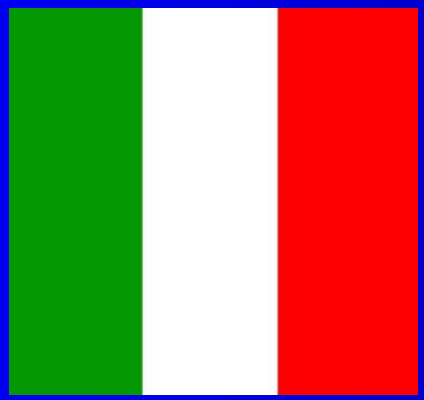
OIE LABORATORY TWINNING ON AVIAN INFLUENZA
2008-2009
IZSVe – FGI ARRIAH



Paris

15 September 2009

Victor N. Irza

Federal Governmental Institution Federal Center for Animal Health (FGI ARRIAH)

National Reference Laboratory for Avian Influenza

- First OIE Twinning project
- Pre-Twinning visit
- Twinning guidelines

LENGTH OF PROJECT: 12 MONTHS

DATE START: 1 st FEBRUARY 2008

DATE END: 31 st JANUARY 2009*

* Postponed to 31 st JULY 2009

MAIN OBJECTIVE:

upgrading of diagnostic capabilities in AI and ND of Canditate

Laboratory to a level comparable to OIE RL standards

PARTICIPANTS

Parent Laboratory:

IZSVe
Istituto Zooprofilattico Sperimentale delle Venezie,
OIE/FAO RL for AI and ND

Candidate Laboratory:

FGI-ARRIAH
Russian Federal Centre for Animal Health
OIE RL for FMD

Pre-Twinning collaboration

- January-February 2007. IZSVe mission with an OIE mandate to FGI ARRIAH to assess capabilities in AI and ND diagnosis and develop an upgrading programme
- March 2007. FGI ARRIAH delegation visited IZSVe and its section in Verona to look in situ how to upgrade
- Pre-Twinning missions – a base for success
- A basis for the plan for the twinning project

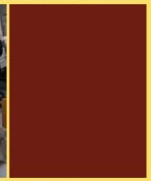
Recommendations and their implementation:

- Necessity to have all reagents, equipment, standard procedures for screening and identification AI and ND viruses.
- Training, network and exchange
- Necessity to upgrade virology unit or to build a new one
- Participation in International ring trials
- Internal controls, standardization, validation
- We made all SOPs for all procedure
- All serologic reactions were harmonized and standartized by printed and officially approved SOP`s
- Necessity to improve the system of sample management

Description of project phases

- 1 st training period: classical virological techniques – 2 FGI-ARRIAH staff members – April –May 2008 (4 weeks)
- In loco mission – September 2008
- 2 nd training period: molecular techniques - 2 FGI-ARRIAH staff members – October 2008 (4 weeks)
- In loco mission – March 2009
- Ring trial

Istituto Zooprofilattico Sperimentale delle Venezie



Welcome to IZSVe!

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Fax +39-049-658685
Web: www.belludi37.it
E-mail: info@belludi37.it

TRANSPORT

RADIO TAXI PADOVA ph. 049-651333

CONTACTS IZSVe (Legnaro)

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35020 - Legnaro (PD)

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Fax: +39 06 494-1031
E-mail: ambrus@ambrussia.it
Web: http://www.ambrussia.it

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address: Via Nomentana, 116, 00187, Roma - Italia
Ph.: +39 06 442-34149, 442-35625
Fax: +39 06 442-34031
Working Hours: Mo-Fr 8.00 – 12.00; 15.00 – 19.00
Visiting Hours: 8.30-11.30

Milan
Consolato Generale della Russia
address: Via Sant'Aquilino, 3, 20148, Milano - Italia
Ph.: +39 02 400-92113, 487-06041, 487-05912
Fax: +39 02 400-90741
E-mail: consul.rus.mi@iol.it, consolato.russo.mi@iol.it
Web: http://users.iol.it/consolato.russo.mi
Working Hours: 8.30 – 13.00; 15.00 – 18.30
Visiting Hours: 9.00 - 12.45

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Consolato Generale della Russia
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Ph.: +39 01 372-6047, 372-6304
Fax: +39 01 374-1361
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EMERGENCY NUMBERS

POLICE 113
AMBULANCE 112
FIRE DEPARTMENT 115

TOURISTIC INFORMATION

Venice Airport
Ph. +39-041-260 6111
Fax +39-041-2606260
Web: www.veniceairport.it

