Kick-Off meeting
FP6 RIVERS Project
February 12-13, 2007 - Institut Pasteur - Paris

25 rue du Docteur Roux
Metchnikoff Building

Meeting minutes

The meeting started on Monday 12th of February at the scheduled time and lasted until the next day 12h30. It was held at Institut Pasteur, Paris, France.

Monday 12 of February: Meeting room 2nd Floor, Metchnikoff building

Jean-Claude Manuguerra, the RIVERS project coordinator welcomed all participants. All partners were represented by at least one collaborator- see the list of participants. He then presented the agenda outline and explained the goals of the meeting, which were:

1/ to kick off the project
2/ to allow personal introduction between attendees
3/ to give an overview of the project; its content and its management and discuss organisational and scientific topics.

9h45 - 10h30 Presentation of the participants

Each participant had 15 minutes to introduce him/herself and to present his/her institution.

- 9h45 - 10h00 Partner 1 - IPP Jean-Claude Manuguerra (presentation available on the RIVERS web site)
- 10h00 - 10h15 Partner 2 – IC Emilia Lupulescu (presentation available on the RIVERS web site)

Dr Lupulescu described her institute and their involvement during the 2 wave outbreaks in Romania between November 2005 and June 2006. Besides animal cases of HPAI H5N1, 35 suspected human cases were virologically investigated. None of them were found positive for influenza A(H5N1) virus.
The Institute is involved in:

- European Influenza Surveillance Scheme (EISS) and the WHO flunet network
- DG SANCO funded project FluSecure: "Combatting influenza in a combined action between the industry and the public sector to secure adequate and fast intervention in Europe"

- 10h15 - 10h30 Partner 3 – MICB Angel S. Galabov

The Stefan Angelov Institute was briefly presented. Pr Galabov explained the role of his institute and how it is articulated with its veterinary partners. The latter, as National Influenza Centre for Avain Influenza (AI), were involved in the Bulagrian outbreaks of HPAI H5N1. Unlike their human counterparts, the veterinary blaboratory has a BSL3 facility. It also has the ability to perform qRT-PCR.

- 11h00 -11h15 Partner 4 - IPC Philippe Buchy (presentation available on the RIVERS web site)

IPC has been working on highly pathogenic avian influenza (HPAI) H5N1 for quite a while especially within the RESPARI project (Panasian and oceanian IP subnetwork focused on the laboratory diagnostic of infectious respiratry diseases). They have been doing so in close

partnership with the veterinary services. IPC is involved in a number of other research projects such as Dengframe (on Dengue).
IPC has already started to collect environmental samples: 30 samples (water, soil .....) were found positive for avian influenza virus (AIV) H5N1. Fresh water snails were also tested but all were negative. Fresh water mussels are to be tesed (results pending).

- 11h15 -11h30 Partner 5 – IPS Vincent Deubel (presentation available on the RIVERS web site)

After a broad presentation of the IPS, Dr Deubel informed the group that IPS is a contributor to the EU funded project entitled Flu Innate.

- 11h30 - 11h45 Partner 6 – CIRAD Flavie Goutard (presentation available on the RIVERS web site)

The CIRAD is involved in the following projects:

- EDEN and Rex Epizone
- a project submitted to the Welcome Trust on AI
- EcoFlu (French National Research Agency) with and IRD Research Unit located in Thailand and CNRS
- TCP FAO
- FSP GripAvi (with AFSSA and INRA)

Maurtitania, Mali, Nigeria and Tchad wetlands sites where the above projects are in progress will participate to RIVERS.
They already have a standardised RT-PCR based on the detection of the M gene, which was tested in a European interlaboratory QC.
In total, to date, 5200 samples were collected from wild birds: all tested negative for H5N1 AIV.

