Introductory statement from the OIE Director General
for publishing an OIE Mission report to the People’s Republic of China

In April 2002, the OIE Director General, Dr Bernard Vallat, was invited by the Chinese Government to visit the People’s Republic of China (PRC). During this visit, a memorandum was signed with the Minister of Agriculture, stating the PRC’s strong interest in the work of the OIE and its willingness to take an active part in that work, notably in connection with the PRC’s new membership of the World Trade Organization (WTO).

Following this visit, a very interactive relationship was established with the PRC, notably in the provision of supplying Standards (translation by Chinese Authorities of the Terrestrial Code, and the Terrestrial Manual into Chinese), the participation of the PRC in Technical regional meetings, and the invitation to the OIE Director General to a Regional Conference on Influenza in Beijing in March 2004. More recently, following a request from the Delegate of the PRC to the OIE, experts were sent to China to assess some of its national reference veterinary diagnostic laboratories and the compliance of the production and quality of avian influenza vaccines with OIE international standards.

For this latter purpose, the OIE convened a team of world renowned experts in agreement with the PRC authorities to assess the suitability of the laboratories included on the list proposed by the Chinese veterinary authorities to meet the requirements and obligations to be designated by the OIE and its Member Countries as Reference Laboratories. The team also assessed the potential international expertise of the scientists at these laboratories, provided advice to the Chinese veterinary authorities on how to become an OIE Reference Laboratory when relevant, and gave an overview of current OIE policies.

Dr Vallat announced this mission in China to all OIE Delegates during the 72nd General Session in Paris (May 2004). During May 2004, OIE experts visited the Chinese Institute of Veterinary Drug Control (IVDC), Beijing, the Harbin Veterinary Research Institute (HVRI), Harbin, and the National Animal Quarantine Institute (NAQI), Qingdao.

The mission was successfully accomplished and its Report is now disclosed herewith for all OIE Delegates and International Organizations with the agreement of the PRC’s OIE Delegate.

The OIE welcomes this collaboration which significatively improves transparency on the worldwide animal health situation and the efficiency of the permanent fight against animal diseases and zoonoses in the world.

Paris, 03-08-04
MISSION REPORT
OIE Mission to the People’s Republic of China
10–19 May 2004

Aim of the meeting

Following receipt of a request from the Delegate of the People’s Republic of China for the OIE to send experts to that country to assess some of its national reference veterinary diagnostic laboratories, and the production and control of avian influenza vaccines, the OIE convened a team of experts in agreement with Chinese authorities to carry out these tasks. An expert team, composed of Dr James Pearson and Dr Philippe Vannier, undertook the first request, that of assessing the laboratories. Another OIE expert team, composed of Dr David Swayne and Dr Michel Lombard, undertook the second request, that of evaluating the manufacture and control of avian influenza vaccines in China.

Reference Laboratories’ evaluation

To assess the suitability of the laboratories, proposed by the Chinese veterinary authorities, to meet the OIE Reference Laboratory (RL) requirements and obligations to be designated by the OIE as Reference Laboratories; to examine the national and international expertise of the potential OIE experts within these laboratories; to provide advice to the Chinese veterinary authorities on how to become an OIE Reference Laboratories when relevant; and to provide an overview of the OIE programmes.

Evaluation of the avian influenza vaccine (inactivated) manufacturing and controls

To assess the quality of the avian influenza vaccines produced in laboratories in the People’s Republic of China in relation to the standards contained in the OIE Manual of Diagnostic Tests and vaccines for Terrestrial Animals with special emphasis on seed management (characteristics of the seed, method of culture), validation as a vaccine, method of manufacture, in-process control, batch control (sterility, safety, potency, duration of immunity, stability, preservatives, precautions), tests on final products (safety, potency, purity), and to provide advice to the Chinese veterinary authorities on the above.

Summary

Visits were made to the China Institute on Veterinary Drug Control (IVDC), Beijing; the Harbin Veterinary Research Institute (HVRI) of the Chinese Academy of Agricultural Sciences, Harbin; and to the National Animal Quarantine Institute (NAQI), Qingdao. The following National Reference Laboratories were reviewed: classical swine fever (CSF) and rinderpest at the IVDC, avian influenza (AI) and contagious bovine pleuropneumonia (CBPP) at the HVRI and Newcastle disease (ND) and bovine spongiform encephalopathy (BSE) at the NAQI.

The following discussions and demonstrations on AI vaccine production were provided: measures taken to ensure the quality of inactivated AI vaccines produced in China at the IVDC, the GMP laboratory that was responsible for manufacturing and QC of inactivated AI vaccine at the HVRI, GMP laboratory for manufacture and QC of inactivated AI vaccine of Yebio at NAQI.

A detailed summary of the visits is enclosed.
Reference Laboratories’ evaluation

The CSF, AI, NDV and BSE national reference laboratories are functioning as centres of expertise and standardisation, storing and distributing diagnostic reagents, developing new diagnostic procedures, gathering epizootiological data, and providing training to other laboratories within China. These laboratories have well trained staffs. The NDV and BSE laboratories have excellent facilities and equipment. The AI laboratory facilities are adequate but old; a plan is being developed to replace this old portion of the HVRI. The laboratory is generally well equipped.

The CSF laboratory is also in an older facility and the equipment appears to be marginal for the support of the sophisticated studies expected of an OIE RL; no plans for replacement or upgrading of the facility or the equipment were provided to the team.

All of the laboratories are very familiar with the OIE Standard diagnostic techniques and are attempting to use them. The Provincial laboratories are responsible for routine CSF, AI and ND surveillance, with samples for AI and ND virus isolation forwarded to the national reference laboratories.

The CSF reference laboratory receives very few tissue samples for virus isolation but does receive isolates for characterisation. Most of the BSE surveillance samples, about 5000 per year, are tested at the BSE national reference laboratory. The NDV and BSE laboratories have established a quality assurance programme, been audited by a third party and received quality assurance accreditation.

The function of the CBPP and rinderpest national reference laboratories is to perform surveillance testing to confirm that China is free of these diseases. An extensive serological surveillance programme was initiated about 4 years ago for both diseases using the OIE prescribed tests with confirmation by the OIE alternative tests. China is planning to submit the data to the OIE as part of the application to be designated free of these diseases. There has been no report of rinderpest since 1955 and the last of isolation of CBPP was in 1989. The CBPP laboratory has limited capability to identify isolates and has not recently received tissues from any suspect cases. The rinderpest laboratory has also not received any tissues from suspect cases and does not have the capability to perform complete characterisation of isolates.

The complete procedure for applying to become an OIE RL was supplied to each laboratory and to the Ministry of Agriculture in Beijing.

