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### EMERGENCY ASSISTANCE FOR THE CONTROL OF AVIAN INFLUENZA

### (CAMBODIA)

### OSRO/CMB/403/FRA

### **Final Technical Report**

by

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List of acronyms : DAHP : Department of Animal Health and Production NAHPIC : National Animal Health and Production Investigation Centre TF1 : Task force in charge of HPAI investigation, surveillance and diagnosis TF2 : Task Force in charge of HPAI communication TF3 : Task Force in charge of HPAI control measures

### **Executive Summary**

### Support to HPAI investigation and surveillance activities

### 1. Active Surveillance program preparation

A surveillance program for avian influenza has been prepared in collaboration with NAHPIC, as well as the work plan and budget for its implementation.

### 2. Participation to the technical exchanges on HPAI surveillance methods.

An informal discussion group has started to exchange on technical issues related to the surveillance approach of the current H5N1 strain in the region. Those exchanges helped me to draft the surveillance programme and were also of use for the experts consultation held from the 21 to 23 July in Bangkok in the frame of the TCP/3006.

This informal discussion group included the following persons :

- Carolyn Begnino and Hans Wagner, FAO/ Bangkok,
- Vincent Martin, EMPRES program, FAO/Rome,
- Ron Jackson, consultant epidemiologist, Epicentre, Massey University, New- Zealand,
- Les Sims, consultant in China,
- Ray Webb, epidemiologist consultant in Lao,

#### 3. Retrospective study of the outbreak places

A detailed action plan has been developed to assist the implementation of a retrospective study of the past outbreak as proposed by T.Rawdon in his report following his mission to Cambodia in April.

The central team has been trained for its implementation and I have supported them during the field visits (7 places out of 12).

The objectives of this survey were to collect more information about the past outbreak and to assess the current situation. The Government used the results of this study to release the outbreak declaration for the 12 places investigated, without declaring them free of disease.

During the field visits, samples were collected, especially when suspected mortality was reported during the past months. Due to difficulty to catch animals during the day, the total number of samples is lower than expected.

The data related to the outbreak places have been collected and sent to FAO/Rome in September for a transfer to Epicentre (New Zealand).

The preliminary analysis of the retrospective study shows that the virus has certainly circulated outside of the declared outbreak places. Out of the 52 villages visited, around 40 % are highly suspected of having HPAI outbreak in the past months, and around 23 % moderately suspected. Out of the 23 farms visited, less than 10 % are highly suspected and 13 % moderately suspected. Those preliminary results show that spread of the virus may be higher in the village poultry population than between the farms.

According to the results of this study as well as the analysis of environmental factors related to the production systems, the climate and the control measures, it is possible to conclude that the virus has not been totally eliminated from the environment and it is probably still circulating at a low level without causing outbreak at the present time. This means that it is not possible to exclude the re-emergence of H5N1 virus in the country.

### 4. Strengthening passive surveillance

The passive surveillance system is still not totally reliable. The only 3 suspected cases investigated from May to July were reported to the Department by the Ministry of Health because of their possible link with human suspected cases. After investigation, the link between the poultry mortality was excluded and H5N1 was not confirmed.

Furthermore, when provincial officers report mortality to the central teams, the information are always very imprecise. Sometimes because they don't have the complete history, but often because they have no methodology for completing a good survey.

For this reason, I proposed to organise a follow-up mission in each province to make sure that the provincial teams improve mortality reporting, in quantity and quality. For some provinces, the visit was also used for market and farms monitoring presentation (only 7 provinces involved in this monitoring)

### 5. Launching of market monitoring

An important part of the active surveillance program is the market monitoring, since this is one of the easiest way to assess the sanitary status of the village poultry population.

The methodology has been discussed and explained to the central epidemiology team for them to be able to inform the provincial teams. I also participated to the first field visits for provincial staff support. The distribution of material has also been prepared, as well as the logistic organisation for samples sending and transport media replacement.

The market monitoring has finally started early August. The provinces, even after receiving the complete explanations and equipments were not very responsive. Only after few weeks, they started to send samples.

