



LABORATOIRE DE
PLOUFRAGAN /
PLOUZANE

Site de Ploufragan

**Contract Anses / OIE
(reference Anses P950A)**

Duration of contract : 01/08/2013 – 31/07/2016

Extension with constant budget: 01/08/2016 - 30/09/2017

UNITE VIROLOGIE
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**Implementation of the
OIE reference laboratory twinning project
between Anses (France) and HVRI (PR China)
for Infectious bursal disease of chickens.**

**Final Report
Part 1: technical report**

Covered period = 01/08/2013 to 30/09/2017

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REPUBLIQUE
FRANÇAISE

Final report on twinning programme between HVRI and Anses

on infectious bursal disease

Scientific and Technical part

1 –Scientific context : Twinning programme and other collaboration actions between HVRI and Anses

Poultry is a cheap source of proteins in both industrialized and developing countries worldwide. Preserving poultry health is therefore a major priority in the “agriculture and veterinary sciences” field. Infectious bursal disease (IBD) is one of the major virus diseases of chickens worldwide, The disease is caused by a virus (Infectious bursal disease virus, IBDV, family Birnaviridae, genus Avibirnavirus) which causes up to 60% mortality and immunosuppression. Immunosuppression in turn promotes secondary bacterial, parasitic or virus superinfections in the birds that survive in IBDV-infected flocks. The control of IBD is therefore critical to maintain poultry health. World Organisation for Animal Health (OIE) reference laboratory and its twinning project play important roles in animal disease control. Asia counties including China have a big poultry industry, which were threatened seriously by IBD. However, there is no OIE reference laboratory in Asian.

The OIE twinning programme is a critical step in setting up cooperation between Anses (French Agency for Food, Environment and Occupational Health Safety) – Ploufragan/Plouzane laboratory and HVRI-CAAS (Harbin Veterinary Research Institute – Chinese Academy of Agricultural sciences, the first veterinary medicine research institute founded in China). Ploufragan/Plouzane laboratory hold the OIE reference laboratory for IBD. The OIE twinning programme is to provide HVRI with training and technical support to comply with OIE standards IBD detection, control, and so on.

Two memoranda of understanding have been signed in 2010, at the national level between the general directors of Anses and CAAS on the 20th November 2010, and at the regional level by representatives of the Anses / Ploufragan-Plouzané laboratory (Dr Salvat) and of HVRI (Dr X. Wang) on the 8th December 2010. These agreements provide a frame to establish further scientific collaboration. The OIE-Twinning programme was approved by OIE for 3 years on Aug 1, 2013. An extension with constant budget until 30th September 2017 was approved by Dr Monique Eloit, General Director of OIE, on Jan 26, 2017. The OIE-Twinning programme registered good progress, and all tasks were performed well.

Annual reports describing the progress of the OIE IBD-twinning programme were sent to OIE on 31 Dec 2015 (year 1, annex B-1), 05 Jan 2017 (year 2, annex B-2) and the present 3-year report (year 3, annex B-3).

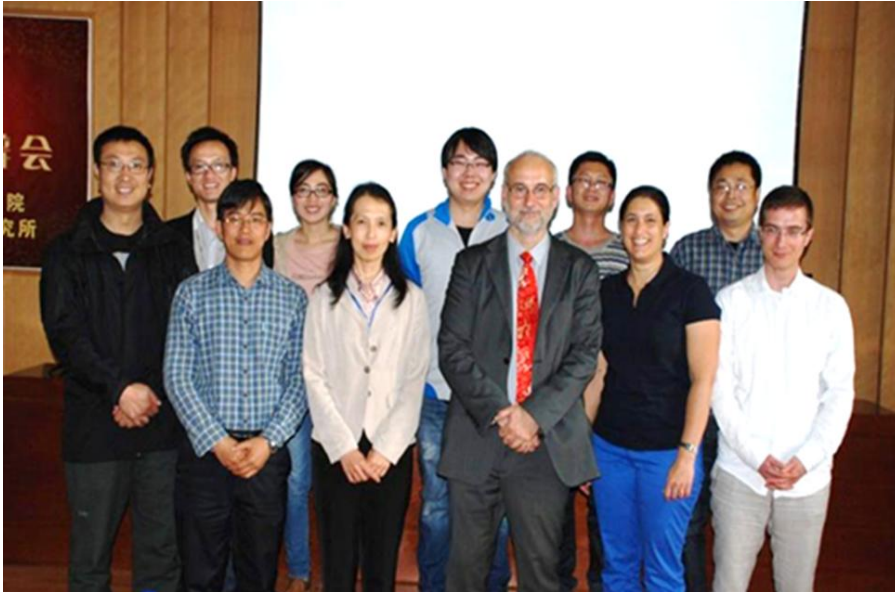
2 - Summary of actions implemented within the twinning programme

2.1– List of visits : who / when / where / training objectives

No	Date	Experts/staff	Place	Training objectives	Person x days
1	2013.8.26-8.27	Wang XM, Gao YL(HVRI)	ANSES	Project launch meeting	4
2	2014.5.18-5.23	Nicolas ETERRADOSSI, Julie REFREGIER PETTON , Sebastien SOUBIES (ANSES)	HVRI	Opening session; the quality management system training	18
3	2014.8.18-8.28	Qi XL, He XJ (HVRI)	ANSES	Production and control of reagents for serological testing	22
4	2015.6.29-7.1	Wang XM, Gao YL (HVRI)	London	Annual meeting for 1st year	6
5	2015.11.16-11.28	Wang YQ, Zhang YP (HVRI)	ANSES	Virus detection and molecular and antigenic characterization.	26
6	2016.11.30-12.8	Li K, Cui HY	ANSES	Training and cooperative study, reporting.	18
7	2017.6.28-6.30	Nicolas ETERRADOSSI(ANSES)	HVRI	Final meeting; International meeting	3



(left to right: Sébastien SOUBIES, Nicolas ETERRADOSSI, Kai LI, Xiaomei WANG, Xiaole QI; Yulong GAO; HVRI, 2014)



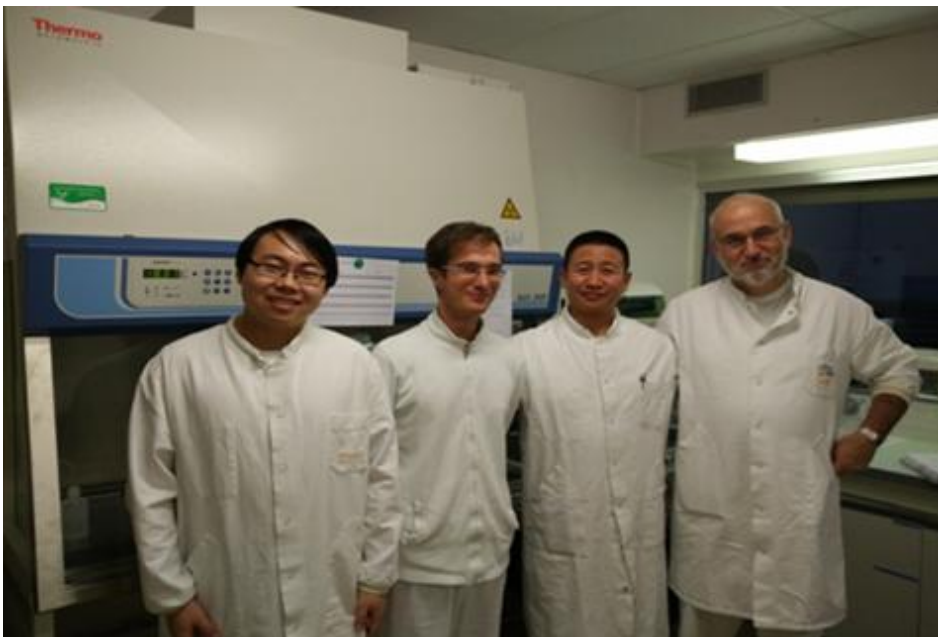
(left to right: Honglei GAO, Lizhou ZHANG, Yulong GAO, Li GAO, Xiaomei WANG, Kai LI, Nicolas ETERRADOSSI, Xiangang REN, Julie REFREGIER PETTON, Xiaole QI, Sébastien SOUBIES; HVRI, 2014)



(left to right: Jean-Erik BLOCHET (Zoopole Developpement, in charge of the animation of Ploufragan Technopark), Julie CALVEZ (Zoopole Developpement), Sébastien SOUBIES, Xiaole QI; Ploufragan-lab, ANSES, 2014)