An overview of the OIE and of laboratory biosafety was provided in Beijing and a seminar with an overview of the OIE was provided in Harbin.

Evaluation of the avian influenza vaccine (inactivated) manufacturing and controls

Institute of Veterinary Drug Control: Initially Outlines of production were presented, as well as general data concerning the method of preparation of the finished products (H5N2 and H9). Subsequently a more complete presentation was made that discussed the quality controls for in-process products and finished products. The subsequent presentation summarised the measures taken to ensure the quality of inactivated AI vaccines produced in China, i.e. product license system, audit system by IVDC inspectors in manufacturing plants, and tests on each batch produced. The IVDC approval for batch release is granted when satisfactory test results by the manufacturer are received. In this case, the Manufacturer's Release Certificate is countersigned and stamped by the IVDC and returned to the sender accompanied by a number of “Approbation labels” equivalent to the number of vaccine bottles filled with the batch volume. Satisfactory batches are identified by the “Approbation labels”, which are additional government labels stuck on the vaccine bottle cap, with the aim to prevent sales of fake vaccines.
Harbin Veterinary Research Institute: The vaccine production procedures were discussed with the OIE experts. A low path AI (LPAI) seed virus was selected for vaccine production in China and the only H5 LPAI virus available was A/turkey/England/N28/1973 (H5N2). Efficacy studies conducted at HVRI have shown this virus strain to be effective in protecting chickens from clinical disease and death following intramuscular challenge with HPAI virus strain A/goose/Guangdong/1/96 (H5N1). We were told, the AI virus H5N2-Weybridge is produced on conventional eggs from farms under contract and submitted to strict serological controls by HVRI. The old buildings of the laboratory were furnished with new equipment, walls, ceilings, floors and stainless steel vessels complying in with most of the Western GMP requirements. Nevertheless GMP and QA did not appear adequate throughout the production steps. Additionally, we noticed that inoculation of eggs and collection of virulent fluids was not performed using good protective measures for technicians. The inactivation process is carried out in one operation (37°C) in a stainless steel vessel but without transfer to a second sterile tank. The antigen is kept at room temperature in the inactivation tank during the 7 days required for the inactivation control test in SPF eggs (three passages). Filtrated antigen is then transferred and emulsified before the filling operations. In process control follows the Chinese requirements for licensing and the results reported by our Chinese colleagues were satisfactory. Batch and final product Quality Controls are performed according to the Chinese regulations and are audited from time to time by inspectors of IVDC. All the tests listed in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals are performed with satisfactory results according to the managers. We were shown a Batch Release Certificate fully fulfilled and countersigned by authorities of the IVDC in Beijing.

The National Animal Quarantine Institute: This institute has the control of a manufacturing company for vaccine production: YEBIO Bioengineering Co. The OIE team visited the GMP laboratory for manufacture and QC of inactivated AI vaccine of this company. The team was told or observed that the production of AI virus, H5N2-Weybridge, is performed on conventional eggs from farms under strict serological control by Yebio. We observed that the buildings were brand new and furnished with the best equipment available in China; walls, ceilings, floors and stainless steel vessels complied completely with Western GMP requirements. Nevertheless GMP and QA did not appear to us to be equivalent in level throughout the production steps. It was noted that the inoculation of eggs and collection of virulent fluids are not performed using good protective measures for technicians. We noted, with satisfaction, the inactivation process includes a transfer to a second sterile tank and storage until the results of the inactivation control tests are obtained. In process controls and batch and final product Quality Controls were as reported for HVRI and were satisfactory.

Key person(s) met and subjects of discussion

The procedures for OIE RL approval and for a country to be declared rinderpest and CBPP free by the OIE was discussed in detail with Dr Zhang Zhonqiu, Deputy Director General, Bureau of Animal Production & Health, Ministry of Agriculture. The relations between China and the OIE were discussed with the OIE Delegate Dr Shen Zhenzhao, Director General, Bureau of Animal Production & Health, Ministry of Agriculture.

Conclusions and possible follow-up

Reference Laboratories’ evaluation

The national reference laboratories for CSF, AI, BSE and NDV have attempted to meet all the items in the OIE RL requirements. These laboratories have well trained and capable staffs; the BSE and NDV laboratory has received quality assurance accreditation. The BSE, AI and NDV laboratories routinely receive tissue samples for isolation and characterisation and they appear to be proficient at these procedures. The AI and NDV laboratories have limited interaction with the other OIE Reference Laboratories and the BSE interaction has been primarily with the OIE Reference Laboratory in Switzerland. The volume of BSE samples has increased from about 1000 in previous years to 5000 this year; the volume of samples for AI and NDV diagnosis is low for a country the size of China. The CSF laboratory does very little or no routine diagnostic testing; consequently, there was concern about their capability to fulfil the OIE RL mandate to provide diagnostic testing for the OIE Member Countries. As China is apparently free of CBPP
and rinderpest, these national reference laboratories are not receiving tissues from suspect cases and have limited experience in agent identification; consequently, it would be difficult for them to meet the complete mandate of OIE Reference Laboratories (RL).

**Recommendations for becoming OIE Reference Laboratories:**

1. As stated previously, the NDV, BSE and AI laboratories are well equipped, have a capable staff, have diagnostic reagents that they have validated, have at least one well qualified expert, have biosafety level 3 facilities and follow the procedures outlined in the OIE *Terrestrial Manual*. The opinion of the OIE experts is, if they address the following suggestions, they would qualify to be considered by the OIE Biological Standards Commission to become OIE Reference Laboratories. They should increase their expertise and testing competency by establishing or increasing their interaction with the other OIE RL. This close interaction should include exchange of isolates and proficiency tests; the exchange, standardisation and validation of reagents, standardisation of techniques and test interpretation; an in-depth visit by the proposed OIE expert to the appropriate OIE RL; future training of junior scientists at OIE RL; and schedule periodic visits and critique of the Chinese laboratory by an OIE RL expert.

2. The NDV and AI surveillance programme should be expanded to increase the competency of the laboratory to test a larger number of more diverse samples and to better estimate the prevalence of the diseases.

3. The plan to construct a new more biosecure AI laboratory and animal facilities is to be commended and should be expedited. The biosecurity procedures presently being used should be increased and all the virus isolation, as well as all work with H5 and H7 isolates, should be performed in biosafety level (BSL) 3 facilities. The work with other AI isolates in BSL 2 facilities should be re-evaluated.