Railways
Ferrovie dello Stato
Web: www.trenitalia.com

Bus
SITABUS
Ph. +39-049-8206811

Important
25th April and 1st May are public holidays in Italy

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Weekly meetings with tutors/staff/participants to evaluate progress of training

Twinning: find a twin at each level

- Exchange of information at each level
- Tutoring: to facilitate this process
- Build cross sectional relations
- Identification of main topics on molecular and classical diagnostic procedures (“short term projects”)

- **PROJECT 1**
- **SCREENING OF FIELD SERA FROM AVIAN SPECIES**
- **DIFFERENT FROM CHICKENS AND TURKEYS**
- **AIM = IDENTIFICATION OF POSITIVE SAMPLES TO AI (NOT H5/H7)**
- **METHODS = ELISA TEST AGAINST THE NP**
- **IF POSITIVE AGID AND HI**

- **PROJECT 2**

- **COMPARISON OF CHEMICAL AND PHYSICAL FACTORS**
- **IN PREVENTING CROSS REACTION IN SERUM SAMPLES**

- **METHODS = USE OF TEMPERATURE, RDE AND**
- **KAOLIN TO ASSESS**
- **THEIR ACTIVITY**

- **PROJECT 3**

- **TO EVALUATE THE STABILITY OF PRE TREATED SERA**
- **BY CHEMICAL AGENTS**

- **METHOD= HI (LOSS OF TITER)**

Virological tecniques

CHARACTERISATION OF ATYPICAL AI STRAINS

PURIFICATION OF AI ISOLATES

EVALUATION OF PATHOGENICITY BY IVPI

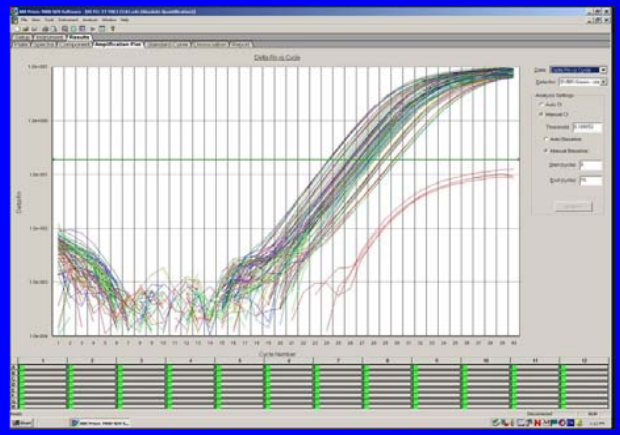
ISOLATION OF AVIAN VIRUSES ON CEL

EVALUATION OF AI INACTIVATION DURING THE NI TEST

Short term projects- Molecular diagnosis

- Extraction/Isolation of viral RNA
  - Protocols
  - Isolates
- Conventional and Real-Time PCR
  - One step procedure
  - Two step procedure
- Sequencing and phylogeny
  - Alignment
  - Comparison

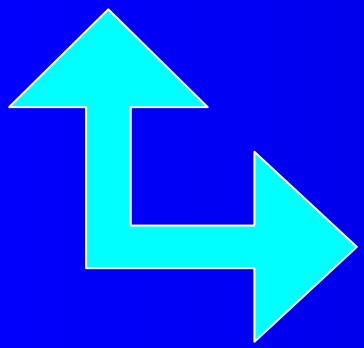

NucleoSpin



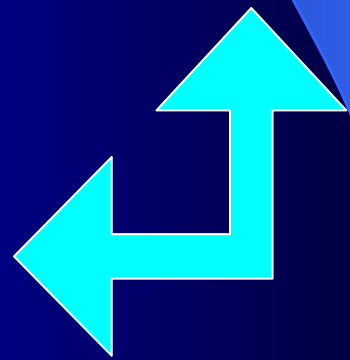
Ring Trial between IZSVe and FGI-Arriah: to harmonize diagnostic procedures

Serology- Virology
- **Virus isolation**
- **Virus characterization**
- **IVPI**

Molecular diagnosis
- **RNA isolation**
- **PCR**
- **Sequencing**



Sensitivity
Specificity
Reproducibility



Teleconference

1 - Second mission to FGI-ARRIAH: aims, personnel involved, report

2 - Ring trials: molecular and classical techniques

3– Virus shipment from FGI-ARRIAH to IZSVe – problems encountered and options for solving