(left to right: Sébastien SOUBIES, Yanping ZHANG, Céline COURTILLON, Yongqiang WANG, Nicolas ETERRADOSSI, Mouna ABED; Ploufragan-lab, ANSES, 2015)



(left to right: Kai LI, Sébastien SOUBIES, Hongyu CUI, Nicolas ETERRADOSSI; Ploufragan-lab, ANSES, 2016)

2.2– List of protocols that have been exchanged

- [1] A guide to chicken embryo lesions induced by different prototypic IBDV strains (serotype 1 and 2).
- [2] Standard protocol for Viral titration on embryonated chicken eggs with inoculation onto the chorio-allantoic membrane.
- [3] Standard protocol for Viral titration on chicken embryonic fibroblasts (CEF).
- [4] Standard protocol for antibodies quantification: viral neutralization assay on CEF.
- [5] Standard protocol for viral antigen inactivation by Beta-propiolactone for AGID.
- [6] Standard protocol of agar preparation for AGID.
- [7] Standard protocol of antibodies detection by AGID.
- [8] Recommendations for reporting to OIE and OIE delegates

2.3– List of reagents exchanged : reference / nature / purpose of exchange / result

2.3.1 Virus (IBDV) exchanged from HVRI to ANSES

No	reference	Nature	purpose of exchange	Result
1	Gx	Frozen	study	-
2	SH99	Frozen	study	The genome and antigenic characterization was studied.
3	HLJ-0504	Frozen	study	The virulence and antigenic characterization was studied.
4	HeB10XS04	Frozen	study	The virulence and antigenic characterization was studied.
5	HuN11-01	Frozen	study	The genome and antigenic characterization was studied.
6	Gt	Freeze-drying	study	Serotype 1 IBDV antigen used by HVRI in VN assays. The titer and specificity were confirmed in Anses

2.3.2 Virus (IBDV) exchanged from ANSES to HVRI

No	reference	nature	purpose of exchange	Result
1	89163	Frozen	study	The virulence was detected in SPF chickens. French reference strain for typical very virulent IBDV, can be used as challenge strain.
2	Faragher 52/70	Frozen	study	The virulence was detected in SPF chickens. European reference strain for classical IBDV, can be used as challenge strain.
3	CT	Frozen	study	The virulence was detected in chicken embryo

4	GLS-5	Frozen	study	fibroblasts. French reference serotype 1 IBDV antigen. Can be used in virus neutralization assays. The virulence was detected in chicken embryo fibroblasts. US antigenically modified IBDV strain. Cause rapid immunosuppression but no mortality. Can be used as an example of antigenic variation within serotype 1.
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2.3.3 Regents exchanged from HVRI to ANSES

No	reference	nature	purpose of exchange	Result
1	IBDV positive serum for AGID	Freeze-drying	study	The neutralizing activity was detected.
2	IBDV negative serum for AGID	Freeze-drying	study	No reactivity was detected versus 6 precipitating antigens in use in Anses.
3	IBDV reference precipitating antigen for AGID	Freeze-drying	study	The reactivity was detected only versus a serum containing antibodies against IBDV when 7 monospecific antisera containing precipitating antibodies to different chicken pathogens were tested in Anses.

2.3.4 Regents exchanged from Anses to HVRI

No	reference	nature	purpose of exchange	Result
1	IBDV positive serum	Freeze-dried	Study	Sharing of Anses reference antiserotype 1 IBDV antiserum.
2-16	15 anonymized serum samples containing different amounts of anti IBDV-neutralizing antibodies	Freeze-drying	Ring trial in virus-neutralization (VN) and agar-gel immunodiffusion (AGID)	The samples were used by HVRI and Anses for proficiency testing in VN and AGID, according to principles of ISO/CEI 17043. ¹
17-20	Three virus suspensions (attenuated CT strain)	Freeze-drying	Ring trial in virus titration in embryonated eggs and chicken embryo fibroblasts	The samples were used by HVRI and Anses for proficiency testing in virus titration in embryonated eggs and chicken embryo fibroblasts. ¹

¹ : See report of ring trial in annex of annual report for year 2

2.4– List of jointly published scientific papers

- [1] Qi X, Lu Z, Wang N, Chen Y, Zhang L, Gao L, Li K, Ren X, Wang Y, Gao H, Gao Y, Nicolas E, Wang X. Analysis of the function of D279N mutation of VP2 of infectious bursal disease virus. *Journal of Integrative Agriculture*. 2015, 14(12): 60345-60357.
- [2] Li K, Courtillon C, Guionie O, Allée C, Amelot M, Qi X, Gao Y, Wang X, Eterradossi N. Genetic, antigenic and pathogenic characterization of four infectious bursal disease virus isolates from China suggests continued evolution of very virulent viruses. *Infect Genet Evol*. 2015, 30:120-127.
- [3] Kai Li, Céline Courtillon, Olivier Guionie, Chantal Allée, Michel Amelot, Xiaole Qi, Yulong Gao, Xiaomei Wang, Nicolas Eterradossi. Genetic, antigenic and pathogenic characterization of infectious bursal disease viruses isolated in China. 2015 WVPA, Cape Town, South Africa.

3 – Perspectives for continuing collaboration between Anses and HVRI beyond twinning programme

3.1- Regular meetings to promote collaboration with other OIE reference laboratories for infectious bursal disease of chickens

After HVRI applies and hopefully becomes one of the IBD OIE reference laboratories, Anses and HVRI will invite OARDC (Wooster, Ohio, USA), the third OIE reference laboratory for IBD, to participate in an “Annual meeting of OIE reference laboratories of chickens”. This meeting will allow i) to share research trends, ideas and reagents, ii) to coordinate reference work on IBD in the different reference laboratories, in order to avoid redundancy in the production of specific reagents (monospecific antisera to specific strains) or to collaborate for validation of new methods, iii) to share views and reach a consensus in the changes that should possibly be proposed when OIE revises the IBD chapters of its reference documents (Manual of standards for terrestrial animals / Terrestrial code). These annual meetings will help the three laboratories in meeting term of reference (ToR) 10 of the OIE reference mandate “To establish and maintain a network with other OIE reference laboratories designated for the same pathogen and organize regular interlaboratory proficiency testing to ensure comparability of results”. The venue for these meetings will be decided jointly depending on the agenda of international conferences on poultry diseases, so that the IBD meetings can be held as “satellite meetings” in order to minimize the transportation costs.

3.2– Reactivating the “international symposium on infectious bursal disease, chicken anaemia virus and other immunosuppressive viral diseases of poultry”.

The topic of immunosuppressive viral diseases of poultry is more important as ever in modern poultry production, that heavily relies on vaccination to control poultry diseases (some of which with a devastating impact or potential for zoonotic transmission) and should strive to maintain the best possible immune-competency in chickens so as to avoid increased susceptibility to bacterial diseases and costly antibiotic treatments that contribute to generate antimicrobial resistance in bacterial pathogens. For many years, the scientific community of researchers working on immunosuppressive diseases of chickens could enjoyed the well-known “Rauschholzhausen symposium” organized by Justus Liebig University in Giessen, Germany, however these symposium have been discontinued in 2001, although they provided an environment that was critical to foster new ideas, promote consortium organization between researchers and coordination of research worldwide. It is Anses and HVRI wish, once HVRI applies and hopefully becomes one of the IBD OIE reference laboratories, to reactivate these meetings by hosting the next one and invite worldwide research groups and companies about IBD to this meeting (Beijing China, 2019). This will enhance the collaboration and promote the research on IBD and benefit to the poultry production worldwide.

3.3-Jointly research and co-publish.

HVRI and Anses have a long standing history of scientific collaboration (since 1998), and have developed strong links and friendship within programmes supported at the European level (INCO-DC programme ERBIC18CT980330), by national (French Ministry of agriculture ADECIA) or binational (Cai YuanPei Hubert Curien Programme) grants, or by international organizations such as the current OIE-funded twinning programme. It is the commitment of the two groups leaders (Prof. X. Wang and Dr N. Eterradossi) that these strong relationships will be maintained and further developed with the different possible sources of co-funding. Based on the discussion at the final meeting of this IBD OIE Twinning programme, we identify possible further scientific collaborations in investigating i) the mechanisms of reassortment (an important evolutionary mechanism of avibirnavirus, that has consequences both for the emergence of new pathogenic strains and to evaluate the stability of vaccines) and ii) further studying the basis for antigenic variation (an important topic with respect to vaccine development).

