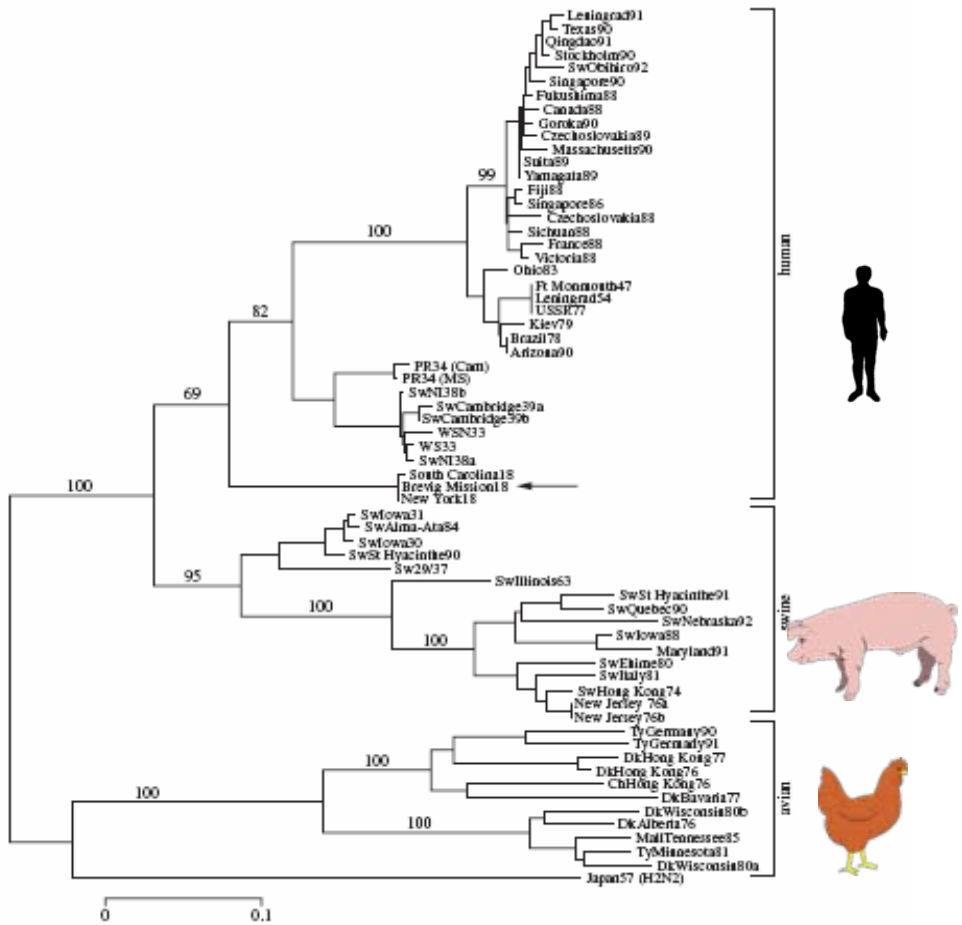
Virologie

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 sapnish influenza virus, *The royal society*, **2001**.