4 - Quality system upgrading at FGI-ARRIAH: IZSVe proposals on how to proceed on that objective

5 - Final workshop

6 – Final report

Activities by “distance sharing…….. “

Shipment of H7 viruses (HP and LP) to FGI –ARRIAH

Shipment of a complete panel of antigens and antisera

Shipment of H5N1 HPAI viruses to IZSVe

Sharing of protocols

Sharing of information on equipments

Sharing of expertise

Dissemination……

Website
Booklet
Generation of the “Tips & Tools” document
Final workshop


ФГУ «ВНИИЗЖ»
IZSVe
TWINNING LABORATORIES
OIE
TWINNING
PROJECT
ON AVIAN INFLUENZA 2008-2009
IZSVe
Istituto Zooprofilattico Sperimentale delle Venezie
ФГУ СИНКО ВНИИЗЖ

In loco missions – to assess the successful transfer of knowledge and skills

- Registration and documentation system
- Procedures
- Protocols: RNA isolation, PCR, gel detection, sequencing
- Quality assurance system
- Procedure validation
- Safety

Virus research unit for AI and NDV

The laboratory consists in four rooms. In the first one, disinfection chamber and lock-chamber are present. In the second room, one freezer (-70C), one refrigerator (+4C) and a centrifuge are situated.

Virus research unit for AI and NDV

- A safety cabinet (BSL 2) for museum AIV and NDV isolates
- A data recording system with a central database and identification of samples based on bar codes

System of registration PBA (PACS Black&Witch, Canada)

Virus research unit for AI and NDV

- There are two other rooms where virus isolation and other virological works are carried out. One is equipped with two biosafety cabinets for post-mortem examinations, samples preparation and eggs inoculation.

Virus research unit for AI and NDV

- Incubator for eggs, water bath, centrifuge for samples clarification, etc.



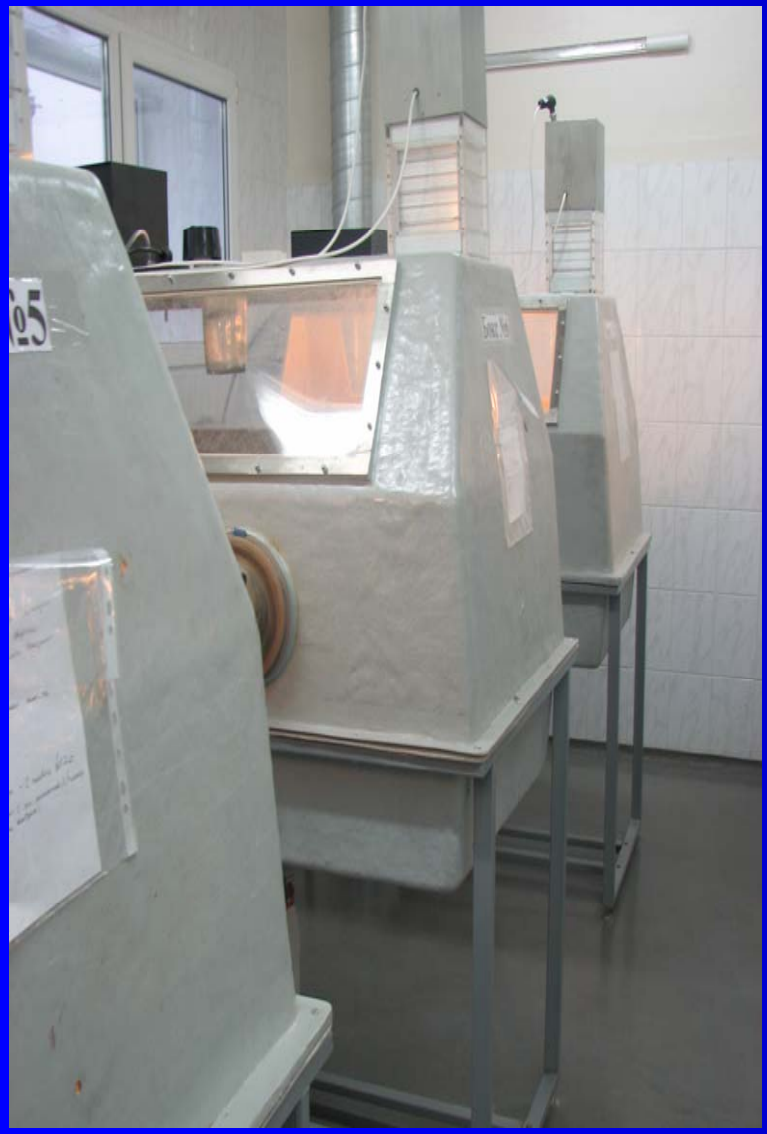

Virus research unit for AI and NDV

- The second virologic room equipped with biosafety cabinet and CO2-incubator for MDCK-cells cultivation, centrifuge and refrigerator.