4 – Comments of participating laboratories on twinning programme

4.1 – HVRI

The IBD OIE twinning programme offers the valuable opportunity to HVRI. The IBD OIE Twinning programme provides with training and technical support to comply with OIE standards about IBD detection, IBD diagnosis, IBD control, quality insurance, and so on. Besides, it extends the global IBD surveillance and establishes a link that facilitates the exchange of knowledge, ideas, and experience between China and OIE. The IBD OIE twinning programme will contribute more to IBD control, which will greatly promote the sound development of chicken farming and food safe.

4.2 - Anses

Context: The OIE-supported twinning programme between Anses and HVRI on infectious bursal disease of chickens has been an important scientific programme for our institute. It is the most recent in a series of scientific collaborations between our two laboratories, and was an outstanding opportunity to spend many weeks in each other laboratories, thus contributing to the good understanding between our two teams. Second, it fits perfectly within the frame of a larger collaboration programme between Anses and China Academy of Agricultural Sciences, and can be cited as a good example of longlasting collaboration growing into good understanding and friendship.

Practical aspects: The OIE-supported twinning programme could be developed efficiently as first international grants allowed some exchange of experts and good working sessions to build the application. The time schedule of the action itself has been modified (delayed) because of the most unexpected passing away of some scientists originally involved (IBD researcher, qualitian) and because of highly pathogenic influenza outbreaks occurring in France and seriously diverting the scientist in charge of the programme from IBD to influenza. Although such events are out of our reach, we must apologize for the delays caused by the subsequent reorganization in Anses, and our Chinese partners in HVRI as well as OIE officials must be acknowledged for their understanding and help to accommodate these events. These problems notwithstanding, all critical steps in the twinning programme were achieved, thanks to the commitment of both partners.

International, scientific and OIE aspects of the twinning: the OIE-funded twinning has been the opportunity for Anses to further collaborate with a group that is now acknowledged in the international scientific community as one of the most – if not the most – prominent groups in IBD research internationally. The level of scientific expertise and commitment of HVRI staff, their virological skills, the outstanding facilities where they work (both in the virology laboratory, the experimental confined animal facilities, and the accommodation space for international conferences) make it very clear that they can play a major role in supporting OIE expertise in the IBD field for the benefit of OIE member countries. For all these reasons, the parent laboratory in this twinning programme brings its unrestricted support to HVRI application to the mandate of OIE reference laboratory for IBD and a recommendation letter (dated July 2nd, 2017) was sent to this effect by Dr Nicolas Eterradossi to Prof. Xiaomei Wang.

Beyond twinning: It is our sincere hope that collaboration can be maintained and developed in the future between Anses and HVRI in the field of infectious bursal disease of chickens. Scientific trends have been identified that should be beneficial for both institutes. The commitment of the two group

leaders in international initiatives (Prof Wang is a member of the scientific committee in the Global Alliance for Research for Avian Diseases, Dr Etteradossi is Vice President of the World Veterinary Poultry Association) creates a good opportunity for this future collaboration to generate again an international movement into research on immunosuppressive virus diseases of chickens.

5-IBD researches of the IBD lab in HVRI

Harbin Veterinary Research Institute (HVRI) was founded in June, 1948 as the first and largest veterinary medicine research institute in China. As an important part of HVRI, IBD Lab in HVRI was founded in the 1980s, and focused on the diagnosis, epidemiological studies, prevention and control, and basic research of IBD.

The IBD Lab in HVRI has established systemic experimental methods and techniques for IBD diagnosis and study. The enzyme-linked immunosorbent assay (ELISA) and the agar gel immunodiffusion assay (AGID) methods for IBDV antibody detection, RT-PCR, real-time RT-PCR, RT-LAMP (loop-mediated isothermal amplification), and IFA (immunofluorescence assay) for IBDV detection have been also developed in the IBD Lab. IBD Lab directly tests approximately 200 tissue samples and 4,000 sera samples every year. Until now, IBD Lab has provided diagnostic reagents for 500,000 AGID and 100,000 ELISA samples to other laboratories and poultry farming companies. In addition, the Animal Infectious Disease Diagnostic and Service Center of HVRI, including IBD detection, has been quality-certified by China National Accreditation Service for Conformity Assessment (CNAS L6928).

The IBD Lab has established five major techniques for IBD investigations: (1) Virology technologies, including virus isolation and identification, virus culture and purification, viral load determination, virulence evaluation, and microscopic pathology detection; (2) Molecular biology technologies, including cloning, sequencing, genetic variation analysis, prokaryotic or eukaryotic expression of virus proteins, and gene engineering; (3) Immunology technologies, including analysis of humoral immunity (including neutralizing antibody detection) and cell immunity (including specific CD4+ and CD8+ T cell proliferation and cytokine secretion); (4) Monoclonal antibody (mAb) technologies, including the development of mAb to IBDV VP1, VP2, VP3, VP5, and the identification of the antigen epitopes.

On August 1, 2013, the OIE Twinning Program for IBD was launched and signed by the OIE Reference Laboratory for IBD, French Agency for Food Environment and Occupational Health Safety (ANSES) and HVRI. Dr. Nicolas Etteradossi is the director of the OIE Reference Laboratory for IBD in ANSES. Prof. Xiaomei Wang is the director of IBD Lab in HVRI. After four years of implementation, IBD Lab in HVRI has significantly improved in research and complied with OIE standards IBD detection, IBD diagnosis, IBD control, and quality insurance. Now all tasks of the OIE Twinning Program for IBD are performed well. Final meeting of the IBD Twinning Program held in HVRI on 28-29 June, 2017. In the future, the IBD Lab in HVRI will continue to maintain IBD detection ability of the OIE standards and will contribute more for IBD control and OIE affairs.

Annual reports for years 1

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**Implementation of the
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between Anses (France) and HVRI (PR China)
for Infectious bursal disease of chickens.**

First Annual Report

Covered period = 01/08/2013 to 31/07/2014

**Nicolas ETERRADOSSI, Céline COURTILLON, Sébastien SOUBIES
(Anses)**

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Implementation of an OIE reference laboratory twinning project between Anses (France) and HVRI (PR China), for Infectious bursal disease of chickens.

1 – Objectives of the twinning programme

As stated in paragraph 2.3 of the twinning contract signed on the first of August 2013, the objectives of the twinning project are (numbering of the objectives as per the twinning contract):

2.3.1 - To provide Harbin Veterinary Research Institute with training and technical support to comply with OIE standards , for producing reagents and performing assays necessary for the serological diagnosis of infectious bursal disease. To produce a panel of validated Chinese reference reagents and exchange it with reagents from parent laboratory.

2.3.2 - To provide Harbin Veterinary Research Institute with training and technical support to comply with OIE standards, for producing reagents and performing assays necessary for the detection of infectious bursal disease.

2.3.3 - To provide Harbin Veterinary Research Institute with training and technical support, for producing reagents and performing assays necessary for the characterisation of infectious bursal disease virus isolates and analysis of isolates antigenic variation and pathogenicity. To produce a panel of validated Chinese reference viruses and exchange it with reference strains from parent laboratory.

2.3.4 - To provide Harbin Veterinary Research Institute with training in the area of Quality Insurance (ISO17025).

2.3.5 - To extend the global infectious bursal disease surveillance and establish a link that facilitates the exchange of knowledge, ideas and experience between French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and Harbin Veterinary Research Institute.

2.3.6 - To encourage Harbin Veterinary Research Institute to establish effective surveillance and to share information in a timely manner with the OIE network.

2.3.7 - To assist Harbin Veterinary Research Institute meet the mandate of an OIE reference laboratory and develop a regional network.

2.3.8 - To jointly publish in scientific journals the results obtained by HVRI with the transferred methods and by Anses with the exchanged Chinese viruses and reagents.

2.3.9 - To create conditions so that the benefits of the twinning project are sustained.

2 – Activities scheduled in the first year of the twinning programme

To fulfill the objectives listed in the previous paragraph, the work plan for first year of the signed twinning programme scheduled the following activities (see paragraph 2.8 in twinning contract, numbering of the activities as per the twinning contract):

2.1 - Opening session of twinning project: Visit of French Agency for Food, Environmental and Occupational Health & Safety (ANSES) expert, quality manager, post-doctoral researcher, and laboratory scientist to Harbin Veterinary Research Institute to assess capacity and to agree how best to structure the courses at the ANSES. Visit will include presentation of seminars by ANSES including topics such as infectious bursal disease

surveillance and diagnostic techniques, investigating the pathogenicity and antigenic variation of infectious bursal disease and working within the ISO17025 frame.

