“CENSA-IZSVE: OIE TWINNING PROJECT on AVIAN INFLUENZA and NEWCASTLE DISEASE (2008-2010)”

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Background

- **AI: one of the greatest concern for public health and poultry sector in recent times**

- **Impact of AI on poultry sector**
  - 1959-1998: 23 million birds affected
  - 1999-2004: > 200 million birds affected

100 fold increase!!
The **Caribbean region** is considered to be **at risk for AI**: 

1. **Backyard poultry** system widespread

2. Important commercial **poultry industry** present (Trinidad, Barbados, Jamaica)

3. **Surveillance systems** diversely structured in the region

4. Legal-illegal **exchange of animals** is large

5. South America-North America **migratory route**

*(T. Lefrancois et al., 2010)*
Why a Twinning?

- 2007, 2008: LPAI H5N2 (Mexican lineage) outbreaks in Dominican Republic and Haiti respectively

- Need of a centre of expertise for Avian Influenza and Newcastle diseases to assist the Cuban government to combat the threat of HPAI H5N1 and H5N2 infections
OIE TWINNING
PARTICIPANTS

**Parent Laboratory:**

IZSVe  
Istituto Zooprofilattico Sperimentale delle Venezie, OIE/FAO RL for AI and ND  
(18 persons involved)

**Candidate Laboratory:**

CENSA  
National Centre for Animal and Plant Health  
FAO CC for preparedness on animal trans-boundary diseases  
(4 trainees for 4 weeks stages)
GENERAL INFORMATION

LENGTH OF PROJECT: 24 MONTHS

START DATE : 1 September 2008

END DATE : 31 August 2010*
* Postponed to 31 December 2010

MAIN OBJECTIVE:

- Improvement of scientific/ technical expertise for early detection and diagnosis of AI and ND of Candidate Lab
- Laboratory upgrading to a level comparable to OIE RL standards
How to get things get going...

- Clear and adequate objectives!!
- Definition and Organization of activities
- Selection of candidates
- PAT
- Assessment mission

Clear and adequate objectives!!
And how things went on...

Creation of a **Sample bank (American Lineages):**
- 6 AIV isolates (H7, H5) from RL Ames (Iowa-US)
- 1 H3N5 strain isolated in Cuba
- IZSve samples library

1. **Virological and serological diagnostic techniques**
2. **Final workshop**
3. **Ring test**
4. **Molecular diagnostic techniques**
WELCOME at IZSVe!

Induction day

Presentation of IZSVe staff involved in the project (working language English/ Spanish)
MOLECULAR PROCEDURES
Training session 1

Molecular diagnostic techniques

8 June – 7 August 2009

Dr. Heidy Diaz de Arce Landa

Dr. Lester Perez Gonzales

detailed weekly programmes

theoretical sessions

practical sessions

short term project (to let trainees work independently in lab)

evaluation questionnaire
Molecular diagnosis

Theoretical aspects

- Organization of a molecular diagnosis laboratory
- Quality assurance system
- RNA extraction: principles
- Conventional and Real Time PCR procedures (Validation)
- Sequencing procedure
- Phylogenetic trees design and analysis
Molecular diagnosis

Laboratory and sample flow management

• Good laboratory practices

• Sample reception unit: documentation of the samples

• Sample storage

• Sample management during the analytical procedures
Molecular diagnosis: **practical sessions**

**RNA extraction and transcription**

- Preparation of clinical samples
- Isolation of viral RNA (commercial extraction kit instead of TRIZOL-based procedures)
- RT: RNA to cDNA

**Short term project**
Evaluation of efficacy of RNA carrier buffer (Hoffmann *et al.*, 2006) compared to the classical ethanol precipitation

CENSA protocols evaluated according to IZSVe procedures
Objective: to develop, standardize and evaluate “conventional” gel-based RT-PCR for detection and characterization of AI and NDV viruses

Gel detection analysis:
- AIV Gene M detection (Fouchier et al., 2000)
- Protocols for H5 and H7 detection (Slomka et al., 07)

• Agarose, acrilamide
• Purification of the samples

CENSA protocols evaluated according to IZSVe procedures
Objective: **standardize and evaluate real time RT-PCR for detection of targeted viruses**

Real-Time PCR (One step-Two step)
- AIV Gene M detection (Spackman et al., 2002), H5, H7
- NDV gene M detection (Wise et al., 2004)

Short term project
Adaptation and development of Real Time PCR protocol for H5 and H7 detection based on **SybrGreen chemistry instead of Taqman probes**

Validation: sensitivity, specificity (AIV, NDV)