Detection of viral RNA

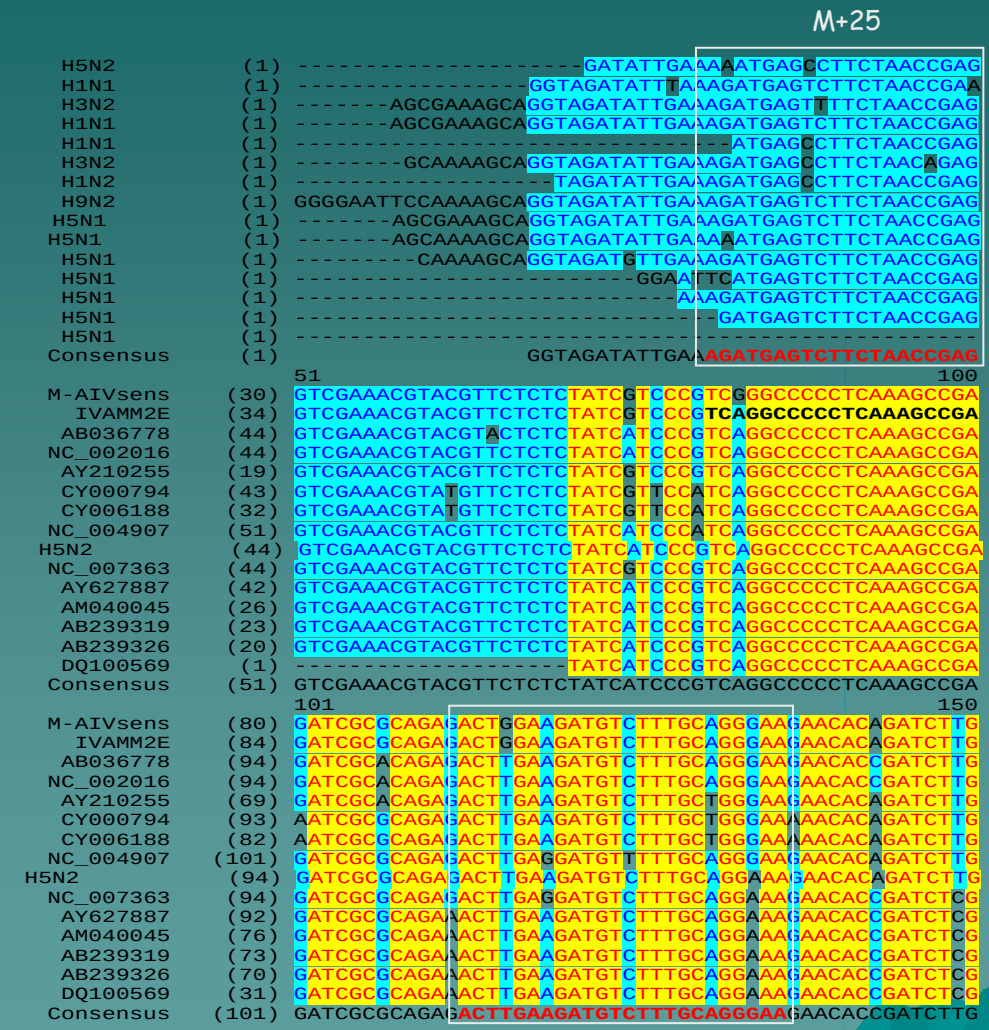
- Automated RNA extraction from samples
- Detection of type A influenza:
  - Matrix (M) gene by real-time RT-PCR
- Detection of H5 or H7 subtypes by real-time RT-PCR on AIV A positive samples
- RT-PCR on the cleavage site of HA for H5 or H7 positive samples
- Sequencing of the amplified cleavage site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

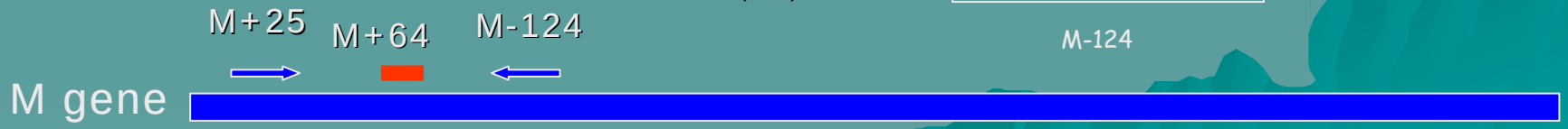
Detection of type A influenza: real-time RT-PCR specific of M gene

