

Avian influenza virological approach



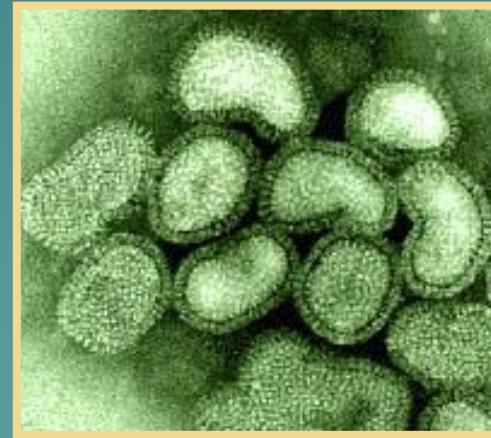
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Outline

- ◆ Influenza virus description
- ◆ Avian influenza diagnostic techniques in use in CIRAD Montpellier
- ◆ Current projet: TCP FAO
 - Surveillance of AIV in wild birds in Africa, Middle-East and in Eastern Europe

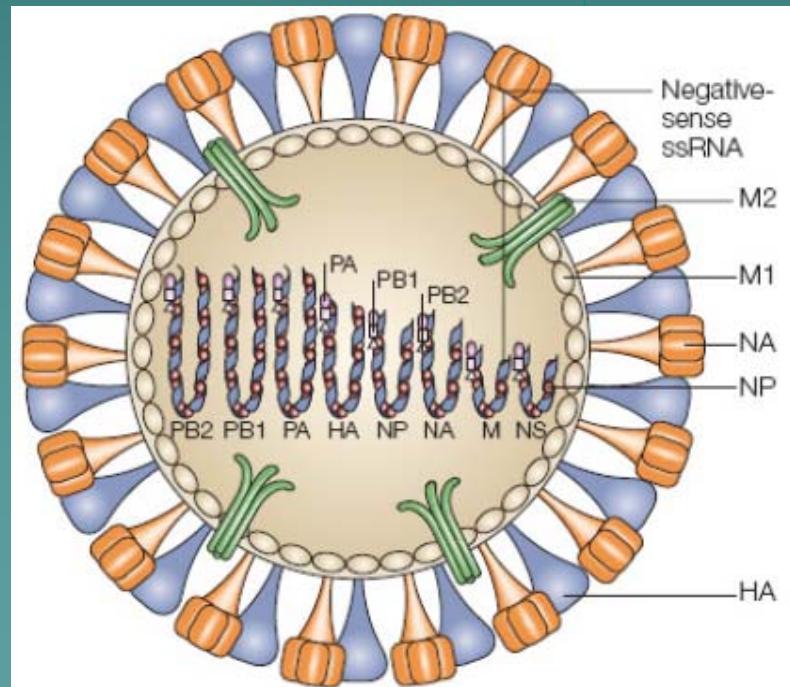


Influenza Virus



Influenza virus

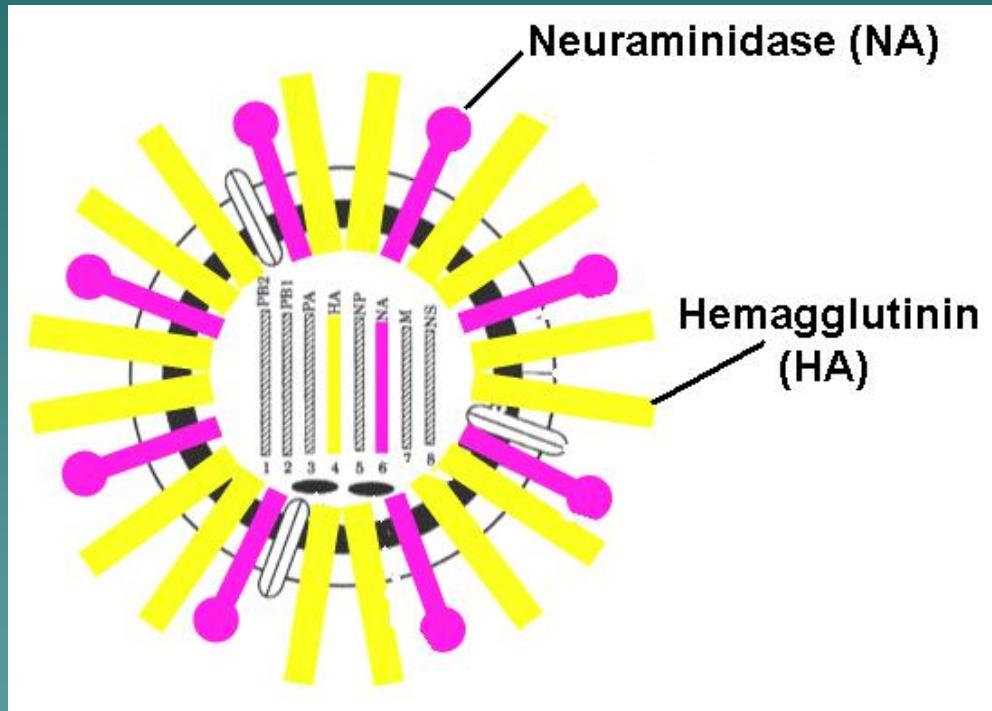
- ◆ Family: Orthomyxoviridae
 - Enveloped
 - 8 segments single stranded (-) sense RNA
- ◆ Three main types
 - Type A
 - ◆ Multi-species
 - Type B
 - ◆ Human
 - Type C
 - ◆ Human and pig