New animal facility was built

- 2 big rooms, BSL-3 level
- 22 isolators for trials with birds
- 4 isolators for multi purpose goals.
- Modern system of air filtration&canalization
- Up to now, more than 30 challenge experiments had been carried out

Virus research unit for AI and NDV
(animal facilities unit)

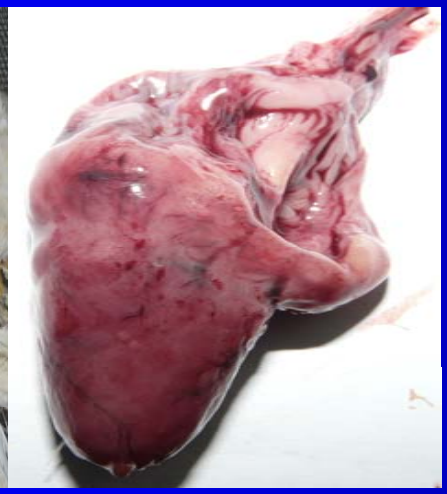


Virus research unit for AI and NDV

AI V and NDV isolates were tested for pathogenicity for susceptible animals (chickens, wild and domestic ducks of different ages)








026
Pa
REGIN
DMD
Differential pressure
transmitter
CE

Virus research unit for AI and NDV

- Written forms (SOP) standardized in accordance to OIE recommendations for all diagnostic procedures

National Reference Laboratory for AI and ND

HI (H5 subtype AIV) and AGID test kits production for a wide use in veterinary practice

Final comment

- **The IZSVe- ARRIAH Twinning project has been a success, and main objectives of the project have been fulfilled**
- **It can be an example and a roadmap for future twinning projects funded by OIE and other international organisations**

National Reference Laboratory – international integration

- Twinning project between Russian NRL and IZSVe (Istituto Zooprofilattico delle Venezie, Italy)
- International Ring Trials for Avian Influenza virus & antibody detection (VLA-Weybridge, UK; IZSVe, Italy). Proficiency tests on serology and PCR have been performed in 2007-2009 with satisfactory results
- Annual EU NRLs meetings
- Integration to Flu lab net, GISAID, OFFLU
- International FAO/IAEA training course for AI diagnostics, ARRIAH, September 2008
- AI monitoring programs in wild birds in RF– a project aimed at sampling in Wrangel Island and joint research in collaboration with US scientists realized in 2008

Avian Influenza related activities of Russian NRL in 2005-2009

4000+ samples submitted to ARRIAH from different regions of Russia and CIS in cases HPAI suspected (poultry, wild & domestic birds) and for monitoring

622 - 2005
1014 - 2006
841 – 2007
1598 – 2008
257 - 2009

- 170 A/H5N1 isolates recovered
- 130 A/H5N1 isolates partially sequenced and phylogenetically compared with isolates from Europe, Asia and Africa deposited in public databases
- The whole genomes of 5 A/H5N1 isolates were sequenced and phylogenetically compared

Diagnosis in domestic & wild birds in 2008 (NRL)

- In total 1570 samples obtained from wild birds (60 species) and 28 samples from poultry have been tested
- Avian Influenza type A virus genome detected in 15 samples:

  -14 – wild birds, 1 - poultry
- Avian Influenza subtype H5 detected in 9 samples from domestic &wild birds
- H5N1 virus was detected in 2 samples: 1 sample from rook (submitted in the end of 2007) and in 1 sample from chicken
- HPAI H5N1 isolation – 1 from chicken (A/chicken/Primorsky/0085/2008)