Taqman Technology

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

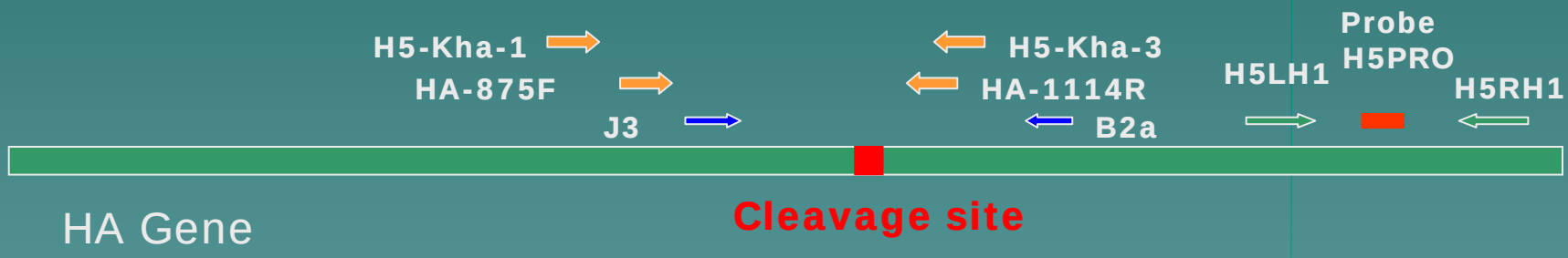
Q-RT-PCR OIE protocole





Detection of H5 subtypes

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



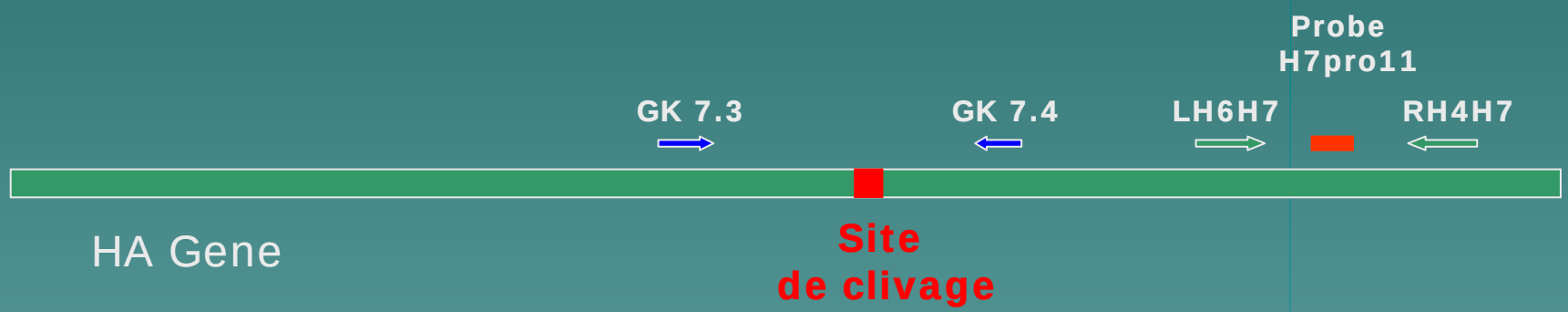
– Conventional RT-PCR: primers H5 from VLA, Weybridge, UK
  - ☞ HA-875F / HA-1114R
  - ☞ J3 / B2a
  - ☞ H5-Kha1 / H5-Kha3

Different sensitivity and specificity → Complementary RT-PCR

Sequencing of the amplification product = pathotyping

Detection of H7 subtypes

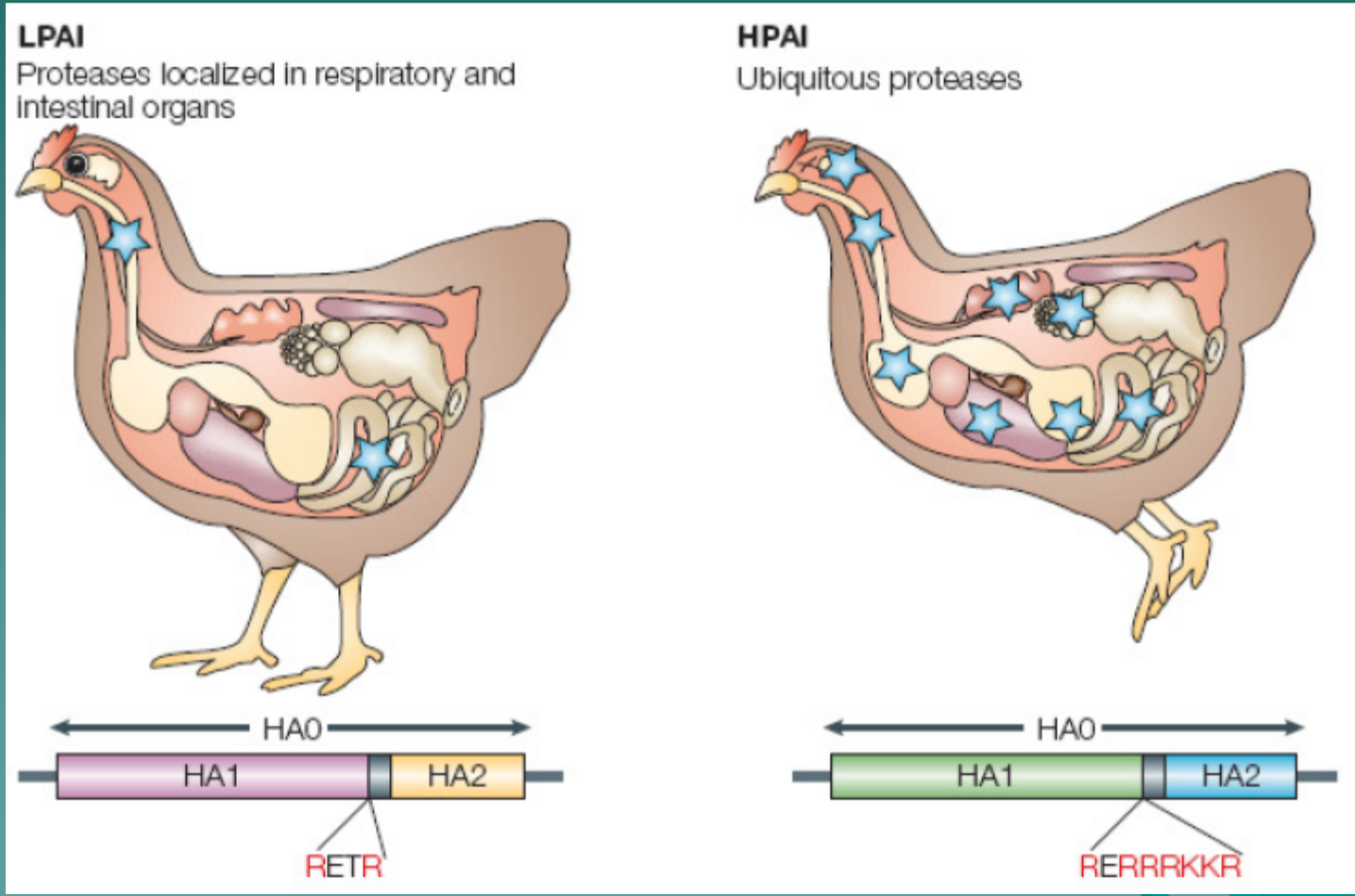
– Real-time RT-PCR
  - ☞ Primers LH6H7 / RH4H7 (VLA, Weybridge, UK)
  - ☞ Taqman probe H7pro11 –5'(FAM) /…/ (BHQ1)-3'