Influenza A Virus

- ◆ Multi-species
 - Mammals (human, pig and horse)
 - Birds
- ◆ The most virulent group
- ◆ Classification in subtypes by surface antigens
 - Hemagglutinin (H or HA) : 16
 - Neuraminidase (N or NA) : 9
 - All HxNy types in waterfowl

Surface antigens and subtypes



Cleaves sialic acids and permits the liberation of viral particles

Attachment site on host cells
(receptor = sialic acid)

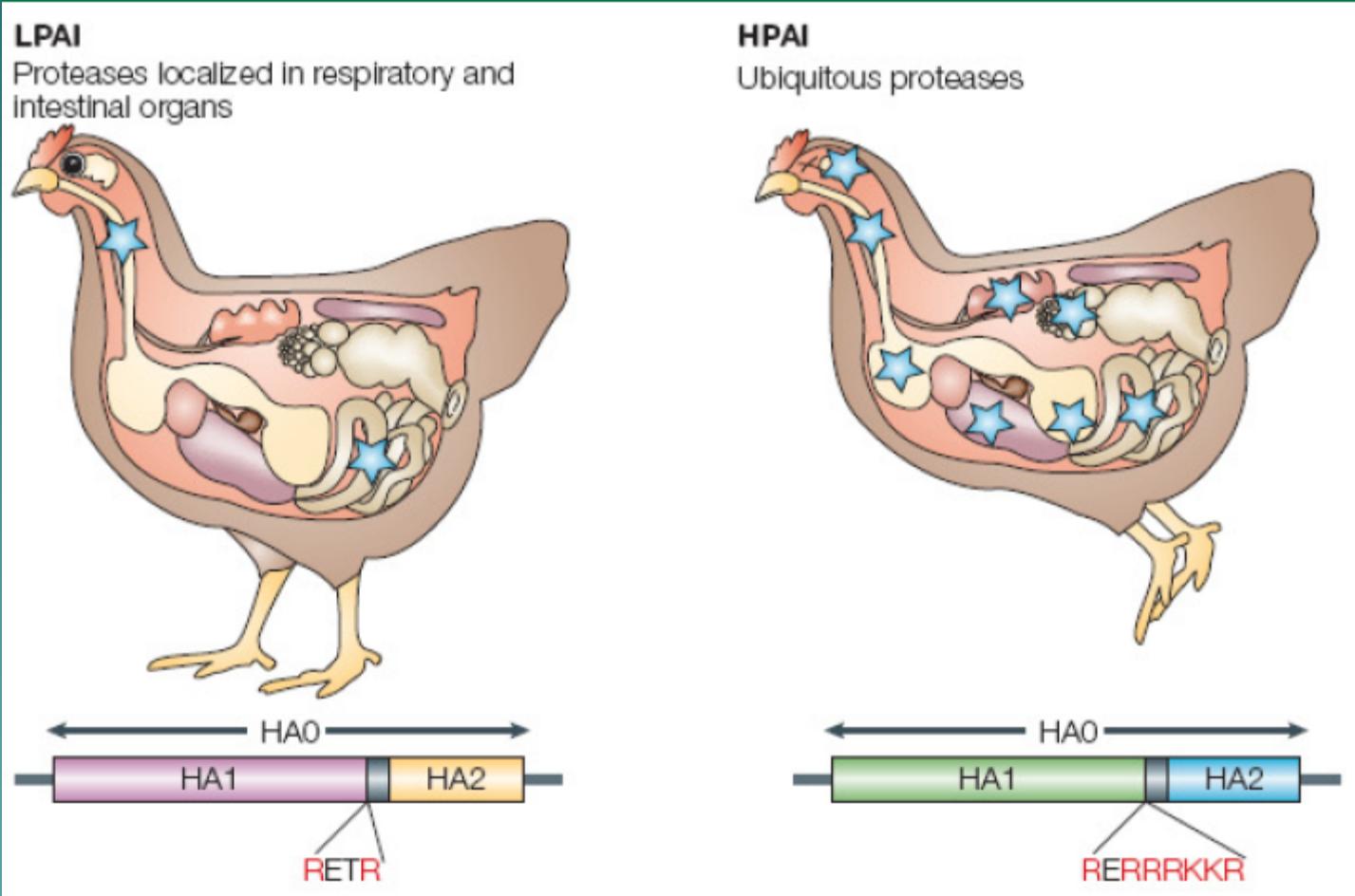
HA subtypes and host distribution

HA subtypes	Primary reservoir		Secondary reservoir		
	wild birds	water birds	birds	mammals	
		chickens	human	pig	horse
H11		+	+		
H13		+	+		
H16	+				
H9		+	+		
H8		+	+		
H12		+			
H6		+	+		
H1		+	+	+	+
H5		+	++		
H2		+	+	+	
H3		+	+	+	+
H4		+	+		
H14	+				
H10		+	+		
H15		+			
H7		++		+	

Avian influenza

- ◆ Pathogenicity based on disease severity on poultry and associated to molecular determinants (unwell known)
 - Low pathogen (LPAI)
 - ◆ Subtypes H1 to H16
 - High pathogen (HPAI)
 - ◆ Some strains of H5 or H7 subtypes (possibly H6 or H9)
 - ◆ Some LPAI strains of H5 or H7 subtypes may mutate in HPAI

Hemagglutinin and HPAI





Avian influenza diagnostic techniques in use in CIRAD Montpellier



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

RNA extraction

- ◆ Samples (processed in BSL3)
 - Tracheal or cloacal swabs in transport medium
 - Organs (dead birds)
 - Conservation at low temperature : +4°C for few days or -80°C, dry ice, liquid nitrogen
- ◆ Automated extraction using silica columns (Macherey Nagel kit)
 - 400 samples in 50 min