4. A quality assurance programme should be initiated at the HVRI

5. CSF laboratory:
   a) The CSF surveillance programme should be modified so that the laboratory receives a large number of diverse diagnostic samples for virus isolation and serology in order to insure that they can provide competent results to Member Countries if they become an OIE RL.
   b) They should increase their expertise and testing competency by establishing or increasing their interaction with the other CSF OIE RL. This close interaction should include exchange of isolates and proficiency tests; the exchange, standardisation and validation of reagents; standardisation of techniques and test interpretation; an in-depth visit by the proposed OIE expert to a CSF OIE RL; future training of junior scientists at OIE RL; and schedule periodic visits and critique of the Chinese laboratory by a CSF OIE RL expert.
   c) Plans should be developed to upgrade the facilities and equipment.
   d) The biosafety procedures should be re-evaluated to determine if the virus isolation and characterisation work should be moved to the BSL 3 facility.
   e) The quality assurance programme should be expanded and the laboratory should work toward third party accreditation under ISO 17025.

6. The CBPP and rinderpest laboratories appear to be competent at performing the surveillance tests expected of them to determine disease freedom. If there is a desire to be an OIE RL, the following should be addressed:
   a) They should increase their expertise and testing competency by establishing or increasing their interaction with the other OIE RL. This close interaction should include
exchange of isolates and proficiency tests; the exchange, standardisation and validation of reagents, standardisation of techniques and test interpretation; future training of junior scientists at OIE RL; and schedule periodic visits and critique of the Chinese laboratory by an OIE RL expert.

b) Develop the expertise and reagents to isolate and completely characterise the agents.

c) Establish a programme to obtain diagnostic samples for agent isolation, this should also include positive samples for agent identification and characterisation.

d) Provide in depth training of the proposed OIE expert in an OIE RL on all the diagnostic procedures for the disease.

e) A review of the resulting improved laboratory capability by an OIE expert for the disease.

**AI vaccine evaluation**

The OIE team concluded that their Chinese colleagues are very knowledgeable and capable. After only a few months of AI vaccine control, they have performed scrupulously their job of the market release of AI vaccines in China.

The OIE team concluded that each batch of AI vaccine sold in China has satisfied all listed QC tests in the manufacturer QC laboratory. These results are validated on the Release Certificate by the additional signature and stamp of the Head of IVDC for the AI vaccine Control department. The procedure to stick “Approbation labels” from IVDC on each bottle cap is an additional stringent procedure to prove that the vaccine has been quality approved by the Government. The Chinese are complying with the level of manufacturing, QC, and QA for AI vaccine requested by the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* Chapter I.1.6, Principles of Veterinary Vaccine Production.

**Recommendations:**

1. **Seed Virus:** The seed virus needs to be re-evaluated by HVRI every 2–3 years to determine if it is still useful for protecting poultry in the field.

2. **Challenge Model:** To determine efficacy, the potency challenge model needs to be changed to more an effective the seed stock selection process:

   o Because influenza viruses change antigenically by the natural process of mutation or drift, the challenge virus should be changed to a current circulating virus or viruses. This should be done by an on going national surveillance programme to isolate AI viruses from poultry, especially ducks and geese, and to conduct genomic sequence analysis of such viruses to determine if a single or multiple lineages are present. If a single lineage is present, a single challenge virus is appropriate, but if multiple lineages are identified, several challenge viruses may be needed depending on how close they are phylogenetically to vaccine strain.

   o The potency challenge should be changed from intramuscular route to intranasal route of inoculation with the minimum dose being either $10^6$ EID$_{50}$ (mean embryo infectious doses) or $10^2$ CLD$_{50}$ (mean chicken lethal doses) of virus per bird.

   o Parameters of protection in the potency challenge model should continue to use the prevention of clinical signs and death. In addition, evaluation of the reduction in replication of the challenge virus in vaccinated birds is needed to better demonstrate reduced environmental shedding and thus potential for reduced virus transmission. The reduction in average virus titre at the peak replication time should be a minimum of $10^2$ EID$_{50}$/ml of media from respiratory and gastrointestinal tract.
Protection should be determined not only for chickens but also for other important poultry species including quail, ducks and geese. The latter may show no mortality on challenge, but are important hosts in maintaining and disseminating the H5N1 HPAI virus, thus emphasising the need to evaluate reduction in shedding of challenge virus from respiratory and gastrointestinal tract to determine efficacy and potency.

3. **Master Seed Virus (MSV).** The immunogenicity test (2.1.4.b5) challenge model should be changed to intranasal with a recent H5N1 HPAI virus. A HI titre of >6 log₂ is a reasonable alternative assessment method for immunogenicity control test of master seed virus.

**Working Seed Virus (WSV).** The storage temperature of WSV (2.1.4.d.) should be changed from –20°C to –70°C.

4. **Manufacturing Process and In-Process Controls:**
   a. Protection of technicians during inoculation of eggs and collection of AI virus should be improved in the two manufacturing sites.
   b. Inactivation process should include a transfer to a second sterile vessel in the Harbin VRI.
   c. A Quality Assurance department should be created in each of the two sites for better management of the Standard Operating Procedures, internal audits, and comprehensive recording of the environmental measures.

5. **Finished Product:**

The current policy for potency test (a SN titre 21 days PV of >6 log₂) and for identification of approved batches (official label on the vaccine bottle cap) should be continued.

- The vaccine should have sufficient antigen to produce a strong protective immune response. This should be a minimum of 50 mean protective doses (PD₅₀), which approximates 5 g of haemagglutinin protein per dose. Such a requirement is not codified in current documents.

- The Immunogenicity Test (2.1.4.b5.), which recommends the use of a challenge model, should be changed to intranasal challenge with a recent H5N1 HPAI virus. A HI titre of >6 log₂ is a reasonable assessment method for potency of final product.

- Other H5 LPAI viruses used throughout the world for vaccines have been shown to be efficacious against the current Asian H5N1 HPAI viruses, as demonstrated in experimental studies conducted in Hong Kong and the USA. These vaccine strains include the H5N2 vaccine virus used in Mexico and vaccine viruses contained in vaccine banks in the USA and Europe. Importation of such vaccines into China should meet Chinese National Vaccine Standards or equivalent; the efficacy and potency of these imported vaccines against current H5N1 Asian viruses should be demonstrated. Likewise, China should only export AI vaccines that meet their National Standards for purity, safety and potency as codified in their regulations. Unlicensed AI vaccine products should not be imported or exported.

- **H9N2 LPAI Vaccine Standardisation:** A single page was provided in the packet from the Institute of Veterinary Drug Control on Quality Standard of the inactivated AI vaccine (Subtype H9). Although, evaluation of H9N2 AI vaccine was not part of the official request to OIE, the following are recommendations submitted for consideration:
  - Seed strain selection should be based on virus surveillance for H9N2 LPAI viruses in all of China and subsequent phylogenetic analysis of isolated viruses to identify the predominant lineage or lineages of virus
• Efficacy testing of seed strain should be similar to the H5N1 HPAI virus. However, the LPAI viruses do not cause clinical signs or death in experimental studies thus determination of efficacy should be based on reduction in replication of the challenge virus in vaccinated birds.