This monitoring is planned to continue until the end of the project.

Before to choose the sampling places in Phnom Penh, a qualitative survey has been made to identify the main live birds markets in the town and to understand the animals' movements within the city. The average number of birds sold in market was also assessed to fix the number of samples needed. The work on animal movement will be continued by the national consultant under TCP/CMB/3002.

#### 6. Monitoring of repopulated farms

A monitoring has been proposed for the former infected farms and the farms located 3 km radius from the outbreak places. This monitoring will authorise the Government, after 6 months, to declare those places free of disease. This monitoring will be also an important tool to strengthen the surveillance network, with some farms acting as sentinels farms in the country.

The methodology for this monitoring has been explained to the central epidemiology team, and tools for its implementation have been prepared (forms, communication system, follow-up).

Every provinces involved in this monitoring, received the visit of the central team for explanation of the methodology and material distribution (at the same time as the market monitoring presentation).

So far, the monitoring has started in Phnom Penh and Kandal provinces. The central team must strengthen its follow-up to push the provincial teams to implement this activity on time.

# 7. Initiation of the identification and geo-referencing of commercial and semi-commercial farms in the outbreak areas

In order to facilitate the implementation of an active surveillance program in this compartment, I proposed to start with the referencing of every commercial farms in the surveillance zone (3-4 km around the outbreak) and then, to extend this census to the whole country.

A questionnaire has been developed in order to collect basic information related to the production system and the biosecurity level of the farm. Central and provincial teams have been trained to the use of this form.

In order to start with a mapping localisation of the production sites in the country, I also proposed to take the geo-reference of the farms visited. Five additional GPS equipments have been purchased and distributed in priority to the provinces with the highest poultry population. Central and provincial teams have been trained how to mark position.

The investigation team has completed this referencing around the outbreak places, but the first coordinates taken have been lost (wrong manipulation of a trainee). This census will be extended to the whole country by another team (TF3) at the same time as the planned disinfection demonstration to the farmers (started early August with some delay). It was also proposed, in a medium term, to do this work to the pig production farms.

The data collected, will have to be shared with the statistic department of the Ministry of Agriculture.

### Support to HPAI diagnosis activities

### 1. Procurement of equipments

All laboratory equipments, ordered under the FAO implemented projects have been correctly delivered to the laboratory. It seems that almost everything has been delivered from the general store room to the laboratory. Nevertheless, the general store management system does not work properly, and it is quite difficult to have an accurate picture of the remaining items. The support of a logistician is planned to improve this situation.

The new list of equipments proposed to be ordered under the Japanese funds has also been checked to control its consistency with the real needs of the laboratory. It was noted that many of the items requested are intended for food hygiene analysis. Among those new equipments, a biosafety cabinet as well as two additional incubators could be dedicated to the egg inoculation technique planned to be introduced by the end of the year in the laboratory.

### 2. Improvement of the traceability

In order to improve the traceability of samples collected in the frame of the surveillance program, a new form for sample identification and submission has been introduced. A regular control was made to check its appropriate use as well as a consistency of the results released.

Staff in the sections involved in the reception and manipulation of samples as well as the field teams, have been trained to the use of this form and the procedure for registration of the samples.

The recording and traceability of the samples collected in the frame of HPAI investigations or surveillance activities is now satisfactory.

### 3. Control of the avian flu data base

The data-base created in February for the avian influenza follow-up has been checked. Some problems have been fixed in France after the mission.

A complete assessment of the database system used in NAHPIC will be the part of a follow-up mission planned for the end of the year.

### 4. Follow up of diagnosis activities

Regular contacts were made with Pasteur Institute in charge of the training of two serology/virology NAHPIC staff (3 weeks training on HI test and IFAT and continuous follow-up on the IFAT slides interpretation).