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

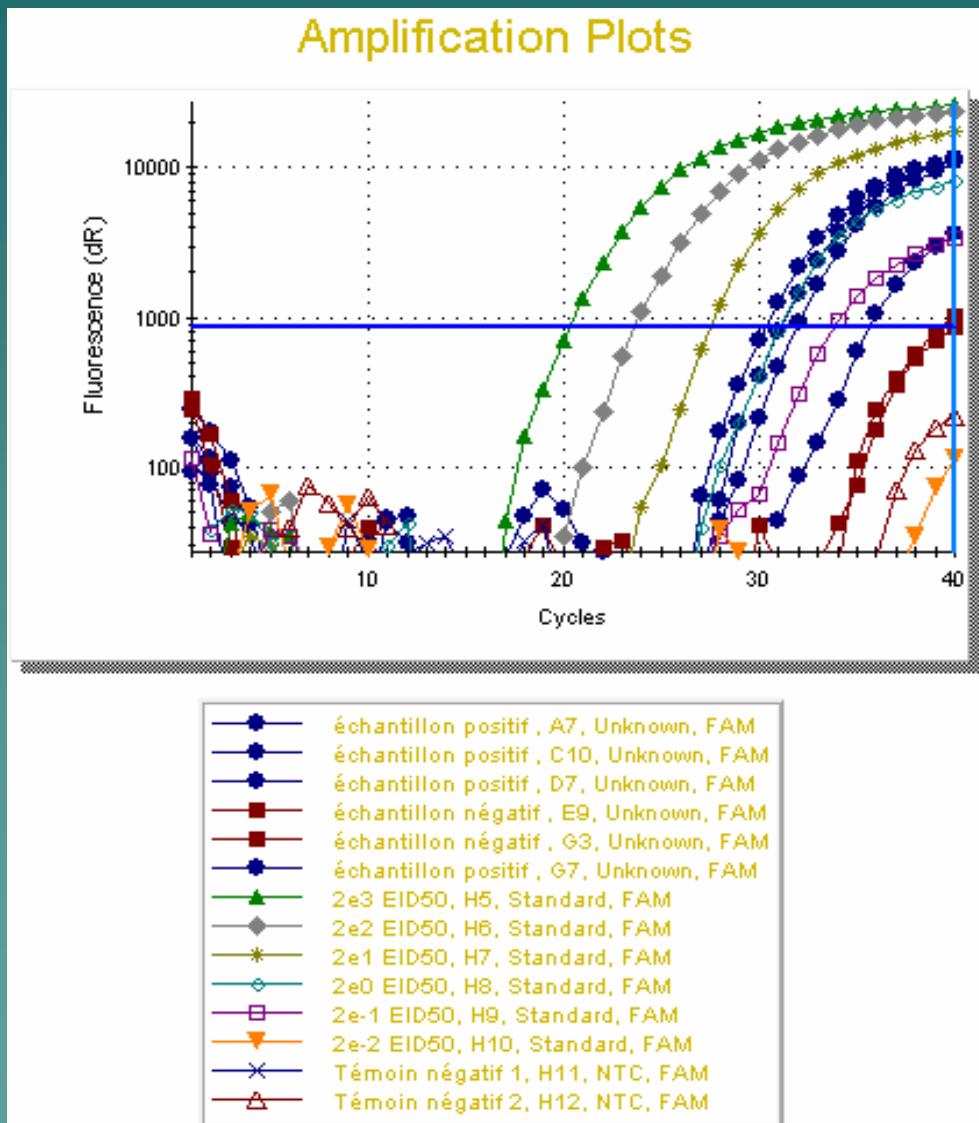
		M+25	M+64	M-124	M-124
H5N2	(1)			GATATTGA	AAATGAG
H1N1	(1)			GGTAGATATTG	AAAGATGAGTC
H3N2	(1)			AGCGAAAGCA	GGTAGATATTG
H1N1	(1)			AGCGAAAGCA	GGTAGATATTG
H1N1	(1)			AGCGAAAGCA	GGTAGATATTG
H3N2	(1)			GCAAAAGCA	GGTAGATATTG
H1N2	(1)			GGGGAAATTCCAAAAGCA	GGTAGATATTG
H9N2	(1)			GGGGAAATTCCAAAAGCA	GGTAGATATTG
H5N1	(1)			GGGGAAATTCCAAAAGCA	GGTAGATATTG
H5N1	(1)			GGGGAAATTCCAAAAGCA	GGTAGATATTG
H5N1	(1)			GGGGAAATTCCAAAAGCA	GGTAGATATTG
H5N1	(1)			GGGGAAATTCCAAAAGCA	GGTAGATATTG
H5N1	(1)			GGGGAAATTCCAAAAGCA	GGTAGATATTG
Consensus	(1)			GGGGAAATTCCAAAAGCA	GGTAGATATTG
		51			100
M-AIVsens	(30)	GTCGAAACGTACGTTCTCTCATCGT	CCGTCG	GGCCCCCTCAAAGCCGA	
IVAMM2E	(34)	GTCGAAACGTACGTTCTCTCATCGT	CCGTCG	GGCCCCCTCAAAGCCGA	
AB036778	(44)	GTCGAAACGTACGTTCTCTCATCGT	ACTCTCATCGT	CCGTCG	GGCCCCCTCAAAGCCGA
NC_002016	(44)	GTCGAAACGTACGTTCTCTCATCGT	ACTCTCATCGT	CCGTCG	GGCCCCCTCAAAGCCGA
AY210255	(19)	GTCGAAACGTACGTTCTCTCATCGT	ACTCTCATCGT	CCGTCG	GGCCCCCTCAAAGCCGA
CY000794	(43)	GTCGAAACGTACGTTCTCTCATCGT	ACTCTCATCGT	CCGTCG	GGCCCCCTCAAAGCCGA
CY006188	(32)	GTCGAAACGTACGTTCTCTCATCGT	ACTCTCATCGT	CCGTCG	GGCCCCCTCAAAGCCGA
NC_004907	(51)	GTCGAAACGTACGTTCTCTCATCGT	ACTCTCATCGT	CCGTCG	GGCCCCCTCAAAGCCGA
H5N2	(44)	GTCGAAACGTACGTTCTCTCATCGT	ACTCTCATCGT	CCGTCG	GGCCCCCTCAAAGCCGA
NC_007363	(44)	GTCGAAACGTACGTTCTCTCATCGT	ACTCTCATCGT	CCGTCG	GGCCCCCTCAAAGCCGA
AY627887	(42)	GTCGAAACGTACGTTCTCTCATCGT	ACTCTCATCGT	CCGTCG	GGCCCCCTCAAAGCCGA