• Manufacturing standards should be modelled after those for H5N1 HPAI.

Team Members: J. Pearson, M. Lombard, D. Swayne, and P. Vannier

Documents joints / Documents enclosed: A detailed summary of the visit to each laboratory is enclosed.

Diffusion : Directeur général, Chefs de service et adjoints, Commission administrative, Coordinateurs régionaux, Chargés de mission, Vanessa Leverkuehn, Documentaliste.
Detailed Summary

Mission of OIE Experts to the People’s Republic of China

The following is a detailed summary of visits to the Chinese laboratories and vaccine production facilities

Evaluation of Laboratories

The China Institute of Veterinary Drug Control (IVDC), Beijing

This institute was established in 1952 and is located in Beijing. The IVDC consists of 18 departments plus the classical swine fever and rinderpest national reference laboratories. These reference laboratories are appointed by the Ministry of Agriculture.

National Classical Swine Fever Reference Laboratory (NCSFRL)

The NCSFRL has a staff of twelve persons (three professors, three associate professors, four assistant researchers, two technicians); in addition five graduate students (two PhD and three MSc) are doing research in the laboratory. The primary activities of the laboratory are research, providing reagents and training. The role of the laboratory is as follows:

• evaluating and, if necessary, arbitrating the results of CSF diagnostic testing done in other laboratories in China;

• preparing, storing, and distributing diagnostic reference reagents;

• gathering, processing, and analysing CSF epidemiology data in order to provide information to the Government and farmers;

• developing new methods and reagents for diagnosis, prevention, and control of CSF;

• establishing standard technique;

• training the technical staff from the regional laboratories;

• organising CSF scientific meeting.

The primary diagnosis of CSF is done in the Provincial laboratories (about 31) and the primary method used is the fluorescent antibody test on frozen sections of tissues as described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual). CSF vaccination is compulsory; consequently, serology is not a primary diagnostic method; if serology is conducted, the competitive ELISA kit, produced and supplied by the NCSFRL, is used. The NCSFRL has received 80 strains of CSF virus over the last several years from the Provincial laboratories. The strains have been partially sequenced showing the existence of two main genotypes in China. Strains seem to be regularly isolated from the field and supplied to the NCSFRL.

The laboratory is BSL-2 and composed of eight rooms. In another building, the CSF seed viruses are handled and stored in a BSL-3 laboratory with controlled ventilation (negative pressure) and HEPA inlet and exhaust filtration.
Conclusions

• The staff is well trained and competent.

• The laboratory equipment and facilities are generally adequate but plans should be developed to upgrade the facilities and to purchase state of the art equipment.

• The laboratory is technically competent to characterise the isolates it receives.

• The procedures used are those outlined in the OIE Terrestrial Manual.

• Most of the CSF diagnosis is performed in the Provincial laboratories; consequently there is very limited CSF diagnostic testing on tissues from suspected cases performed in the laboratory.

• There appears to be a need for a more formal relationship with the provincial laboratories for such things as laboratory approval, coordination of quality assurance testing, proficiency testing, confirmation of test results, etc.

• There has been scientific collaboration with other scientists; however, it is recommended that there be greater interaction with the OIE CSF reference laboratories.

• The research performed has been published; however, it is recommended that more of the publications be in English so that it is available to the scientific community.

National Reference Laboratory for Rinderpest (NRLR)

The NRLR has a staff of twelve persons (five senior scientists, five assistant professors and two technicians).

The primary activities and role of the laboratory are similar to those of the NCSFRL. Epistemiosurveillance is being carried out by the NRLR in the framework of a FAO programme. 20,000 kits are provided each year by FAO to test 37744 sera collected from cattle in 12 border Provinces considered at high risk. This survey was initiated in 2001 and the results have all been negative. Rinderpest has not been reported in China since 1955 and the disease apparently has been eradicated. The laboratory is not receiving samples for virus isolation or identification and the capability to characterise Rinderpest isolates is limited. The Chinese government would like the free status of the country to be recognised officially by OIE.

The live strains of the virus are stored in the BSL3 laboratory.

Conclusions

The staff is well trained and the laboratory is adequately equipped to perform the surveillance testing that is being conducted. It does not appear appropriate for China to initiate a procedure for the recognition of the laboratory as an OIE reference laboratory as every indication is that the disease has been eradicated. Consequently, the scientists, even thought well trained, cannot have the practical experience to be OIE experts.

The Harbin Veterinary Research Institute (HVRI) of Chinese Academy of Agricultural Sciences, Harbin

This research institute is the biggest veterinary laboratory in China and has a staff of 509 people with seven divisions. It also has a large farm situated on the outskirts of Harbin. The HVRI has been designated the National Key Laboratory of Veterinary Biotechnology. It also has a Department of Animal and Poultry Infectious Diseases, Avian Influenza Centre, Centre for Diagnosis and Epidemiology of Animal Infectious Diseases, Experimental Animal Centre,
Bioproduct Industrialisation Centre, and Scientific Information Centre. The Ministry of Agriculture has appointed HVRI as the national reference laboratory for avian influenza and for contagious bovine pleuropneumonia (CBPP).

**Avian Influenza (AI) National Reference Laboratory**

Dr Hualan Chen directs the AI national reference laboratory. She has a staff of 30, which includes scientists, technicians and graduate students. She received her PhD in China and has completed 3 years of post-doctoral training at the CDC in Atlanta, Georgia, US. Research quality is superb and the studies have been published in international journals in English as well as National Chinese Journals. She employs the latest biotechnology in her research, including use of eight-plasmid infectious clone system for generation of reassortment AI viruses for production of vaccines and to study disease pathogenesis.