H5 and H7 different lineages (Euroasiatic, American)
Molecular diagnosis

Sequencing procedures

- Protocols, equipments
- Analysis of sequences coding for HA protein
- Molecular epidemiology and phylogenetic data interpretation
VIROLOGY & SEROLOGY
Training session 2
Serological and virological diagnostic techniques
22 November – 19 December 2009

Dr. Carmen Laura Perera
Dr. Abdulahi Alfonso Morales

- detailed weekly programmes
- theoretical sessions
- practical sessions
- short term project
- evaluation questionnaire
IN DETAILS... VIROLOGY

Virus isolation and titration

- Isolation of avian viruses in **embryonated chicken eggs** (AI VIRUSES, AMPV-1)

- Isolation of avian viruses on different **cell culture systems** (embryo fibroblasts, embryo hepatocytes, tracheal rings and MDCK)

- Virus titration by **plaque assay**.
IN DETAILS... VIROLOGY

Pathogenicity evaluation:

- In vivo pathogenicity tests (IVPI) carried out for H5N1 HPAI (A/cygnos olor/Italy/742/2006)

- ICPI for 2 NDV strains
IN DETAIL ....... SEROLOGY

- Pre-treatment of avian sera collected from species different from chicken
- Pre-treatment of mammalian sera (pigs, horses)
- Preparations of RBCs
- HI test, Neuroaminidase Inhibition test

Short term project: To armonize the NI macro assay and to set up the micro methods
IN DETAIL.......SEROLOGY

DIVA strategy concepts

Differentiating infected from vaccinated animals strategy

METHOD = indirect Immunofluorescent antibody test (iIFA) Test for the detection of anti N1 antibodies
Production of reference reagents

- Production of NDV antigen
- Procedures on production of hyperimmune sera
- Evaluation of cross reactivity of hyperimmune sera
RING TRIAL
Ring trial: to harmonize diagnostic procedures

Ring Trial between IZSVe and CENSA (MARCH 2010)

Serology (HI)
Results: 8/8 (100%) of sera samples correctly detected (small differences in HI titers)

Molecular diagnosis (Real Time PCR)
Results: 8/8 (100%) of the viral antigens correctly detected

Sensitivity
Specificity
Reproducibility
Dissemination...

Web page
Dissemination

Posters

EU Media briefing

OIE Twinning Project 2009
for Avian Influenza
Legnaro (Italy) – San José de las Lajas (La Habana, Cuba)

Twinning - an integral concept to capacity building for the improvement of veterinary services.
Twinning between the laboratories of OIE (OIE/FAO and National Reference Laboratory for Avian Influenza and Newcastle Disease) in Italy and CIENIA - Cuba is to strengthen and share expertise and experience on Avian Influenza managing and control.

OIE Proyecto de Hermanamiento 2009
Por la Influenza Aviar
Legnaro (Padova, Italy) – San José de las Lajas (La Habana, Cuba)

Hermanamiento - un concepto esencial para la construcción de capacidades y la mejora de los servicios veterinarios.

Hermanamiento entre las laboratorios de OIE - Italia y ciencia de referencia CIENIA, Cuba y Nacional por la Influenza Aviar y la Entomopatía del Nancay (CIENIA) - Cuba para consolidar y compartir conocimientos y experiencia en el manejo y control de la influenza aviar.

OIE laboratory twinning projects
OIE/TFAO and National Reference Laboratory for Avian Influenza and Newcastle Disease, OIE and National Collaborating Centre for Newcastle Disease and the Humen Animal Health Institute Zooprophylaxis (Sparvettoria della Venezia, Legnaro, Italy

BACKGROUND
Twinning projects are a fundamental part of OIE’s strategy to foster the professional development of veterinary services and share experiences and expertise between OIE Reference Laboratories and collaborating countries. Twinning projects provide support to OIE Reference Laboratories in all areas of animal health and veterinary services. They are designed to improve the quality and level of expertise of laboratories in scientific, teaching, and to improve the OIE Referral Network (CN), as laboratories are part of the wider OIE vision to improve the capacities of veterinary services.

ACTIVITIES
The Twinning projects in this area focus on methodological issues, as well as to foster the exchange of experiences and expertise. These projects are conducted under the OIE’s Twinning projects to build on existing laboratories. Earning Twinning projects, the OIE Twinning projects aim to establish a close working relationship. During the project period, the lab of the laboratory provides the necessary services and support to the collaborating laboratory to maintain good practices.

Acknowledgements
OIE
The IZSVe – CENSA Twinning project has been a success, and the main objectives of the project have been fulfilled.

Importance of established contacts and relationship!!

Now: define together the way forward !!!!!
Thank you

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