– 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



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

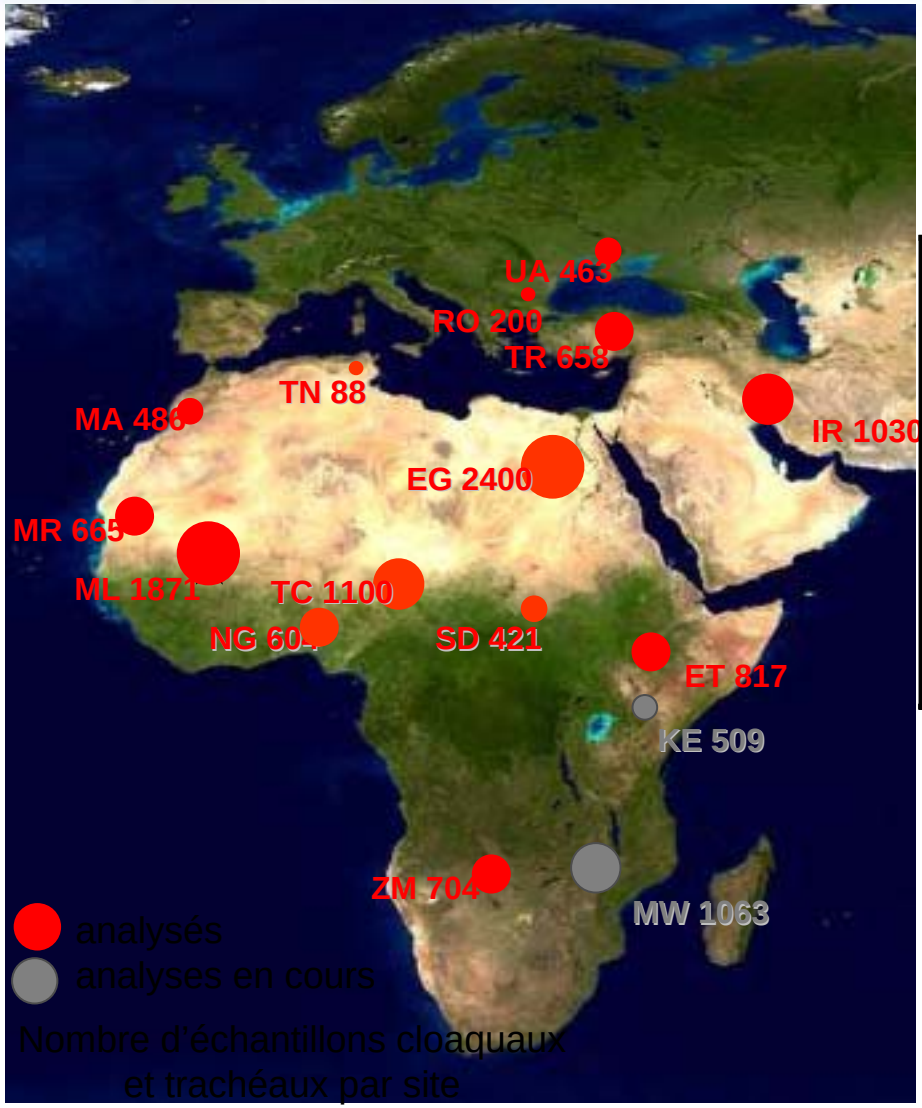
Caractérisation moléculaire

- 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



CIRAD

WETLANDS INTERNATIONAL

Résultats d'enquêtes 2006 & 2007

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

3.5 % pendant la première campagne