**AI Reference Laboratory Facilities**

- The laboratory is located in three buildings:
  - **Biosafety level 1 (BSL-1) laboratory**: Includes multiple rooms where the majority of studies are conducted; no work with infectious virus is conducted in these laboratories. The BSL-1 facilities include laboratories for molecular and serological studies, clean tissue culture and a shared equipment laboratory (centrifuges, spectrophotometers & gel image analyser). The AI reference laboratory has the following specialised equipment: a dedicated eight capillary sequencer (Beckman-Coulter, Genetic Analysis System CEQ 8000 – eight samples/1.5 hours, 96 samples/22 hours), and four thermocyclers (three 96-well and one 24-well) for molecular studies. The limited software and information technologies available have restricted phylogenetic analysis studies that can be conducted by the laboratory. The equipment available is functional and productive research is being performed, but molecular studies are limited because of low capacity and insufficient analysis capabilities. The following would increase the molecular capability of the laboratory: 1) Replacement of existing sequencer with an ABI 3100 or 3730 type sequencer with 48 or 96 capillary systems, 2) Purchase an additional two 96-well thermocyclers and associated robotics for processing molecular samples for the sequencing, 3) Purchase new software and computer systems for phylogenetic analysis of sequence data, and 4) Purchase two light-cyclers to develop, validate and use real-time RT-PCR for rapid avian influenza diagnostics.
  - **BSL-2 Laboratory**: A single moderate-sized laboratory where H9N2 low path AI (LPAI) virus and diagnostic specimens for H9N2 isolation are handled. The laboratory has two “biosecure inoculation rooms”, with HEPA filtered exhaust; it includes two clean benches and serological testing equipment. These rooms are used to inoculate embryonating chicken eggs with LPAI and to harvest and test the fluid from the inoculated eggs. This laboratory had low usage because there has been a decreased emphasis on H9N2 LPAI research and more time and resources are being devoted to H5N1 HPAI virus work, which must be conducted in a BSL-3+ laboratory. The laboratory complex needs to be upgraded to at least BSL-2. Biosafety cabinets have been ordered to replace existing clean benches. However, the facility needs a major change in operating protocol to meet access restriction standards for of BSL-2 and the egg incubators should be moved from the hallway into the laboratory. The processing of field samples under these conditions poses a risk as it is possible that tissues from a flock thought to be infected with LPAI could turn out to be HPAI and the virus would then be isolated in this laboratory that has minimal biosafety. It is recommended that plans be developed to do all the virus isolation from field samples in a BSL 3 laboratory.
**BSL-3+ Laboratory:** There is a single shared BSL-3 laboratory facility, of approximately 200 sq metres, that is used by all groups at the HVRI. Individual groups schedule time in the facility, but only one research group at a time is allowed to use the facility. The facility occupies part of one floor in the pre-existing building. The laboratory was constructed in 1999 as a renovation of BSL-1 space and follows the box-with-in-a-box concept (i.e. a clean corridor circles the laboratory and is within the existing outside walls of the building). The BSL-3 Laboratory has no windows. Entry is through separate male and female change rooms with shower facilities. We did not enter the BSL-3 facility but observed it from the clean corridor. The facility has single HEPA filtered intake and double HEPA-filtered exhaust air systems. The sewage is treated by heat and chemicals. A guard is stationed at the entry point 24 hours a day 7 days a week. The guard has a list from the HVRI Director of the persons that are allowed into the laboratory and the individual employee must sign-in and out. Materials exit the laboratory through a pass-through airlock with formaldehyde fumigation (separate ventilation from rooms) – no dunk tank pass-through is available and the airlock is not large enough to fumigate large equipment leaving the laboratory. Items leaving the laboratory are double-bagged; the outside disinfected and carried through the shower with the employee for movement to the animal facility or for removal of non-infectious material. A Safety Officer is responsible for facility, maintenance and testing. Entry into the BSL-3 laboratory requires training for individual employees. In 2003, this facility was used by the Director (Dr Kong) for SARS-coronavirus experiment in civet cats.

- Other support systems:
  - A shared fluorescent-activated cell analyser and sorter was located in the hallway adjacent to BSL-3 laboratory in BSL-1 space
  - Animal experimental facilities are located in a separate building that had been renovated in 1990 under a joint China-Australia project.
    - These facilities are in need of replacement since they do not meet international standards for animal welfare and biosecurity/biosafety
    - The isolation cabinets used for primary containment of the AI virus are adequate, but should not be the only structural systems for biocontainment, especially for HPAI virus experiments. The isolators (primary barrier) should be located within a secondary barrier (building) that meets BSL-3Ag or OIE containment group 4 standards.
    - Animal isolators should be obtained that will allow studies in ducks and geese.
  - Specific Pathogen Free facilities for production of SPF chickens and eggs:
    - This is a very valuable resource for the institute, but the production levels during some periods may not be adequate to supply the research and diagnostic needs of the AI and other poultry disease research groups. Expansion of the SPF poultry production facility may be necessary.

- Laboratory functions:
  - The AI reference laboratory developed the antigen (baculovirus expressed AI H5) and positive control sera, which is manufactured by the Institute as test kits. These kits are sold to Provincial laboratories for serological testing of poultry for AI infection (agar gel precipitin – AGP). Also, reagents were developed and are manufactured as ELISA kits to detect AI infection (type A influenza) in chickens, but ELISA has little use in the field. The laboratory provides H5, H7 and H9 reagents for HI testing to a few provincial laboratories. The procedure to confirm Provincial laboratory AGP positive samples was not clear. Confirmation of H and N typing and follow up investigation of H5 and H7 positives is an important part of an AI control programme. In order to
insure valid results a quality assurance programme for the Provincial laboratories, including proficiency testing, should be established. HVRI should develop and validate assays to detect H5N1 infection in ducks and geese – current AGP and ELISA tests are inadequate to provide proper testing.

- AI reference laboratory is responsible for isolation and characterisation of H5N1 AI viruses in all of China. The competence is very high in this activity, but the number of surveillance samples supplied to the AI reference laboratory is limited. It would seem that a reliable HPAI surveillance programme for a nation the size of China should generate a much greater number of virus isolation and positive serology samples. It is suggested that the China Epizootiology Centre, National Animal Quarantine Institute develop a surveillance-sampling plan for China in order to better establish the distribution of AI viruses. This programme could include training Provincial and county animal health authorities in the sampling plan so that adequate samples are provided to HVRI for assaying. Also, sero-surveillance should be designed and conducted for all poultry species, especially ducks and geese, to get a better understanding of the current AI situation in China.

- AI Reference laboratory should consult international experts on development and validation of RRT-PCR test to detect the H5N1 infection. The current primary test used for detection of AI virus, i.e. virus isolation and identification is slow but is probably adequate for the small number of samples received. If the surveillance is increased RRT-PCR would allow efficient testing of a larger volume of samples and give timely information on active infections, which will facilitate control and eradication strategies.

- The laboratory apparently does not have or has a very minimal quality assurance programme.

**Conclusions**

- The laboratory has a very technically competent and experienced staff performing excellent diagnostic and research work.

- The laboratory has adequate equipment to perform the current volume of AI diagnostic testing, but additional equipment is needed if the volume of diagnostic testing increases.

- Additional equipment would facilitate the molecular research that is being conducted.

- The biosecurity of the facilities is marginal; the construction of new facilities is planned.

- The laboratory has the technical expertise to evaluate AI viruses from other Asian countries.