Duration: 1 week. 4 persons. Approximate date: Project term 1 (Exact time to be scheduled by HVRI and Anses)

2.2. Training course at ANSES for two laboratory staff from Harbin Veterinary Research Institute for Techniques : production and control of reagents for serological testing.

Production and control e.g. of reference antisera, precipitating antigen, agar for gel diffusion, reference virus stock for virus neutralization, virus titration for virus neutralization. Performing and interpreting serological assays (Agar gel diffusion, virus neutralization, ELISA). Comparison with protocols performed in HVRI. Identification of possible aspects that require harmonization.

Duration: 2 weeks, 2 persons. Approximate date: Project term 2 (Exact time to be scheduled by Anses and HVRI)

2.3. Provision of antigens and other reagents to Harbin Veterinary Research Institute to start the project as required. Interlaboratory/Proficiency tests to ensure competency at Harbin Veterinary Research Institute.

Approximate date: Project term 2 (Exact time to be scheduled by Anses and HVRI)

2.4. Production by HVRI of home-made reference reagents for serological testing, and provision of these reference reagents to Anses for comparative testing.

Duration: - . Approximate date : Project term 3-4.

2.5. Visit of chinese referent scientists in Anses (France)

Duration: 1 week, 2 persons

2.6. French expert in Paris

Duration: 3 days, 1 person

3 – Advancement of the twinning programme

The implementation of the twinning programme has been approximately delayed by six months, due to the sudden passing away, in July 2013, of one of the scientists in charge of the programme in the parent laboratory. Another scientist (veterinarian, PhD in molecular virology) has been recruited on the 1st April 2014, to contribute to scientific and technical work on Infectious bursal disease, including the OIE twinning programme.

The scheduled activities are listed below and advancement for each of them is indicated in Italics.

3.1 - Opening session of twinning project: Visit of French Agency for Food, Environmental and Occupational Health & Safety (ANSES) expert, quality manager, post-doctoral researcher, and laboratory scientist to Harbin Veterinary Research Institute to assess capacity and to agree how best to structure the courses at the ANSES. Visit will include presentation of seminars by ANSES including topics such as infectious bursal disease surveillance and diagnostic techniques, investigating the pathogenicity and antigenic variation of infectious bursal disease and working within the ISO17025 frame.

Duration: 1 week. 4 persons. Approximate date: Project term 1 (Exact time to be scheduled by HVRI and Anses)

This visit was organized from the 18th to the 23rd May 2014 (project term 3), instead of project term 1 as originally scheduled. The reason for this six month delay was the necessity for the parent laboratory to recruit and inform the new scientist in charge of IBD programmes (Dr Sebastien SOUBIES, recruited April 2014) about the twinning project. Three scientists from Anses visited HVRI. These included Dr Nicolas ETERRADOSSI (OIE expert for IBD and AMPV), Mrs Julie REFREGIER PETTON (assistant head of Quality Management in parent laboratory) and Dr Sebastien SOUBIES (in charge of IBD research and technical work). The programme of the visit is attached as Annex 1, it included:

- i) attending the PhD defences of HVRI students working with IBDV and other viral disease of poultry,*
- ii) lecturing HVRI student and staff about the twinning programme and about current research in Anses on IBDV and AMPV,*
- iii) discussing with HVRI students their research on IBDV,*
- iv) meeting with HVRI staff in charge of the new diagnostic laboratory for animal diseases,*
- v) introducing HVRI staff to the quality management system implemented in parent laboratory (2 lectures),*
- vi) reviewing the progress of the twinning programme and planning future steps with HVRI expert Pr Xiaomei Wang,*
- vii) visiting HVRI new diagnostic facility*
- viii) visiting HVRI new laboratory site, currently under construction.*

1.2. Training course at ANSES for two laboratory staff from Harbin Veterinary Research Institute for Techniques : production and control of reagents for serological testing.

Production and control e.g. of reference antisera, precipitating antigen, agar for gel diffusion, reference virus stock for virus neutralization, virus titration for virus neutralization. Performing and interpreting serological assays (Agar gel diffusion, virus neutralization, ELISA). Comparison with protocols performed in HVRI. Identification of possible aspects that require harmonization.

Duration: 2 weeks, 2 persons. Approximate date: Project term 2 (Exact time to be scheduled by Anses and HVRI)

This visit was organized from the 19th to the 28th August 2014 (project term 5, actually at the very beginning of the second year of the twinning project), instead of project term 2 as originally scheduled. It is however reported here, as it was originally part of the programme for year 1. As explained before, the six month delay was necessary due to the reorganization of the IBD team in parent laboratory. Two scientists from HVRI, namely Drs Xijun HE (Head of Department of Veterinary pathology, animal disease diagnostic center, HVRI) and Dr Xiaole QI (HRVI) visited Anses. The programme of the visit is attached as Annex 2. The programme included:

i) Comparison of lesions induced in chicken embronated eggs by the different strains of IBD virus (serotype 2 virus and different strains of serotype 1 viruses : cell culture adapted vaccine, North-American antigenic variant E, classical reference strain Faragher 52/70, very virulent IBDV (vvIDV), recent strain of Chinese vvIBDV.

ii) Technical demonstration of virus neutralization in eggs and cells. and cross virus neutralization in cells (a technique that is critical for the sound assessment of antigenic variation in IBDV)

iii) Technical demonstration of chloroform inactivation of possible enveloped contaminants in bursal homogenates submitted for IBDV typing (a technique that is critical to avoid introducing enveloped viral contaminants such as avian influenza viruses or paramyxoviruses when receiving field samples from abroad for IBDV characterization)

iv) ELISA analysis of sera from AMPV infected flocks. Scientific discussion about subgroup specificity of AMPV ELISA antigens

v) Visiting the « Zoopole » Technopark of Saint-Brieuc Armor, devoted to animal production. Meeting and discussion with Dr Frederic Bernard, Head of Zoopole

vi) Visiting the facilities of Laboceca 22, one of French largest diagnostic laboratories for animal diseases, Food hygiene, and environmental analysis. Introduction to the MALDI-TOF approach of bacterial identification. Meeting and discussions with Drs Hervé MORVAN and Luc MIELLI, heads of Laboceca 22.

vii) Discussion to organize the future tasks within the programme (see below paragraph 4)

1.3. Provision of antigens and other reagents to Harbin Veterinary Research Institute to start the project as required. Interlaboratory/Proficiency tests to ensure competency at Harbin Veterinary Research Institute.

1.3.1 – Provision of antigens and other reagents

Four reference virus stocks representing different pathotypes of serotype 1 IBDV have been provided by Anses to HVRI (see annex 3). A reference antiserum (Four 0.5 ml vials of freeze-dried serum) had been provided previously (2012), while preparing the twinning contract.

HVRI provided in 2012 five virus suspensions to Anses for IBDV characterization (see below paragraph 4.). Four of these contained IBDV and three of them were propagated in 2013 in Specific Pathogen Free chickens in Anses contained animal facilities. Aliquots of the resulting bursal homogenates were returned to HVRI (See Annex 3).

1.3.2 – Proficiency tests to compare results obtained at HVRI and Anses

A list of standard operating procedures has been discussed and is currently being translated into English at Anses and HVRI in order to be exchanged between the two institutes before the end of 2014.

The format of a proficiency tests is currently being discussed within the parent institute. Propositions will be made to HVRI to organize proficiency testing for

- i) titration of IBDV suspensions in cell culture and embryonated eggs (critical protocols for the titration of vaccines and VN antigens, or the adjustment of challenge doses, respectively*
- ii) virus neutralization assay in cell culture and eggs (critical protocols for the measurement of post vaccine antibody responses and for performing cross VN assay to assess antigenic variation).*

The protocols for the proficiency tests will be discussed before March 2015 so that the proficiency tests can be performed first semester 2015.

1.4. Production by HVRI of home-made reference reagents for serological testing, and provision of these reference reagents to Anses for comparative testing.

Duration: - . Approximate date: Project term 3-4.

HVRI provided in 2012 five virus suspensions to Anses for IBDV characterization. Four of these suspensions contained IBDV strains that have been further characterized in Anses (see below paragraph 4.).

1.5. Visit of chinese referent scientists in Anses (France)

Duration: 1 week, 2 persons

HVRI expert, Prof. Xiaomei WANG, and her assistant with the OIE twinning project Dr Yulong GAO, attended the XVIIIth congress of the world Veterinary Poultry Association in Nantes, France, 19th to 23rd August 2013 (this congress was organized by the French branch of WVPA, whose chairman is Dr Nicolas ETERRADOSSI. The congress covered all aspects of poultry diseases and gathered 1236 participants from 72 countries). Prof WANG and Dr GAO then visited Anses Ploufragan – Laboratory, on the 26 and 27 August 2013.