- 11h45 - 12h00 Partner 7 – IPL Nathalie Deboosere (presentation available on the RIVERS web site)
- 12h00 - 12h15 Partner 8 – ICM Jan Radomski (presentation available on the RIVERS web site)

Dr Radomski preseted its institution and his team. They are involved in the following projects:

- CrossGrid
- EuroGrid
- AIN
- EGEE
- Cost717
- GRIP
- ChemoMentum

- 12h15 - 12h30 Partner 9 – WIV Ze Chen (presentation available on the RIVERS web site)

So far, they collected about 6000 samples from domestic and wild birds. They have H5N1 isolates from chicken and white swans. They also conducted serological surveys in humans. They collected serum specimens from common urban populations in Wuhan City, Hubei Province and Taiyuan City, Shanxi Province. The H5N1 antibody positive rates in two groups of common urban population were 1.25% and 0.94%, respectively" to replace "They also conducted serological surveys in humans and found that 2.25% were seropositive for AIV H5N1.

14h00 – 15h00 : The Rivers Project - Jean-Claude Manuguerra (presentation available on the RIVERS web site)

15h00 – 15h45 Organization of the network / Internal and external reports - Financial Plan - Jean-Pierre Broyart/ Bureau Europe (presentation soon available on the RIVERS web site)

Dr Broyart gave a complete presentation of many organisational aspects of the project, in relation with the EU relevant offices, in particular regarding financial issues. Dr Radomski insisted to receive a letter from the coordination stipulating that he can spend his EU money for personal, equipment and consumables.

15h45 - 16h00 Biosafety issues, exchange and availability of biological material - Alain Barnier et Jean-Claude Manuguerra

Biosafety issues were briefly discussed and the Stefan Angelov Institute not having a BSL3 facility is particularly interested by the results to be yielded by WP0 and Dr Galabov insisted on the importance of determining a BSL2 "bio-equivalent strain" that should be distributed to all partners to ensure comparability of data across the project. It has been decided that the WHO Biosafety Guidelines would be made available on the RIVERS website as a pdf file.
A round table allowed the group to list the H5N1 AIV strains existing in the laboratories within RIVERS.

- IPC: Have some human and animal H5N1 AIV strains; they are all clade 1
- IC works with the vaccine strain NIBRG-14, which has been engineered by reverse genetics from the A/Vietnam/1194/2004(H5N1) strain. It belongs to the clade 1. IC has asked their veterinary counterparts to obtain some clade 2 animal strains isolated during the outbreaks in Romania.
- MICB has 4 clade 2 animal strains.
- WIV has some avian H5N1 isolates
- CIRAD has no H5N1 strain except A/Scotland/1/59(H5N1)
- IPL and IPP have one clade 1 strain obtained from IPC indirectly or directly respectively.
- IPS has worked on engineering H5N1 viral pseudoparticles with different clade 2 H5. These pseudoparticles could be used in RIVERS.

16h30– 17h30 Discussion of workprogramme (WPG) 1 "Survival of avian influenza viruses in waters and aquatic biological systems" - Introduction by WP leaders and discussion with participants

Nathalie Deboosère (IPL) agreed to send a questionnaire to ask everybody about their molecular detection assays (protocols, standards etc.....). A selection of these will be compared by IPL and the best results should allow the group to choose one single protocol for the whole RIVERS project (**WP1**).
The discussion deviated towards **WP2** and **WP3** and mainly dealt with sampling issues in relation to WP1. The need of concentrating water from 100 L (a bathtub content) to 1 L in the case of low viral load waters should be answered but it is not clear at this point of time through what steps and what kind of techniques (immunochromatography .....).
Flavie Goutard (CIRAD) asked how to transport water and under which volume, especially from foreign remote countries (in Africa for example). She also asked how to collect and recognise molluscs. It has been envisaged to record relevant data when collecting waters: e.g. bird (wild and domestic) density on the pond, proximity or not of farms, GPS coordinates, weather conditions (air temperature, hygrometry, light .....) and pictures of the water. CIRAD operates on 5 main geographical spots. Dr Radomski stated that satellites or weather stations respective resolutions power might not be sufficient to account for micro phenomena.
Jan Radomski explained that gathering data through the web will force us to collect and enter critical data we might forget otherwise. A web based database could also help detect aberrant data keyed in by error.