AM040045	(26)	GTCGAAACGTACGTTCTCTCATCGT	ACTCTCATCGT	CCGTCG	GGCCCCCTCAAAGCCGA
AB239319	(23)	GTCGAAACGTACGTTCTCTCATCGT	ACTCTCATCGT	CCGTCG	GGCCCCCTCAAAGCCGA
AB239326	(20)	GTCGAAACGTACGTTCTCTCATCGT	ACTCTCATCGT	CCGTCG	GGCCCCCTCAAAGCCGA
DQ100569	(1)			TATCGATCCGTC	GGCCCCCTCAAAGCCGA
Consensus	(51)	GTCGAAACGTACGTTCTCTCATCGT	ACTCTCATCGT	CCGTCG	GGCCCCCTCAAAGCCGA
		101			150
M-AIVsens	(80)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA
IVAMM2E	(84)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA
AB036778	(94)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA
NC_002016	(94)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA
AY210255	(69)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA
CY000794	(93)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA
CY006188	(82)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA
NC_004907	(101)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA
H5N2	(94)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA
NC_007363	(94)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA
AY627887	(92)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA
AM040045	(76)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA
AB239319	(73)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA
AB239326	(70)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA
DQ100569	(31)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA
Consensus	(101)	ATCGCC	CAGA	ACTGGAAGATGT	TTTGCAGGAA