- The laboratory should exchange reagents and virus strains with other OIE Reference Laboratories and experts.

- A quality assurance programme based on ISO 17025 should be put in place

**Contagious Bovine Pleuropneumonia (CBPP) National Reference Laboratory**

The studies on the disease started in 1955 and a lapinised live strain, adapted to the ovine species, was prepared as a vaccine in Harbin and used successful to control the disease; vaccination was stopped 20 years ago. The last isolation of the CBPP agent was made in 1989 and there has been no evidence of infection since that date. China wishes to obtain OIE recognition as being CBPP free. Serological surveillance is carried by HVRI and sera from the Provinces considered to be high risk are being tested. Starting in 2000, 15 000 sera have been tested each year and the programme will continue on the same basis until 2005.
The staff of the national laboratory is composed of twelve people: one professor, two associate professors, six assistant professors and three technicians; plus five students (two PhD and five MSc). The complement fixation test is the primary test used and all suspect animals are slaughtered. A competitive ELISA, developed by CIRAD-EMVT in France and commercialised by a private company, is used for confirmation of suspect reactions, all of the suspects have been negative. An ELISA using a recombinant antigen is under development. For agent characterisation, when colonies are identified on agar plates, biochemical tests are performed to identify the *Mycoplasma* isolated. If the CBPP agent is suspected, the colonies are cloned and DNA is extracted for PCR allowing a characterisation using validated primers.

There has been scientific collaboration with OIE reference laboratories in Portugal and France.

**Conclusion**

The survey appears to be carried out in a proper way. Even though the laboratory is well equipped and has trained personnel, the lack of experience in working with isolates for the last 15 years would limit their expertise.

**National Animal Quarantine Institute (NAQI), Quingdao**

This Institute is under the authority of the Chinese Ministry of agriculture and is composed of 140 people and includes:

- The China Epizootiology Centre;
- The National Exotic Animal Disease Centre composed of the BSE national reference laboratory and Newcastle national reference laboratory;
- The Animal Health and Animal Product Safety Control Centre;
- The National Animal Disease Diagnostic Reagent Centre.

The NAQI has the charge of the secretariat of the national standard committee of animal quarantine technology.

The NAQI has the control of a manufacturing company for vaccine production: YEBIO Bioengineering Co., which is located on the NAQI.

The China Epizootiology Centre is providing technical supports for the government by carrying out surveillance and epidemiological investigation on the major animal diseases according to national epidemic control programmes for diseases such as AI, CSF, West Nile Fever and others. The centre is collecting data to support the health status of the country and to record notifications of diseases. Also an attempt is being made to register the geo-spatial position of the farms.

The Diagnostic Centre for Exotic Animal Diseases (27 people) was created in 2001 and consists of the national reference laboratories for BSE and Newcastle diseases.

The laboratories quality assurance system and methodology is accredited by a third party. The quality assurance accreditation was obtained in 2003 and the laboratory works in compliance with ISO 17025. The laboratory biosafety is managed by a specific department.

**The Newcastle National Reference Laboratory**

The laboratory is in a BSL-3 facility with HEPA filtered inlet and exhaust. The mission is research, diagnostic testing, characterisation of the viruses isolated, standardisation of diagnosis, providing reagents and training. One hundred sixty viral strains have been characterised and sequencing has demonstrated that the genotype 7 was predominant in
China chickens whereas the genotype 6 is dominant in pigeons. All the techniques used to identify the ND viruses are as described in the OIE Terrestrial Manual: HA, HI, virulence determination by intracerebral inoculation of day old chicks and real time-PCR (RT-PCR). ND virus RT-PCR and monoclonal antibodies have been developed in this laboratory and the gene of the F protein was cloned and expressed. Over the last three years, 20,000 samples have been submitted to the centre for isolation and characterisation; over 19,000 of these samples were to qualify poultry for export; the remainder were for diagnostic purposes; a small number of samples for a country the size of China. The laboratory has excellent facilities, very good equipment and a well trained staff. It is recommended, that if a programme is initiated to increase the number of NDV surveillance samples, the laboratory should initiate plans to buy the equipment and start the validation of RRT-PCR.

The BSE National Reference Laboratory

This laboratory is in charge of the epidemiovigilance of the BSE in China. For that purpose, 5000 brains per year coming from the whole of China are being examined; no evidence of BSE has been detected. The standard techniques used are those described in the OIE Terrestrial Manual and include histopathogy and immunochemistry. The laboratory has evaluated and used, on a limited basis, the commercially available rapid tests. The western blot test has also been used for confirmation testing. The staff of the laboratory has an ongoing cooperative relationship with the OIE Reference Laboratory in Switzerland to help insure competency is being maintained. They have also cooperated with a number of other laboratories in various countries. For research, the PrP gene was expressed into E. coli and monoclonal antibodies have been developed. The laboratory has excellent facilities with very good equipment and a well trained staff.

Conclusions, ND and BSE national laboratories

• The facilities and equipment of the Institute and particularly of the two national reference laboratories are excellent and the staff is very competent. If the volume of Newcastle samples were increased, additional equipment would be needed.

• The laboratory has established a quality assurance programme and is accredited under ISO 17025, which is a major achievement that few laboratories in Asia have accomplished.

• The number of samples for the BSE surveillance seems to be appropriate whereas for Newcastle disease, the number of samples submitted for disease diagnosis appears to be low.

Evaluation of the Avian Influenza Vaccine (Inactivated) Manufacturing and Controls

The China Institute of Veterinary Drug Control (IVDC), Beijing

This institute was established in 1952 and is located in Haidian district of Beijing. The IVDC is the Institute for inspection, supervision, test and appraisement of the quality of Veterinary Drugs as empowered by “Regulation of Veterinary Drug Administration” issued by the State Council. IVDC consists of 18 departments and also has the responsibility for the national reference laboratories for classical swine fever and rinderpest. All laboratories and departments are appointed by the Ministry of Agriculture.
The main responsibilities of IVDC is 1) inspecting, supervising, sampling, testing, and final arbitration for the quality of Veterinary Drugs sold in China, 2) setting and revising the National Standards of Veterinary Drugs, 3) reviewing standards for new and imported Veterinary Drugs, 4) supplying reference substances, master seeds and cell lines, 5) collecting the national veterinary microorganisms, 6) setting and revising the methods for surveillance of Veterinary Drug residues in animal-derived food, and 7) collecting adverse effects of Veterinary Drugs.