Tuesday 13 of February : Meeting room 4th Floor, Metchnikoff building

9h15 – 10h15 Discussion of WPG3 "Modelling and recommendations" - Introduction by WP leader and discussion with participants

Jan Radomski presented a model of diffusion of influenza in humans in Thailand. He presented 3 papers: 1/ data driven based modelling. Problems of scale, the need to bridge different levels (molecular, genomic, individual hosts, populations (hosts and viruses), global) were mentioned. Dr Radomski told the group that they are very few kinds of influenza modelling approaches.
2/ modular, multilayered systems: Fergusson 2003 Nature paper (Vol 433, p 422:428). Different strains might have their own characteristics. For example in Thailand vs. in the USA. In Canada, a northern Inuit village during Spanish influenza vs. the rest of the country.....

Various definitions regarding models and computing were discussed such as virus (characteristics: incubation period ....)

Globally, WPG 3 consists in a multiscale, agent-based simulation models of possible determinants for AIV stability, perpetuation etc...

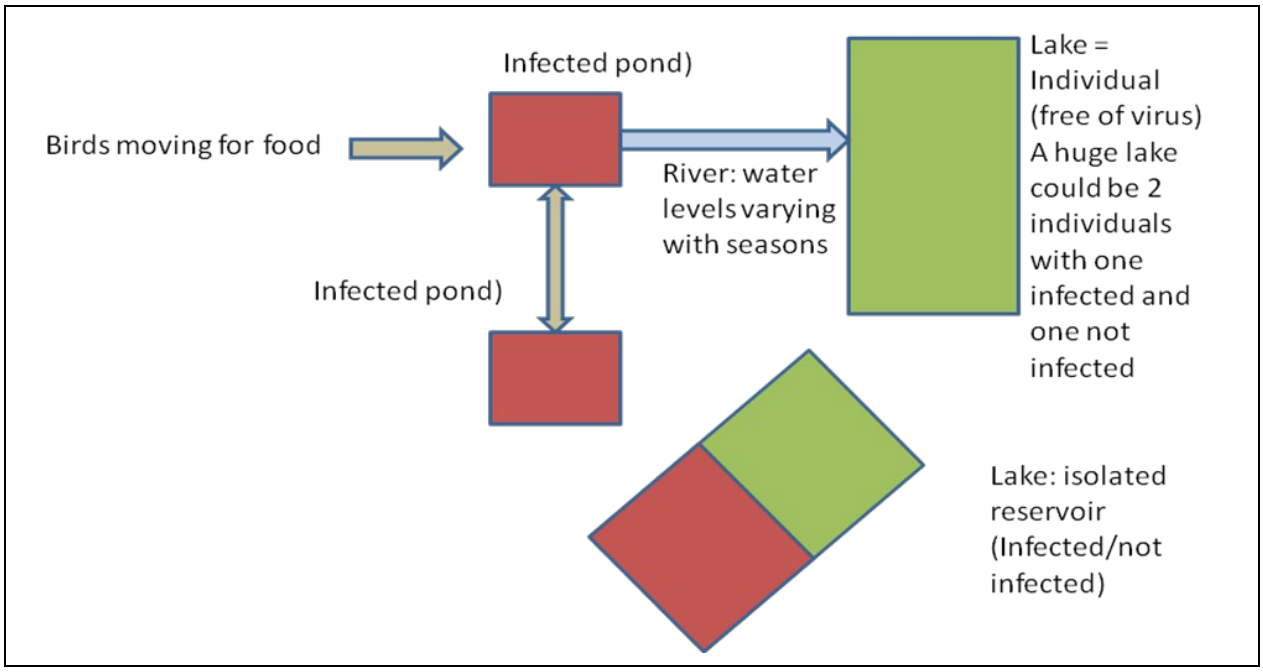
It could be divided into water, air and dry state in both natural and laboratory controlled environments.

The genetic/antigenic differences between clade 1 and clade 2 should be taken into account.