1.6. French expert in Paris

Duration: 3 days, 1 person

Not needed, hence not implemented.

4 – Other Anses-HVRI collaboration activities during the period, not covered by the OIE twinning programme

The first year of the OIE twinning project overlapped (1st August 2013 to 18th November 2013) with a pre-existing collaborative programme between HVRI and Anses within the frame of the Cai Yuan Pei Hubert Curien funding scheme. This programme supported a one-year training and research period for a HVRI PhD student (Mr Kai LI) in Anses Ploufragan, within the frame of a research programme entitled « Extent and biological significance of antigenic and genetic variation in infectious bursal disease virus isolates from China ».

During his stay, Mr Kai LI was trained in the methods used by the OIE reference laboratory, and contributed to the research performed in this group in relation with the antigenic, genetic and/or in vivo characterization of four Chinese strains of IBDV (identification of a Chinese isolate with a significantly increased pathogenicity). Mr Kai LI also contributed to some reverse genetics work related to the cell culture adaptation of very virulent IBDV.

Based on the results obtained during Dr Kai LI's stay, one co-authored paper was submitted (and accepted for publication in December 2014) in the scientific journal "Infection, Genetics and Evolution" (five-year impact factor = 3.34). The paper is now available online under reference:

LI K., COURTILLON C., GUIONIE O., ALLEE C., AMELOT M., QI X., GAO Y., WANG X., ETERRADOSSI N. (2015). Genetic, antigenic and pathogenic characterization of four infectious bursal disease virus isolates from China suggests continued evolution of very virulent viruses. Infection, Genetics and Evolution, 30, 120-127 (accepted and available online December 2014).

Two more scientific papers are to be finalized.

5 – Forthcoming activities within the twinning programme (Year 2)

Activities scheduled for year 2 of the twinning project (as per paragraph 2.8 of the twinning contract) are listed below. For each activity, comments appear in italics wherever necessary.

5.1. Meeting of the expert scientists of Anses and HVRI: annual review of the twinning programme

Duration: 7 days, 2 persons. Approximate dates: Project term 5.

To optimize travelling budget and presentation of the results in international conferences, it was agreed that this meeting will take place in parallel with XIXth WVPA Congress, to be held in Cape Town, South Africa, 7-11 September 2015,

5.2. Training course at ANSES for two laboratory staff from Harbin Veterinary Research Institute for Techniques : Virus detection and molecular and antigenic characterization.

Training e.g. in virus isolation (embryonated egg, cell culture), molecular and antigenic assays for virus detection and characterization, as used in Anses (PCR, sequencing, AC-ELISA, cross VN). Comparison of the protocols in use in HVRI and Anses. Identification of protocols that require harmonization / improvement. Training on the production of reagents and setting up the assays necessary for antigen characterization.

Duration: 3 weeks, 2 persons.

Approximate date: Project term 5-6 (Exact time to be scheduled by Anses and HVRI)

5.3. Provision of reagents to Harbin Veterinary Research Institute as required to start the first assays. Interlaboratory Proficiency tests to ensure competency at Harbin Veterinary Research Institute.

6

Approximate date: Project term 6 (Exact time to be scheduled by Anses)

To be organized as discussed in the forthcoming months, according to the format of the proficiency tests.

5.4. Production by HVRI of home-made reagents for detection and characterization, including reference panel of viruses representative for Chinese IBDV isolates. Exchange with Anses and comparison of results.

Approximate date: Project term 7

To be organized as discussed in the forthcoming months, according to the format of the proficiency tests.

2.5. Visit of quality insurance experts to HVRI: training HVRI scientists to the auditing process within the ISO17025 scheme.

Duration: 1 week, 2 persons, approximate date: project term 7-8

The HVRI diagnostic centre has recently been accredited by CINAS, the Chinese Committee for Quality Insurance. Therefore this second training will not focus on quality insurance itself, but on the process of performing audits in order to ensure long term sustainability of the quality insurance scheme. It was agreed by the partners that this visit will be organized after the first session of proficiency tests has been completed, so that their results can be analyzed and discussed during the visit.

Annual reports for years 2

LABORATOIRE DE
PLOUFRAGAN /
PLOUZANE

Site de Ploufragan

Contract Anses / OIE
(reference Anses P950A)
Duration of contract : 01/08/2013 – 31/07/2016

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**Implementation of the
OIE reference laboratory twinning project
between Anses (France) and HVRI (PR China)
for Infectious bursal disease of chickens.**

Second Annual Report

Covered period = 01/08/2014 to 31/07/2015

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REPUBLIQUE
FRANÇAISE

1

**Implementation of an OIE reference laboratory twinning project
between Anses (France) and HVRI (PR China),
for Infectious bursal disease of chickens.**

1 – Objectives of the twinning programme

As stated in paragraph 2.3 of the twinning contract signed on the first of August 2013, the objectives of the twinning project are (numbering of the objectives as per the twinning contract):

2.3.1 - To provide Harbin Veterinary Research Institute with training and technical support to comply with OIE standards , for producing reagents and performing assays necessary for the serological diagnosis of infectious bursal disease. To produce a panel of validated Chinese reference reagents and exchange it with reagents from parent laboratory.

2.3.2 - To provide Harbin Veterinary Research Institute with training and technical support to comply with OIE standards, for producing reagents and performing assays necessary for the detection of infectious bursal disease.

2.3.3 - To provide Harbin Veterinary Research Institute with training and technical support, for producing reagents and performing assays necessary for the characterisation of infectious bursal disease virus isolates and analysis of isolates antigenic variation and pathogenicity. To produce a panel of validated Chinese reference viruses and exchange it with reference strains from parent laboratory.

2.3.4 - To provide Harbin Veterinary Research Institute with training in the area of Quality Insurance (ISO17025).

2.3.5 - To extend the global infectious bursal disease surveillance and establish a link that facilitates the exchange of knowledge, ideas and experience between French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and Harbin Veterinary Research Institute.

2.3.6 - To encourage Harbin Veterinary Research Institute to establish effective surveillance and to share information in a timely manner with the OIE network.

2.3.7 - To assist Harbin Veterinary Research Institute meet the mandate of an OIE reference laboratory and develop a regional network.

2.3.8 - To jointly publish in scientific journals the results obtained by HVRI with the transferred methods and by Anses with the exchanged Chinese viruses and reagents.

2.3.9 - To create conditions so that the benefits of the twinning project are sustained.

2 – Activities scheduled in the second year of the twinning programme

To fulfill the objectives listed in the previous paragraph, the work plan for second year of the signed twinning programme scheduled the following activities (see paragraph 2.8 in twinning contract, numbering of the activities as per the twinning contract):

2.1. Meeting of the expert scientists of Anses and HVRI: annual review of the twinning programme

Duration: 7 days, 2 persons. Approximate dates: Project term 5.

2.2. Training course at ANSES for two laboratory staff from Harbin Veterinary Research Institute: Virus detection and molecular and antigenic characterization.

Training e.g. in virus isolation (embryonated egg, cell culture), molecular and antigenic assays for virus detection and characterization, as used in Anses (PCR, sequencing, AC-ELISA, cross VN). Comparison of the protocols in use in HVRI and Anses. Identification of protocols that require harmonization / improvement. Training on the production of reagents and setting up the assays necessary for antigen characterization

Duration: 3 weeks, 2 persons. Approximate date: Project term 5-6 (Exact time to be scheduled by Anses and HVRI)

2.3. Provision of reagents to Harbin Veterinary Research Institute as required to start the first assays. Interlaboratory Proficiency tests to ensure competency at Harbin Veterinary Research Institute.

Approximate date: Project term 6 (Exact time to be scheduled by Anses)

2.4. Production by HVRI of home-made reagents for detection and characterization, including reference panel of viruses representative for Chinese IBDV isolates. Exchange with Anses and comparison of results.

Approximate date : Project term 7

2.5. Visit of quality insurance experts to HVRI: training HVRI scientists to the auditing process within the ISO17025 scheme.

Duration: 1 week, 2 persons, approximate date : project term 7-8

2.6. French expert in Paris

Duration: 3 days, 1 person

3 – Advancement of the twinning programme

The implementation of the twinning programme has been approximately delayed by six months, due to the sudden passing away, in July 2013, of one of the scientists in charge of the programme in the parent laboratory.

This delay has been maintained during the second year of the twinning program.

The scheduled activities are listed below and advancement for each of them is indicated in Italics.