The second OIE experts were invited to attend a presentation on the activities of the Department for Management for the production of avian influenza (AI) vaccine (inactivated). Outlines of production were presented, without details, as well as general data concerning the method of preparation of the finished products (H5N2 and H9). A more complete presentation was made later and discussed the quality controls for in-process products and finished products. The subsequent presentation summarised the measures taken to ensure the quality of inactivated AI vaccines produced in China i.e. product license system, audit system by IVDC inspectors in manufacturing plants, and tests on each batch produced. The IVDC approval for batch release is granted when satisfactory test results by the manufacturer are received. In this case, the Manufacturer’s Release Certificate is countersigned and stamped by the IVDC and returned to the sender accompanied by a number of “Approbation labels” equivalent to the number of vaccine bottles filled with the batch volume. According to the speaker, in the Chinese market, satisfactory batches are identified thanks to “Approbation labels”, which are additional government labels stuck on the vaccine bottle cap, with the aim to prevent sales of fake vaccines.

**Conclusions:**

The initial presentation did not provide the figures demonstrating the complete activity of the Department for Management for the production of AI inactivated vaccine in China. But later in Harbin and Quingdao, we had the chance to observe two Batch Release Certificates indicating all QC tests were officially accepted as satisfactory. These certificates had the countersignature and stamp of the Head of the IVDC Department for Management for AI vaccine. We were told this countersignature, with stamp, is required for release of the product on to the market.

We understood also that the audits by IVDC in manufacturing sites are limited to factories with bad internal QC results. The OIE team concluded that their Chinese colleagues are very knowledgeable and capable. After only a few months of AI vaccine control, they have performed scrupulously their job of the market release of AI vaccines in China.

The OIE team has concluded that each batch of AI vaccine sold in China has satisfied all listed QC tests in the manufacturer QC laboratory. These results are validated on the Release Certificate by the additional signature and stamp of the Head of IVDC for the AI vaccine Control department. The procedure to stick “Approbation labels” from IVDC on each bottle cap is an additional stringent procedure to prove that the vaccine has been quality approved by the Government. Unfortunately we were not shown these labels.

**The Harbin Veterinary Research Institute (HVRI) of Chinese Academy of Agricultural Sciences, Harbin, Mandchouria**

This research institute is the biggest in China with 509 people in seven divisions and a farm of 1.4 million of square meters. Among the divisions located at the HVRI is a national key laboratory of veterinary biotechnology, a department of animal and poultry infectious diseases (This department includes the national influenza centre appointed by the Ministry of Agriculture, a centre for diagnosis and epidemiology of animal infectious diseases (also appointed by the Ministry of Agriculture and includes the national reference centre for contagious bovine pleuropneumonia), an experimental animal centre, a bioproduct industrialisation centre; and a scientific information centre. There were also premises for SPF egg production (built through Australia-China collaboration) and the so called “attenuated vaccine and inactivated vaccine GMP standard workshop”.
The OIE team visited the GMP laboratory responsible for manufacturing and QC of inactivated AI vaccine, guided by the respective Heads of these units.

Conclusions:

2. Vaccine Seed Stock Selection:

In 1995, Harbin Veterinary Research Institute (HVRI) received from the OIE Avian Influenza Reference Laboratory (Veterinary Laboratory Agencies, Weybridge, United Kingdom) an H5N2 (A/turkey/England/N28/1973) low pathogenicity (LP) avian influenza (AI) strain for use as an antigen source to conduct haemagglutination-inhibition (HI) testing of avian influenza viruses for the H5 subtype. All the H5N1 AI viruses isolated in China and other Asian countries since 1996 have been high pathogenicity (HP) and inappropriate for vaccine seed stock because most inactivated AI vaccine production in the region is conducted in biosafety level 2 (BSL-2) manufacturing facilities. A LPAI seed virus was selected for vaccine production in China and the only H5 LPAI virus available was A/turkey/England/N28/1973 (H5N2). Efficacy studies conducted at HVRI have shown this virus strain to be effective in protecting chickens from clinical disease and death following intramuscular challenge with HPAI virus strain A/goose/Guangdong/1/96 (H5N1).

At HVRI the master and working seed viruses are kept deep-frozen (~70°C). HVRI provides the working seed viruses for all of the AI vaccine (inactivated) manufacturing sites in China.

2. Method of Manufacture:

The manufacturing unit was not working when visiting the premises. We were told, the AI virus H5N2-Weybridge is produced on conventional eggs from farms under contract and submitted to strict serological controls by HVRI. We observed the old buildings of the laboratory were furnished with new equipment, walls, ceilings, floors and stainless steel vessels complying in with most of the Western GMP requirements. Nevertheless GMP and QA did not appear adequate throughout the production steps. Additionally, we noticed that inoculation of eggs and collection of virulent fluids was not performed using good protective measures for technicians (There were no disease security regulations in force). Collections of AI virus were stored in primary containers which were pooled after filtration but apparently not diluted. The inactivation process is carried out in one operation (37°C) in a stainless steel vessel but without transfer to a second sterile tank. The antigen is kept at room temperature in the inactivation tank during the 7 days required for the inactivation control test in SPF eggs (three passages). Filtrated antigen is then transferred and emulsified before the filling operations.

3. In process control:

This follows the Chinese requirements for licensing and the results reported by our Chinese colleagues were satisfactory.

4. Batch and Final product Quality Controls:

These are performed according to the Chinese regulations (potency test in birds 21 days post-vaccination: SN titre must be > 6 log₂) and are audited from time to time by inspectors of IVDC. All the tests listed in the OIE Terrestrial Manual are performed with satisfactory results according to the managers and we have been shown a Batch Release Certificate fully fulfilled and countersigned by authorities of the IVDC in Beijing. A bottle of AI inactivated vaccine manufactured in HVRI was presented to us. The “Approbation label” (issued by IVDC in Beijing), usually stuck on the bottle cap, was absent.
What the OIE expert team has learned and observed during this visit is that the Chinese are complying with the level of manufacturing, QC, and QA for AI vaccine requested by the OIE *Terrestrial Manual* Chapter I.1.6, Principles of Veterinary Vaccine Production. Recommendations of the team are presented below.

**National Animal Quarantine Institute (NAQI), Quingdao**

This Institute is under the authority of the Chinese Ministry of Agriculture and is composed of 140 people in several divisions:

- The China Epizootiology Centre
- The National Exotic Animal Disease Centre composed of the two national laboratories: BSE reference laboratory and Newcastle reference laboratory
- The Animal Health and Animal Product Safety Control Centre
- The National Animal Disease Diagnostic Reagent Centre

The NAQI has the charge of the secretariat of the national standards committee of animal quarantine technology.

The NAQI has the control of a manufacturing company for vaccine production: YEBIO Bioengineering Co.

The OIE team visited the GMP laboratory for manufacture and QC of inactivated AI vaccine of Yebio, guided by Dr Duyuanzhao PV, General Manager.

