International Laboratory Twinning on Avian Influenza 2008-2009
IZSVe – FGI ARRIAHH

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Federal Governmental Institution Federal Center for Animal Health (FGI ARRIAHH)
National Reference Laboratory for Avian Influenza
First OIE Twinning project
Pre-Twinning visit
Twinning guidelines

LENGTH OF PROJECT: 12 MONTHS

DATE START: 1st FEBRUARY 2008

DATE END: 31st JANUARY 2009*
* Postponed to 31st JULY 2009

MAIN OBJECTIVE:
upgrading of diagnostic capabilities in AI and ND of Candidate
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

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

- Characterisation of atypical AI strains
- Purification of AI isolates
- Evaluation of pathogenicity by IVPI
- Isolation of avian viruses on cell culture
- 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
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

What is a twinning?

Twinning projects aim at creating and supporting exchange of knowledge and partnerships, ideas and experience between two parties, namely the OIE Reference Centre and a Candidate laboratory. Twinning project have been chosen by the OIE as a means for upgrading the quality and expertise of a laboratory in the so called developing and in transition countries and are part of the wider OIE initiative to improve the capacity of veterinary services.

The concept

The rapid spread and wide occurrence of animal diseases, such as highly pathogenic avian influenza (HPAI), bluetongue and foot and mouth disease has highlighted the need for a global approach. It is clear that, with the current level of global movements and trade of live animals and animal products, the outbreak of infectious diseases in a certain area could have effects on a global level.

An effective control of infectious diseases depends on the possibility to achieve an early diagnosis, which is only possible if expertise centres and laboratory apply standardised procedures and tests, as the OIE Reference Laboratories. At present the expertise and diagnostic capacity provided by the OIE Reference Centres is located mainly in Europe and developed countries. Therefore, the OIE promotes the extensions of the veterinary services and the network of laboratories to other areas which might lack the expertise and diagnostic capacity.
OIE TWINNING PROJECT
ON AVIAN INFLUENZA 2008-2009
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 (-70°C), one refrigerator (+4°C) 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)
AI V and NDV isolates were tested for pathogenicity for susceptible animals (chickens, wild and domestic ducks of different ages)
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

- 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.
- 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
Thank you for your attention!