2.1. Meeting of the expert scientists of Anses and HVRI : annual review of the twinning programme

Duration: 7 days, 2 persons. Approximate dates: Project term 5.

Scientists from both laboratories took advantage of the first meeting of the Global Alliance for Research on Avian Diseases (GARAD) held in London from 29th June to 1st July 2015 to meet and plan the tasks from year 2 of the twinning, namely activities 2.2, 2.3, 2.4 and 2.5 described below.

2.2. Training course at ANSES for two laboratory staff from Harbin Veterinary Research Institute for Techniques: Virus detection and molecular and antigenic characterization.

Training e.g. in virus isolation (embryonated egg, cell culture), molecular and antigenic assays for virus detection and characterization, as used in Anses (PCR, sequencing, AC-ELISA, cross VN). Comparison of the protocols in use in HVRI and Anses. Identification of protocols that require

harmonization / improvement. Training on the production of reagents and setting up the assays necessary for antigen characterization

Duration: 3 weeks, 2 persons. Approximate date: Project term 5-6 (Exact time to be scheduled by Anses and HVRI)

This point has been addressed in November 2015, so after the second year of the twinning project. Dr Yanping Zhang and Dr Yongqiang Wang from HVRI were welcome in ANSES Poufragan, from 16th to 28th November 2015.

Hands-on training was offered for virus culture and isolation (egg inoculation via chorio-allantoic route, lesions of infected embryos, preparation of viral stocks starting from bursae of infected chickens), serological diagnostics (viral neutralization assay, agar gel immunodiffusion), molecular biology (extraction of viral RNA from a viral stock, RT-PCR) and pathogenicity assessment (infection and necropsy of SPF chickens).

Documents and protocols provided:

- A guide to chicken embryo lesions induced by different prototypic IBDV strains (serotype 1 and 2)*
- Protocol of Viral titration on embryonated chicken eggs with inoculation onto the chorio-allantoic membrane.*
- Protocol of Viral titration on chicken embryonic fibroblasts (CEF)*
- Protocol of antibodies quantification : viral neutralization assay on CEF*
- Protocol of viral antigen inactivation by β -propiolactone for AGID*
- Protocol of agar preparation for AGID*
- Protocol of antibodies detection by AGID*

Discussion about the advancement of the twinning project and provisional timing.

2.3. Provision of reagents to Harbin Veterinary Research Institute as required to start the first assays. Interlaboratory Proficiency tests to ensure competency at Harbin Veterinary Research Institute.

Approximate date: Project term 6 (Exact time to be scheduled by Anses)

A ring trial (inter-laboratory comparison) organized following the principles of ISO /CEI 17043 was proposed to scientists from HRVI. To do so, two panels of serum (n=15) and viral (n=3) samples were prepared and validated in terms of homogeneity and stability in the parent laboratory. The panels were then transferred in a blinded fashion and given to ANSES and HVRI scientists (on 26th November 2015). Both laboratories analyzed these samples using standard diagnostic techniques as presented in the OIE manual (results received by organizers on 1st February 2016 (ANSES) and 30th March 2016 (HVRI)). Results were analyzed in a blinded fashion. Results were globally consistent between the two laboratories.

The invitation letter to the ring trial is presented in annex 1.

Detailed results of the ring trial are presented in annex 2.

2.4. Production by HVRI of home-made reagents for detection and characterization, including reference panel of viruses representative for Chinese IBDV isolates. Exchange with Anses and comparison of results.

Approximate date: Project term 7

Four isolates from HVRI were obtained for viral characterization during year A of the twinning.

Reference diagnostic reagents from HVRI have not been transferred to ANSES so far. It is anticipated that aliquots of HVRI reference reagents will be exchanged with ANSES during year 3.

2.5. Visit of quality insurance experts to HVRI: training HVRI scientists to the auditing process within the ISO17025 scheme.

Duration: 1 week, 2 persons, approximate date: project term 7-8

This point has not been addressed so far for 2 reasons:

- The quality insurance expert from ANSES Ploufragan, due to health problems, could not visit HVRI.*
- In the meantime, HVRI diagnostic department was audited by CINAS and received the ISO 17025 accreditation (copy of certificate included in annex 3).*

2.6. French expert in Paris

Duration: 3 days, 1 person

Not needed and then not implemented.

4 – Other Anses-HVRI collaboration activities during the period, not covered by the OIE twinning programme

Based on the results obtained during Dr. Kai LI's stay during the first year of the twinning project, one co-authored poster was presented during the XIXth meeting of World Veterinary Poultry Association (WVPA) held in Cape town from 7th to 10th September 2015.

An article including authors from both laboratories was published (reference 1).

5 – Forthcoming activities within the twinning programme (Year 3)

Activities scheduled for year 3 of the twinning project (as per paragraph 2.8 of the twinning contract) are listed below. For each activity, comments appear in italics wherever necessary.

3.1. Meeting of the expert scientists of Anses and HVRI, annual review of twinning project.

This annual review will include a specific session on reporting to i) the OIE scientific and technical service, ii) the OIE delegates from Member States, iii) the other institution that may possibly submit samples for characterization. Hands-on reporting exercise based on the IBDV isolates from China characterized previously (see item 2.4). The meeting will also include scientific discussions to identify the possible frames to support cooperation between HVRI and Anses beyond the completion of the twinning programme.

Duration: 7 days. Approximate date: Project term 9

This meeting of 7 days was originally planned in the first term of 2016. Discussion about the results of the ring-trial should be held. The meeting should also focus on reporting to OIE. The Chinese isolates characterized during the venue of Dr. Li Kai in ANSES, as well as other strains potentially characterized recently by HVRI, will be used as a basis for a hands-on exercise about reporting.

Discussion will also focus about the application of HVRI as OIE reference laboratory. See point 3.5 for a summary.

Additionally, HVRI should provide a set of home-made diagnostics reagents (IBDV reference antiserum, reference precipitating antigen, reference viruses) so that a comparison will be made with ANSES.

NB: This meeting took place from 30th November to 8th December 2016; its content will be detailed in the twinning project third year report. All points, except the transfer of home-made diagnostics reagents (now scheduled during the programme extension), were completed.

3.2. Training course at ANSES for two research staff from Harbin Veterinary Research Institute for techniques: pathotyping IBDV isolates

Establishing protocols to test e.g. the acute pathogenicity of IBDV isolates. Practical comparison (in containment facilities) of the pathogenicity of IBDV isolates from China selected as per 2.4 and 3.1. Comparison with reference strains from Anses

Duration: 2 weeks, 2 persons. Approximate date: Project term 10 (Exact time to be scheduled by Anses and HVRI)

This point has already been addressed during the venue of Dr. Li Kai in ANSES. The focus of the visit could therefore be redefined and it has been proposed to cover the experimental assessment of IBDV immunosuppressive potential. Staff from ANSES mentioned that the visit could be postponed to the 4th term of 2016.

NB: This training course took place from 30th November to 8th December 2016; its content will be detailed in the twinning project third year report. All points were completed.

3.3. Study of IBDV pathogenicity in HVRI

Implementation of the previously defined protocols for testing pathogenicity in pilot experiments in HVRI (references 2 to 6). Exchange of results so as to ensure that Anses and HVRI reach consistent conclusions.

Approximate date; Project term 10-11 (Exact time to be scheduled by HVRI)

Training under ANSES experimental conditions has already been addressed during the venue of Dr. Li Kai in ANSES.

A possibility for assessment under HVRI experimental conditions could be for HVRI to organize a pathotyping session in which Chinese isolates could be compared with European reference isolates (for example strains F52-70 and 89163, already provided to HVRI in November 2013). Ideally the timetable should allow clinical signs to be seen by ANSES scientists during their last visit in HVRI. See point 3.5 for a summary.

3.4 Training in Quality Insurance (in HVRI)

Duration: 7 days, 2 persons

The point 2.5 originally supposed to be addressed during year 2 ("training in auditing technics") will not be addressed during year 3 since HVRI obtained accreditation from CINAS (copy of certificate included in annex 3).

3.5. Conclusion meetings:

3.5.1 - Meeting 1: "International cooperation for an improved surveillance of infectious bursal disease of chickens". This meeting will be held in Harbin Veterinary Research Institute. It will include the two experts from ANSES and Harbin Veterinary Research Institute and delegates from other laboratories in the region. The purpose of the meeting will be to encourage the participation of national reference laboratories to a network for regional surveillance of infectious bursal disease.

Duration: 1.5 days Approximate date: project term 12

3.5.2 - Meeting 2: Final meeting of the twinning programme, involving the key individuals from OIE, ANSES and Harbin Veterinary Research Institute engaged in the twinning and infectious bursal disease research.