The question of what data to collect to get an "aquatic picture" of AIV ecology came back again, in particular in the North. Pr Ze told us that WIV has the possibility to access Qi Hao lake at some of its points (to be decided) but not very often (maybe once or twice during the project).

Control site(s) were also discussed in the North and in Africa during summer time and winter time. Remote sensing (eg vegetation index) could provide with useful tools. The sites where CIRAD currently operates do not seem to be contaminated by H5N1 AIVs. Others sites along the Danube river or in Cambodia can be contaminated at one time and not at another. It was agreed that **the first teleconference should decide on points of sampling: in Cambodia, China (Wuhan area and Qi Hao lake), Danube countries, France and Africa.**

Jan Radomski discussed on how models could be constructed: probably using environments as individuals



Many level scales have to be considered: from very local to local to regional and to country level/
It is important to discuss the above issues together with WP2 in WPG1 and WP9 in WPG3.

The discussion went back to water concentration issues and was led by Nathalie Deboosère (IPL). The first step will probably be using electrostatic filters (standardised method) on up to 100 L. The efficacy of this kind of approach depends on the pHi of the virus. It was tested with vaccinia virus, which is an enveloped virus. With it, 5 to 20% of the total amount of virus was recovered: so if there are 100 pfu in 10 L of water, then the system should collect 5 to 20 pfu. Electropositive filters can also be used. For the second step of concentration, adsorption/elution using erythrocytes is envisaged. Nathalie Deboosère told the group that IPL could prepare all the filters for them.

Jan Radomski hoped that the database could be in place by sometime in May 2008. **This means that a new GENTT diagram should be issued and adapted for the first months of the project.**
As far as remote sensing (RS) is concerned a priority question is how to get RS data.

10h45 – 11h45 Discussion of WPG2 ""Survival of avian influenza viruses in air and surfaces "

WP5: Ana Burguière wished to be creative in this topic. The main factors to be considered are temperature, hygrometry, UV, surface matrices..... Ana Burguière told the group that IPP has already got some experience with collecting air as they are doing it in aircraft: 300 L of air in 5 minutes. Nathalie Deboosère informed us that IPL uses a system to produce virus loaded aerosols.
Jan asked the group whether it was relevant for him to add biomolecular modelling taking into aacount water molecules in relation to hygrometry.

WP6: Angel Galabov insisted upon the fact that his WP was heavily dependent on the results produced by WP 1, 8 and W0, WP1 and WP5. Again, sampling questions came back into the discussion: we need to define where and how to collect samples in farms. Surfaces around farms can be very different from each other: middle vs. small villages. Sampling in the surroundings of formerly infected farms would be very interesting but Pr Galabov wondered

whether this was possible. IPC then said that this would be feasible in Cambodia and hoped that, in the case of future outbreaks, the infected farms could be followed in the course of time (longitudinally). Again it was stressed that the formulation of protocols is very important: points of sampling, data collection (GPS position, date, time .....). Sampling protocols could be inspired by other diseases such as salmonellosis.

WP7: Dr Deubel demonstrated how the use of H5N1 pseudoparticles would be an advantage to answer questions such as: is ethanol 70% enough to destroy the envelope, how long do particles remain on gloves, what is the impact of ultrasound on the particles. The idea would be to start this study with H1N1 and H3N2 particles and then test various serotypes such as H5, H7, and H8. Within H5N1 particles, various clades of HA could also be tested. Furthermore, this approach could be further applied to vaccine production to test the stability of the H5 with various reagents.

11h45 -12h15 General discussion – Practical organisation

Jan Radomski questioned Jean-Pierre Broyart to know whether all expenses for this meeting could be considered as managerial costs: the answer was yes.

12h15 - 12h30 Future meetings, Closing remarks

The date for the next annual meeting should be decided by end of August 2007. The venue will be discussed taking into account the costs of travel and accomodation globally for the whole project as well as the easyness of flights and connexions. All partners can volonteer to host the next annual meeting and all proposals will be welcome.

ANNEX : List of participants

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