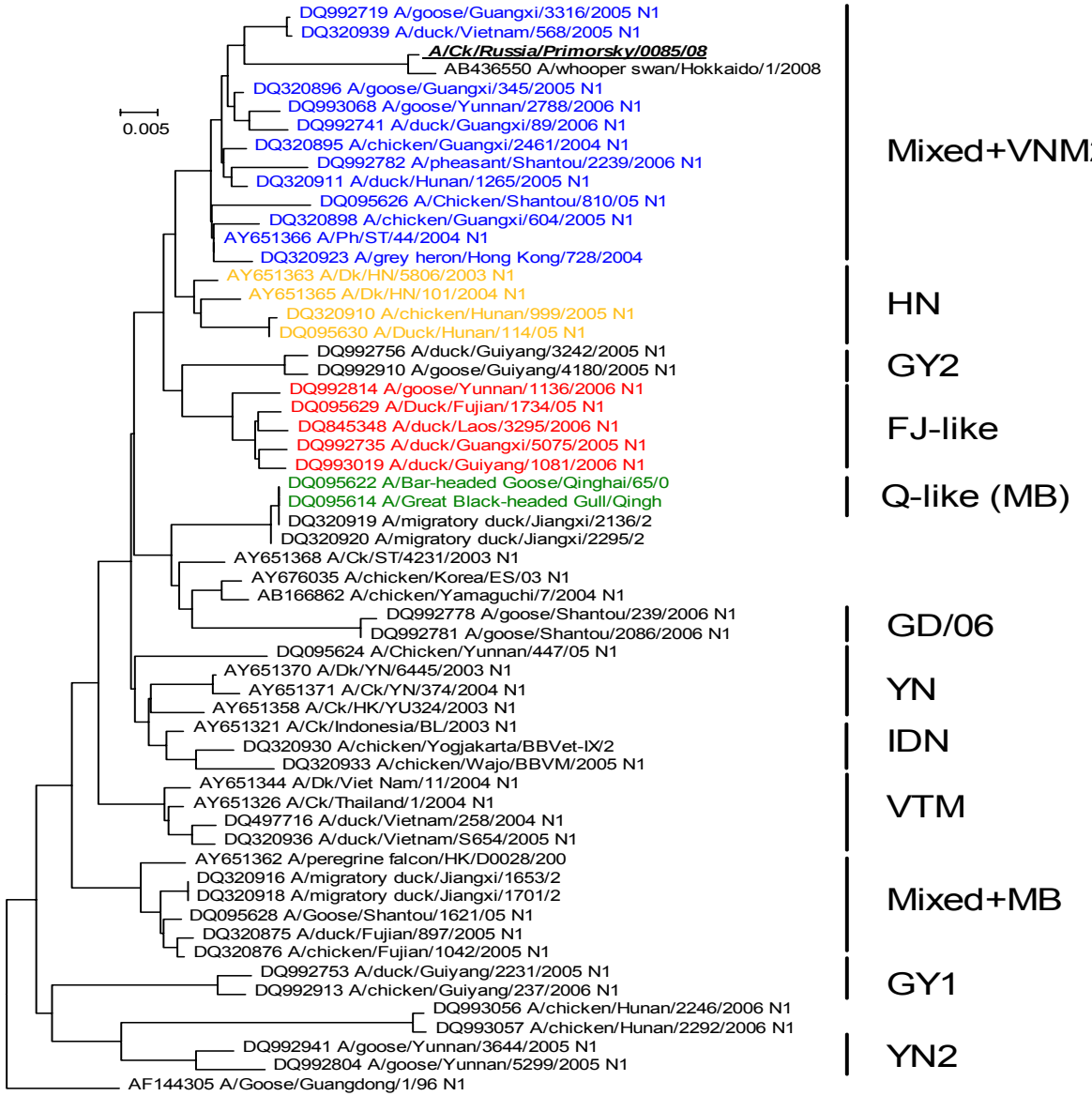
No detection of HPAI H5N1 in wild birds in 2008

HPAI virus 2008

- A/chicken/Primorsky/0085/2008 isolate belonged to the Asian genetic line of HPAI (A/Guandong/1/96 lineage) but differed from avian influenza virus isolates, recovered in the territory of the Russian Federation in 2005–2007
- Dendrograms constructed using NJ method according to identified genome fragments of A/Russia/Primorsky/0085/08 isolate and a number of Qinghai sublineage sequences, demonstrated their differences
- HA-gene - similarity to sublineage Mixed/VNM2
- Other genes – similarity to FJ-like group of isolates including those caused disease in humans (A/Shenzhen/406H/2006, A/human/China/GD02/2006)
- This strain was identical to HPAI virus caused mortality in wild birds in Japan in April 2008 (A/whooper swan/Hokkaido/1/2008 (H5N1) и A/whooper swan/Hokkaido/2/2008 (H5N1), similarity>99%