OIE officials will be contacted to define which location (OIE headquarters, Anses or Harbin Veterinary Research Institute) is best suited for this final meeting. The aim of the meeting will be first to evaluate the outcomes of the project, to draft HVRI application to the OIE reference mandate, including fields in expertise that might remain more specific to HVRI or Anses at the end of the twinning programme. The second aim of the project will be to identify areas for ongoing collaboration and a strong basis for maintaining the link between the two laboratories.

Duration: 2 days Approximate date: project term 12 (Exact time to be scheduled by Anses, HVRI and OIE)

A second meeting (point 3.5.2) involving individuals from OIE, ANSES and HVRI who have been instrumental to the twinning programme, will be organized. Contact will be made by Dr Nicolas Etteradossi with OIE representative to discuss the time and place of this meeting.

A single mission of ANSES scientists in HVRI could possibly take place during the first semester 2017 to allow application of HVRI as reference laboratory for IBD to OIE in January 2017 (in time for submission to the next meeting of the Biological Standards Commission). This mission should therefore address the following points: i) prospects for future collaborations beyond the twinning programme (point 3.1), ii) HVRI application as reference laboratory to OIE (point 3.1), iii) pathotyping session of French and Chinese IBDV references strains (point 3.3), iv) conclusion meeting (point 3.5.2).

At the same period (first semester 2017), the international meeting mentioned in point 3.5.1 could be held in Harbin and organized by HVRI. The exact timing should be defined at least six months in advance. The meeting would be entitled "international cooperation for an improved surveillance of infectious bursal disease of chickens". Representatives from national reference laboratories throughout Asia should be invited to be informed of the mission of HVRI as a future OIE reference laboratory for IBDV. Prof. Dr Aini Ideris, vice-president of WVPA (tnca@upm.my), may be contacted to help advertising the meeting.

This new timing requires an extension of OIE contract (same funding, longer duration) that will be discussed with OIE officials.

3.6: French expert in Paris

Duration: 3 days, 1 person

Not required by OIE hence not implemented.

3.7 Analysis of reference diagnosis reagents

Production, shipment of HVRI home-made reference diagnosis reagents as well as their analysis in ANSES was originally scheduled during the second year of the twinning programme. This task is now planned during the programme extension.

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- 5 - LE NOUEN C., RIVALLAN G., TOQUIN D., DARLU P., MORIN Y., BEVEN V., DE BOISSESON C., CAZABAN C., COMTE S., GARDIN Y., ETERRADOSSI N. (2006) Very virulent infectious bursal disease virus: reduced pathogenicity in a rare natural segment B-reassorted isolate. *Journal of General Virology*, 87, 209-216.
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Annual reports for years 3

LABORATOIRE DE
PLOUFRAGAN /
PLOUZANE

Site de Ploufragan

**Contract Anses / OIE
(reference Anses P950A)**
Initial Duration of contract : 01/08/2013 – 31/07/2016
Extension with constant budget: 01/08/2016 - 30/09/2017

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**Implementation of the
OIE reference laboratory twinning project
between Anses (France) and HVRI (PR China)
for Infectious bursal disease of chickens.**

Third Annual Report

Covered period = 01/08/2015 to 30/09/2017

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REPUBLIQUE
FRANÇAISE

1

**Implementation of an OIE reference laboratory twinning project
between Anses (France) and HVRI (PR China),
for Infectious bursal disease of chickens.**

1 – Objectives of the twinning programme

As stated in paragraph 2.3 of the twinning contract signed on the first of August 2013, the objectives of the twinning project are (numbering of the objectives as per the twinning contract):

2.3.1 - To provide Harbin Veterinary Research Institute with training and technical support to comply with OIE standards, for producing reagents and performing assays necessary for the serological diagnosis of infectious bursal disease. To produce a panel of validated Chinese reference reagents and exchange it with reagents from parent laboratory.

2.3.2 - To provide Harbin Veterinary Research Institute with training and technical support to comply with OIE standards, for producing reagents and performing assays necessary for the detection of infectious bursal disease.

2.3.3 - To provide Harbin Veterinary Research Institute with training and technical support, for producing reagents and performing assays necessary for the characterisation of infectious bursal disease virus isolates and analysis of isolates antigenic variation and pathogenicity. To produce a panel of validated Chinese reference viruses and exchange it with reference strains from parent laboratory.

2.3.4 - To provide Harbin Veterinary Research Institute with training in the area of Quality Insurance (ISO17025).

2.3.5 - To extend the global infectious bursal disease surveillance and establish a link that facilitates the exchange of knowledge, ideas and experience between French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and Harbin Veterinary Research Institute.

2.3.6 - To encourage Harbin Veterinary Research Institute to establish effective surveillance and to share information in a timely manner with the OIE network.

2.3.7 - To assist Harbin Veterinary Research Institute meet the mandate of an OIE reference laboratory and develop a regional network.

2.3.8 - To jointly publish in scientific journals the results obtained by HVRI with the transferred methods and by Anses with the exchanged Chinese viruses and reagents.

2.3.9 - To create conditions so that the benefits of the twinning project are sustained.

2 – Activities scheduled in the third year of the twinning programme

To fulfill the objectives listed in the previous paragraph, the work plan for third year of the signed twinning programme scheduled the following activities (see paragraph 2.8 in twinning contract, numbering of the activities as per the twinning contract):

2.1. Meeting of the expert scientists of Anses and HVRI, annual review of twinning project.

This annual review will include a specific session on reporting to i) the OIE scientific and technical service, ii) the OIE delegates from Member States, iii) the other institution that may possibly submit samples for characterization. Hands-on reporting exercise based on the IBDV isolates from China characterized previously (see item 2.4 of twinning programme). The meeting will also include scientific discussions to identify the

possible frames to support cooperation between HVRI and Anses beyond the completion of the twinning programme.

Duration: 7 days.

Approximate date: Project term 9

2.2. Training course at ANSES for two research staff from Harbin Veterinary Research Institute for Techniques : pathotyping IBDV isolates

Establishing protocols to test e.g. the acute pathogenicity of IBDV isolates. Practical comparison (in containment facilities) of the pathogenicity of IBDV isolates from China selected as per 2.4 and 3.1. Comparison with reference strains from Anses

Duration: 3 weeks, 2 persons.

Approximate date: Project term 10 (Exact time to be scheduled by Anses and HVRI).

2.3. Study of IBDV pathogenicity in HVRI

Implementation of the previously defined protocols for testing pathogenicity in pilot experiments in HVRI. Exchange of results so as to ensure that Anses and HVRI reach consistent conclusions.

Approximate date: Project term 10-11 (Exact time to be scheduled by HVRI)

2.4 Training in Quality Insurance (in HVRI)

Duration: 7 days, 2 persons.

2.5. Conclusion meetings:

2.5.1 - Meeting 1: "International cooperation for an improved surveillance of infectious bursal disease of chickens". This meeting will be held in Harbin Veterinary Research Institute. It will include the two experts from ANSES and Harbin Veterinary Research Institute and delegates from other laboratories in the region. The purpose of the meeting will be to encourage the participation of national reference laboratories to a network for regional surveillance of infectious bursal disease.

Duration: 1.5 days Approximate date: project term 12.

2.5.2 - Meeting 2: Final meeting of the twinning programme, involving the key individuals from OIE, ANSES and Harbin Veterinary Research Institute engaged in the twinning and infectious bursal disease research.

OIE officials will be contacted to define which location (OIE headquarters, Anses or Harbin Veterinary Research Institute) is best suited for this final meeting. The aim of the meeting will be first to evaluate the outcomes of the project, to draft HVRI application to the OIE reference mandate, including fields in expertise that might remain more specific to HVRI or Anses at the end of the twinning programme. The second aim of the project will be to identify areas for ongoing collaboration and a strong basis for maintaining the link between the two laboratories.

Duration: 2 days Approximate date : project term 12 (Exact time to be scheduled by Anses, HVRI and OIE).

2.6: French expert in Paris

Duration: 3 days, 1 person

3 – Advancement of the twinning programme

The implementation of the twinning programme has been approximately delayed by six months, due to the sudden passing away, in July 2013, of one of the scientists in charge of the programme in the parent laboratory. This delay has been maintained during the third year of the twinning program, and was somewhat increased due to the fact that scientists in the parent laboratory (Anses-Ploufragan, France, also National Reference Laboratory for Avian influenza and Newcastle Disease) were strongly diverted to avian influenza work, due to France experiencing two major highly pathogenic influenza outbreaks in 2015-2016 and 2016-2017.