M gene



Real-time RT-PCR specific of M gene: validation criteria

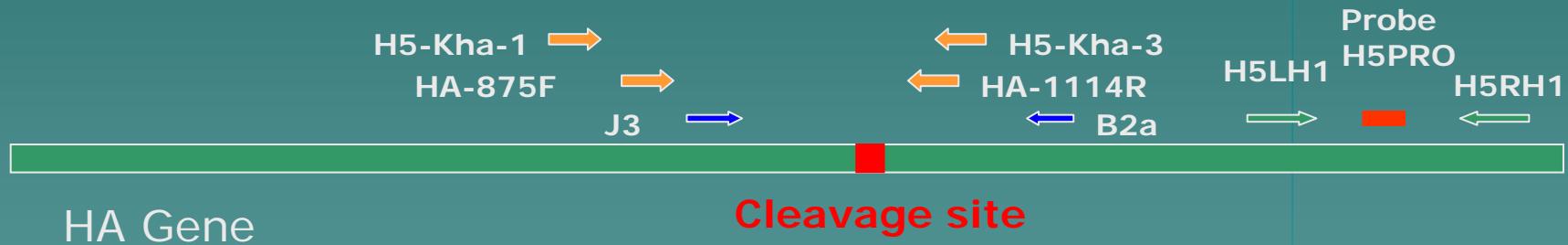
- Dilutions of A/PR/8/34 H1N1 as standard and positive control
- $27 \text{ Ct} < 10^3 \text{ EID50} < 29 \text{ Ct}$
- Positive samples: $< 35 \text{ Ct}$



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

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



Interlaboratory tests

- ◆ Organised by VLA, Weybridge, UK
- ◆ Detection of gene M, H5, H7
- ◆ H5, H7 pathotyping by analysis of the HA cleavage site

Generic AI result (n=9)	H5 consensus result (n=5)	H5 RealTime (n=5)	H5 conventional with pathotyping (n=5)
9/9	5/5	5/5	3/5

H7 consensus result (n=5)	H7 RealTime (n=2)	H7 conventional with pathotyping (n=5)	N1 (n=1)
2/2	2/2	2/2	1/1