HA -gene tree constructed on sequences of fragments 77-1072 , NJ method

Colored red - FJ-like, blue -Mixed+VNM2, yellow –HN, green - QH-like



Diagnosis in wild birds in 2009 (NRL)

- In total 257 samples tested in January-August 2009:
- 244 - from wild birds
- 13 - from poultry
- Avian Influenza type A virus genome detected in 20/24 samples from wild birds delivered from Ubsu-Noor lake, Republic of Tyva:

  H5N1 virus was detected in all 20 samples

  HPAI H5N1 isolation – 9/10 from Great crested grebe

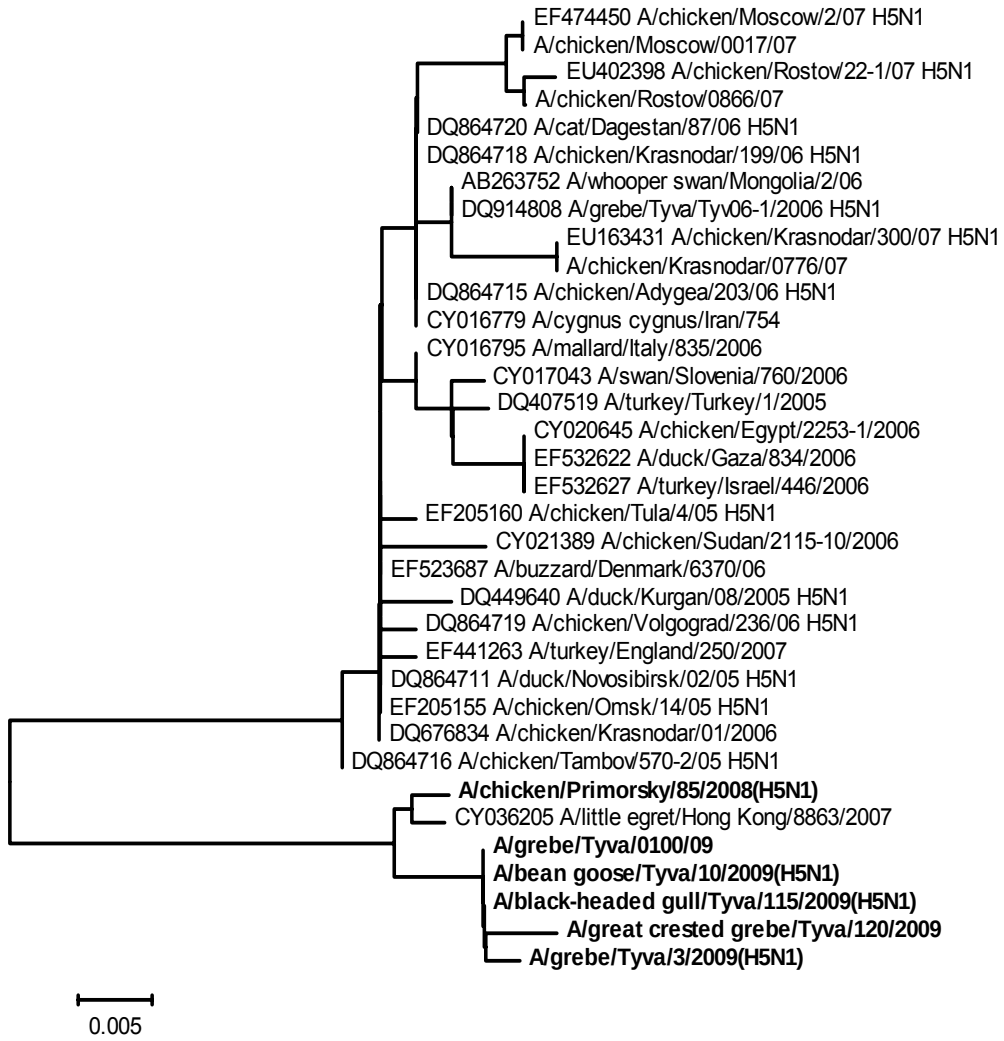
  1/1 from Bean Goose

  1/2 from Black headed gull

A new H5N1 introduction to Russia

June 2009

HA- gene tree of HPAI virus isolate A/grebe/Tyva/0100/09




Thank you for your attention!