As a consequence and in agreement with HVRI, an extension of the duration of the OIE IBD-twinning programme until 30th September 2017 but with constant budget was solicited from OIE (Annex 1). This extension was approved by Dr Monique Eloit, General Director of OIE in a signed letter dated the 26th January 2017 (Annex 2)

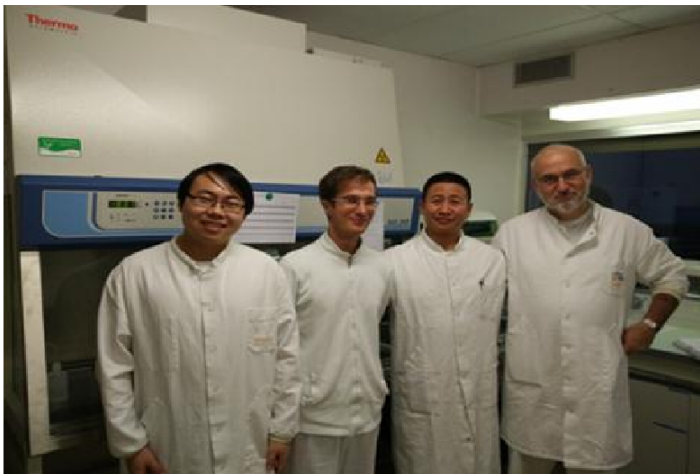
The scheduled activities are listed below and advancement for each of them is indicated in Italics.

3.1. Meeting of the expert scientists of Anses and HVRI, annual review of twinning project, including specific session on reporting to OIE and hands-on reporting exercise (duration 7 days, approximate date project term 9).

Dr Kai Li and Dr Hongyu Cui from HVRI were hosted in ANSES Ploufragan from 28th November to 9th December 2016. Items studied and discussed during this session included

- i) Presentation and visit of Ploufragan ANSES laboratory facilities,*
- ii) Presentation and visit of Laboceca 22, one of the largest French Veterinary diagnosis laboratories,*
- iii) Information and exercise about reporting obligation for an OIE reference laboratory (presentation of OIE organization, reporting to OIE delegates, different types of requests including by private companies, OIE letter on reporting obligations EEV/SL 35.403), iv) hands-on exercise about reporting to OIE taking as examples Chinese strains studied jointly by ANSES and HVRI (results published jointly in international journal Li et al., 2015, MEEGID). The draft report under OIE format used by current OIE reference laboratory for IBD is included as Annex 3.*
- iv) Presentation and discussion of the results of the "ring-trial" organized between ANSES and HVRI during the second year of the twinning project.*
- v) Presentation of protocols evaluating the immunosuppressive potential of IBDV: discussion about official guidelines (OIE diagnosis manual, European Pharmacopeia), presentation of recent results obtained in ANSES and example of a protocol in progress in ANSES laboratory.*
- vi) Review of OIE twinning programme tasks and programming completion of remaining tasks.*

The following documents were provided i) Background information about reporting, ii) OIE guidelines for reporting obligations, iii) Current list of OIE national delegates, iv) Template for reporting to OIE, v) Ring-trial results, vi) Article about ANSES characterization of immunosuppressive potential of IBDV live vaccine, vii) Import permit to organize transfer of reference material from HVRI to ANSES.



(left to right: Kai LI, Sébastien SOUBIES, Hongyu CUI, Nicolas ETERRADOSSI; Ploufragan-lab, ANSES, 2016)

3.2. Training course at ANSES for two research staff from Harbin Veterinary Research Institute for Techniques : pathotyping IBDV isolates (duration 3 weeks, 2 persons, approx.. date = project term 10)

This point has been addressed in November 2015 (already reported in previous annual report). Dr Yanping Zhang and Dr Yongqiang Wang from HVRI were welcome in ANSES Ploufragan, from 16th to 28th November 2015.

Hands-on training was offered for virus culture and isolation (egg inoculation via chorio-allantoic route, lesions of infected embryos, preparation of viral stocks starting from bursae of infected chickens), serological diagnostics (viral neutralization assay, agar gel immunodiffusion), molecular biology (extraction of viral RNA from a viral stock, RT-PCR) and pathogenicity assessment (infection and necropsy of SPF chickens).

Documents and protocols provided:

- *A guide to chicken embryo lesions induced by different prototypic IBDV strains (serotype 1 and 2)*
- *Protocol of Viral titration on embryonated chicken eggs with inoculation onto the chorio-allantoic membrane.*
- *Protocol of Viral titration on chicken embryonic fibroblasts (CEF)*
- *Protocol of antibodies quantification : viral neutralization assay on CEF*
- *Protocol of viral antigen inactivation by β -propiolactone for AGID*
- *Protocol of agar preparation for AGID*
- *Protocol of antibodies detection by AGID*

Discussion about the advancement of the twinning project and provisional timing.



(left to right: Sébastien SOUBIES, Yanping ZHANG, Céline COURTILLON, Yongqiang WANG, Nicolas ETERRADOSSI, Mouna ABED; Ploufragan-lab, ANSES, 2015)

3.3. Study of IBDV pathogenicity in HVRI (Approx. date term 10-11).

The experimental protocols previously shared in previous steps of the twinning programme for the evaluation of IBDV acute pathogenicity were implemented in HVRI to evaluate the pathogenicity of F52/70, 89163, HLJ0405

and HuB-1 IBDV strains, the three former strains being part of the reference strains included in the twinning programme. Percent survival, mean bursa-to-body weight index and mean lesion score were recorded. Consistently with their genetic composition and with previously obtained results, the HLJ0405 and 89163 exhibited a phenotype typical of vvIBDV strains (with HLJ0405 more being pathogenic than 89163) and strain F52/70 exhibited a phenotype typical of classical IBDV under HVRI experimental conditions.

3.4 Training in Quality Insurance (in HVRI) (Duration: 7 days, 2 persons)

This point was not addressed as HVRI diagnostic department was audited by CNAS and received the ISO 17025 accreditation during year 2 of the twinning programme (copy of certificate included in annual report of second year).

3.5. Conclusion meetings "International cooperation for an improved surveillance of infectious bursal disease of chickens" and "Final meeting of the twinning programme" (duration 2 days, approx. date project term 12).

The "International cooperation for an improved surveillance of infectious bursal disease of chickens" and "Final meeting of the twinning programme" were held in HVRI on the 28th June 2017 and 29th-30th June 2017, respectively, in presence of Dr Noriyoshi Ojima, Deputy Regional representative for OIE for Asia and Pacific, Prof Wang Chuanqing from Henan Agricultural University and Prof. Dr. Hermann Müller, formerly Univ. Leipzig, Germany. In the first meeting, the OIE initiative and expectations for the twinning laboratories were presented by Dr Ojima. The previous steps in establishing a scientific collaboration between EU and China in the field of infectious bursal disease of chickens, which paved the way for the HVRI-Anses twinning programme, were presented by Prof H.Müller, who had coordinated these first initiatives. State of the art in IBDV research in Anses and HVRI were presented by researchers from HVRI (Drs Yulong Gao, Xiaole Qi, Yongqiang Wang and Kai Li) and Anses (Dr Nicolas Eterradossi). The achievements of the OIE twinning programme were presented by Dr Eterradossi and Prof Wang and discussed with Dr Ojima. In the second meeting, perspectives for future research were discussed jointly by all participants. Further steps after completing the twinning programme were discussed and will include i) completing the reporting of the twinning contract, ii) submitting to OIE the HVRI application to the mandate of reference laboratory for infectious bursal disease. Further collaboration will include implementing annual meetings between OIE reference laboratories for IBD, re-activating the organization of the international symposium on infectious bursal disease and other immunosuppressive viral diseases of chickens and submitting joint research projects in response to Chinese-French binational or EU international calls for projects.



(sitted left to right: Chuanqing WANG, Xiaomei WANG, Nicolas ETERRADOSSI, Hermann MULLER, Noriyoshi OJIMA, Yuong GAO; standing left to right : Virk ALTAF; Xiaole QI, Mouping WANG, Honglei GAO, Zongxi CAO; Kai LI, Qing PAN, Changjun LIU, Li Gao, Aijing LIU, Tiantian WU, HVRI, 2017)

3.6: French expert in Paris (Duration: 3 days, 1 person)

Not needed, therefore not implemented.

4 – Forthcoming activities within the twinning programme :

Final reporting + HVRI submission of its application to the OIE reference mandate for infectious bursal disease of chickens.

5 – Financial aspects

To be reported separately after the 30th September 2017

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