TCP FAO : Surveillance of AIV in wild birds in Africa, Middle- East and in Eastern Europe

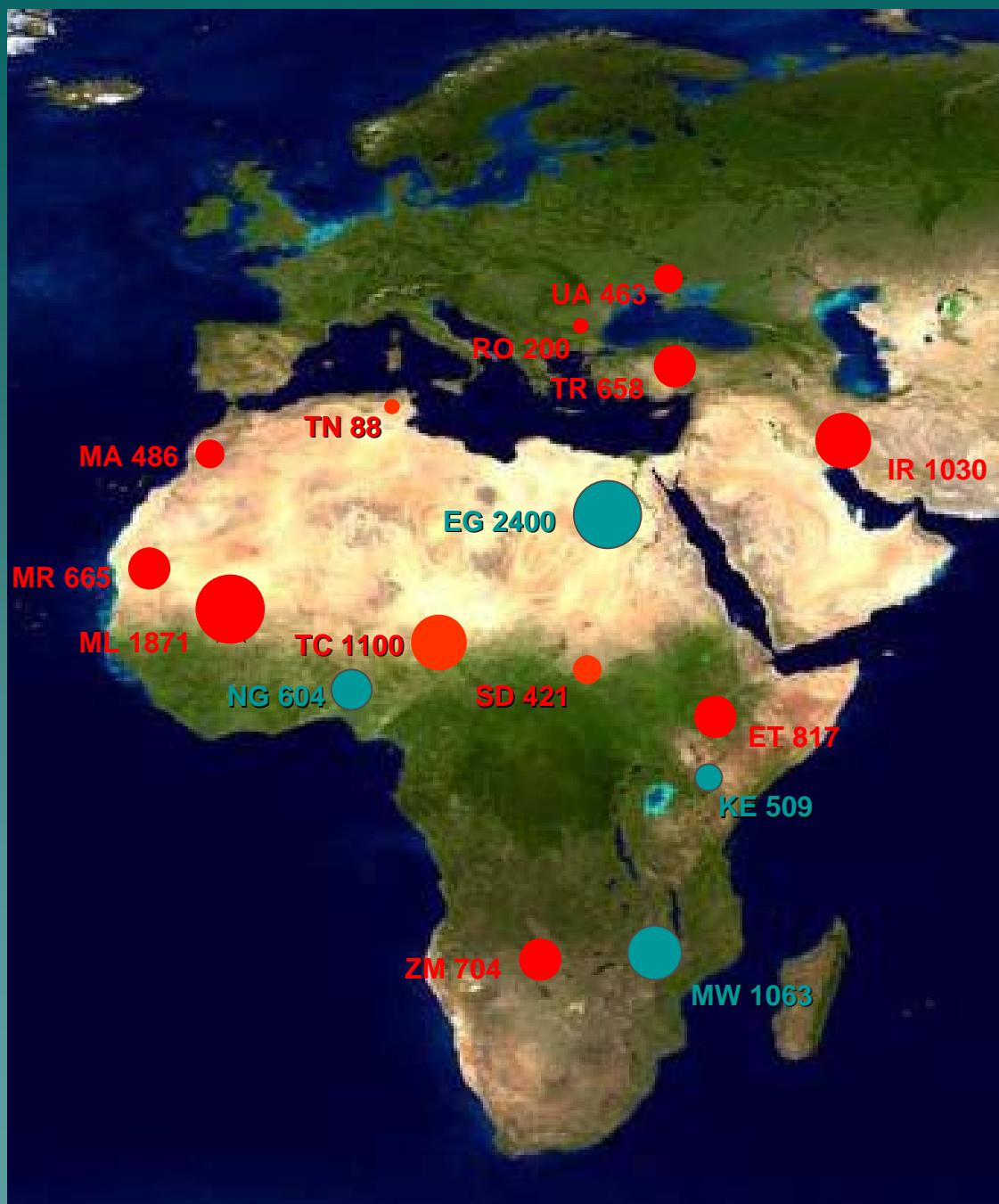


Sampling sites

number of tracheal and cloacal samples collected per site



- analysed samples
- analysis in process



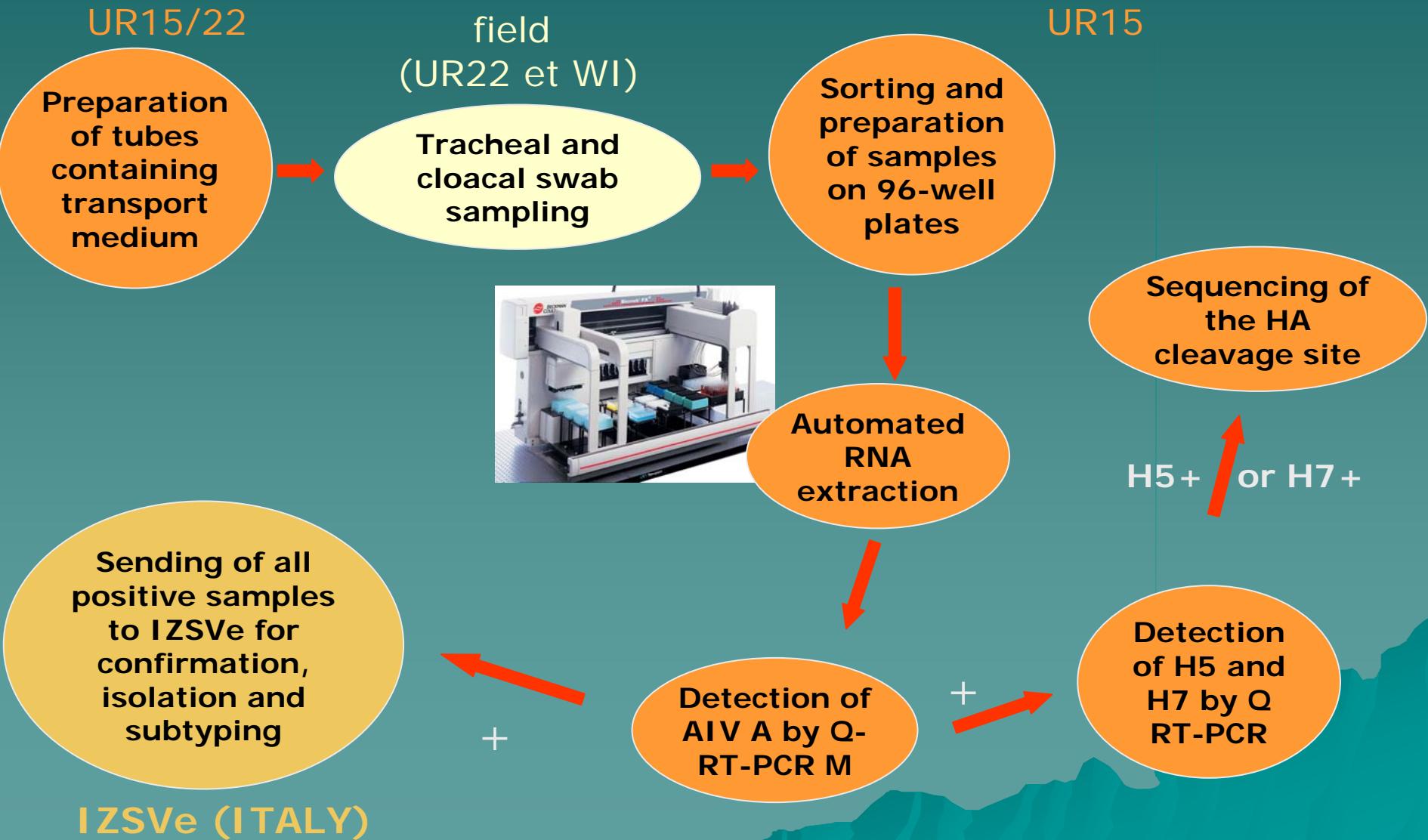
Sampling procedure



Shipment
under dry
ice

-80°C

Surveillance of AIV in wild birds in Africa, Middle-East and in Eastern Europe



Surveillance of AIV in wild birds in Africa, Middle-East and in Eastern Europe

- ◆ Preliminary prevalence of influenza A virus detected by real-time RT-PCR on the M gene: 2.4% among all the samples and 3.8% among the birds
- ◆ In accordance with prevalence during the 1st : 3.5%

Gripavi: virological approach

- ◆ Molecular characterisation of African strains of AIV and NDV (wildlife, poultry and environment)
- ◆ Determination of dissemination profile of the viruses using phylogenetic analyses