**Conclusions:**

2- **Management of seed virus:** Working seed is provided by the Harbin Laboratory and is kept frozen at –20°C.

3- **Method of Manufacture:** The manufacturing unit was not working when we visited the premises. We were told, the production of AI virus H5N2-Weybridge is performed on conventional eggs from farms under strict serological control by Yebio. We observed that the buildings were brand new and furnished with the best equipments available in China; walls, ceilings, floors and stainless steel vessels complied completely with Western GMP requirements. Nevertheless GMP and QA did not appear to us to be equivalent in level throughout the production steps. It was noted that the inoculation of eggs and collection of virulent fluids are not performed using good protective measures for technicians. A modification of the procedures to provide better protection has been recommended. Collections of AI virus are pooled in stainless steel containers (5 litres) then pooled in bigger tanks, after filtration, without dilution. We noted, with satisfaction, the inactivation process includes a transfer to a second sterile tank and storage until the results of the inactivation control tests are obtained. The antigen necessary for the preparation of one vaccine batch is kept in a unique tank adjacent to the emulsification unit before distribution to one of the two emulsification units and then to one of the two filling lines.

4- **In process controls:** These follow the Chinese requirements for licensing and the results reported by our Chinese colleagues were satisfactory.

5- **Batch and Final product Quality Controls:** These procedures are performed according to the Chinese regulations (potency test in birds 21 days post-vaccination: SN titre must be > 6 log₂) and are subject to audit from time to time by inspectors of IVDC from Beijing. All the tests listed in the OIE *Terrestrial Manual* are performed with satisfactory results and we have been shown a Batch Release Certificate countersigned and stamped by authorities of the IVDC in Beijing. No sample of vaccine was available for inspection.
What the OIE expert team has learned and observed during this visit complies with the level of manufacturing, QC, and QA for AI vaccine requested by the OIE Terrestrial Manual, Chapter I.1.6. Principles of Veterinary Vaccine Production.

**RECOMMENDATIONS:**

6. **Seed Virus:** The seed virus needs to be re-evaluated by HVRI every 2–3 years to determine if it is still useful for protecting poultry in the field.

7. **Challenge Model:** To determine efficacy, the potency challenge model needs to be changed to more a more effective the seed stock selection process:
   - Because influenza viruses change antigenically by the natural process of mutation or drift, the challenge virus should be changed to a current circulating virus or viruses. This should be done by an on-going national surveillance programme to isolate AI viruses from poultry, especially ducks and geese, and to conduct genomic sequence analysis of such viruses to determine if a single or multiple lineages are present. If a single lineage is present, a single challenge virus is appropriate, but if multiple lineages are identified, several challenge viruses may be needed depending on how close they are phylogenetically to vaccine strain.
   - The potency challenge should be changed from intramuscular route to intranasal route of inoculation with the minimum dose being either $10^6$ EID$_{50}$ (mean embryo infectious doses) or $10^2$ CLD$_{50}$ (mean chicken lethal doses) of virus per bird.
   - Parameters of protection in the potency challenge model should continue to use the prevention of clinical signs and death. In addition, evaluation of the reduction in replication of the challenge virus in vaccinated birds is needed to better demonstrate reduced environmental shedding and thus potential for reduced virus transmission. The reduction in average virus titre at the peak replication time should be a minimum of $10^2$ EID$_{50}$/ml of media from respiratory and gastrointestinal tract.
   - Protection should be determined not only for chickens but also for other important poultry species including quail, ducks and geese. The latter may show no mortality on challenge, but are important hosts in maintaining and disseminating the H5N1 HPAI virus, thus emphasising the need to evaluate reduction in shedding of challenge virus from respiratory and gastrointestinal tract to determine efficacy and potency.

8. **Master Seed Virus (MSV).** The immunogenicity test (2.1.4.b5) challenge model should be changed to intranasal with a recent H5N1 HPAI virus. A HI titre of $>6 \log_2$ is a reasonable alternative assessment method for immunogenicity control test of master seed virus.

   **Working Seed Virus (WSV).** The storage temperature of WSV (2.1.4.d.) should be changed from $-20^\circ$C to $-70^\circ$C.

9. **Manufacturing Process and In-Process Controls:**
   a. Protection of technicians during inoculation of eggs and collection of AI virus should be improved in the two manufacturing sites.
   b. Inactivation process should include a transfer to a second sterile vessel in the Harbin VRI.
   c. A Quality Assurance department should be created in each of the two sites for better management of the Standard Operating Procedures, internal audits, and comprehensive recording of the environmental measures.
10. **Finished Product:**

The current policy for potency test (a SN titre 21 days PV of $>6 \log_{10}$) and for identification of approved batches (official label on the vaccine bottle cap) should be continued.

- The vaccine should have sufficient antigen to produce a strong protective immune response. This should be a minimum of 50 mean protective doses ($PD_{10}$), which approximates 5 g of haemagglutinin protein per dose. Such a requirement is not codified in current documents.

- The Immunogenicity Test (2.1.4.b5.), which recommends the use of a challenge model, should be changed to intranasal challenge with a recent H5N1 HPAI virus. A HI titre of $>6 \log_{2}$ is a reasonable assessment method for potency of final product.

- Other H5 LPAI viruses used throughout the world for vaccines have been shown to be efficacious against the current Asian H5N1 HPAI viruses, as demonstrated in experimental studies conducted in Hong Kong and the USA. These vaccine strains include the H5N2 vaccine virus used in Mexico and vaccine viruses contained in vaccine banks in the USA and Europe. Importation of such vaccines into China should meet Chinese National Vaccine Standards or equivalent; the efficacy and potency of these imported vaccines against current H5N1 Asian viruses should be demonstrated. Likewise, China should only export AI vaccines that meet their National Standards for purity, safety and potency as codified in their regulations. Unlicensed AI vaccine products should not be imported or exported.

- **H9N2 LPAI Vaccine Standardisation:** A single page was provided in the packet from the Institute of Veterinary Drug Control on Quality Standard of the inactivated AI vaccine (Subtype H9). Although, evaluation of H9N2 AI vaccine was not part of the official request to OIE, the following are recommendations submitted for consideration:
  
  - Seed strain selection should be based on virus surveillance for H9N2 LPAI viruses in all of China and subsequent phylogenetic analysis of isolated viruses to identify the predominant lineage or lineages of virus
  
  - Efficacy testing of seed strain should be similar to the H5N1 HPAI virus. However, the LPAI viruses do not cause clinical signs or death in experimental studies thus determination of efficacy should be based on reduction in replication of the challenge virus in vaccinated birds.
  
  - Manufacturing standards should be modelled after those for H5N1 HPAI.