Contact has been made with Australian OIE reference laboratory for avian influenza (Dr. Paul Selleck) in order to organise a proficiency test for the serological techniques (HI test) performed by NAHPIC. Ten sera

with unknown antibodies titre sent by the OIE reference laboratory were tested by the serology team with the results sent back to the reference laboratory. The results of this external quality control were very positive.

The OIE laboratory also provided reference antigens prepared from the Vietnamese strain for HI test and control slides made from organs smears for IFAT. The use of the local antigen will probably increase the sensitivity of the HI test.

The terms of reference for an additional laboratory expert mission were amended to include the possibility of introducing egg inoculation technique in NAHPIC. Coordination with the regional FAO TCP/RAS/3006 will have to be made in order to avoid overlap and duplication and to assure that regional trainings in-house trainings are complementary

#### 5. Improvement of the data recording and management

Simple tools have been introduced for information transfer and follow-up in order to improve the information management within the Department. Following my mission the national consultant in communication has provided support to the task force in charge of mass media and communications

One database on Excel has been developed and transferred to serology section in order to record all their analysis results and to help them to validate their tests and the type of samples collected.

I have also asked CIRAD to develop an Access database for farm recording and linkage with a mapping system. A first version has been presented to NAHPIC during the mission. Minor changes have been done in France. The definitive version will be transferred during my follow-up mission.

A proposal has been drafted for training of some laboratory staff on Excel and Access to make them familiar with the use of the two software and get more autonomy.

### I. Presentation of the poultry sector

### A Poultry population statistics

The annual animal population statistics, produced by the provincial animal and production offices, gives an idea of the poultry population by province. But those data do not make the difference between the village poultry population and the farming system. In February 2004, the provincial teams have been asked by the DAHP to provide a list of the poultry farms in their province. The list provided gives an indication of the animal population present in those farms at this date, but without their exact localisation and without an idea of their real capacity. This list of poultry farms by province and the 2002 animal population statistics are provided in Annex 1.

According to the official services, the official animal population statistics are not totally reliable. Furthermore, there is no systematic list of farms by provinces to give an idea of the national production of the commercial sector and to apply appropriate control measure in case of outbreak.

For this reason, I proposed to start with a general census and geo-referencing of the "commercial farms" in the country, linked with a mapping system.

This information could be used for both animal disease control or animal production purposes. Indeed, in case of a new outbreak, the veterinary services will get immediately the list of the farms in a specific commune or village, and could apply promptly control measures. It would be also the occasion to clearly quantify the production capacity of the country and to localise the main production zone (for the commercial sector).

Thus, a **new complete census has been launched in July 2004**. The census has started in June with the epidemiology team during the retrospective study around the outbreak places. It will be extended to the whole country by the end of the year or before, by the provincial teams with the support of the TF3 central team.

A form has been prepared to collect the information related to: the size and the type of production of the farm; the bio-security level of the premises; the origin and destination of the animals or the animal products. I have trained the head of epidemiology unit to the use of the form ; he was then responsible to train the TF3 provincial teams. Five additional GPS equipments have been purchased under Japanese funds and distributed in priority to the provinces with the highest poultry population. Central and provincial teams have been trained how to mark position. The investigation team completed this referencing around the outbreak places but the first coordinates taken have been lost (wrong manipulation of a trainee).

A first version of a **national animal production database has been prepared in July 2004** by the programmer of CIRAD. This database still need minor changes, and will be transferred during my follow-up mission in October/November 2004. It is planned to link this Access database to the mapping system already in place in the epidemiology unit (ArcView 3.1). See Chapter 6 for a presentation of the first version of the database.

### **B** Poultry production systems

The poultry production systems can be divided into three main sectors. The definition can vary a little bit according to the main purpose of the classification.

The definitions given here are more based on the production criteria (farm capacity and biosecurity management), it can be different if we use in priority the economical criteria. For this reason, the definitions of the sectors used for the socio-economical impact assessment is slightly different.

According to the criteria set during the FAO expert consultation in Bangkok, the poultry production systems can be categorised as follows:

**Commercial sector** (sector 1) :

- Industrial system with high level of biosecurity.
- More than 10 000 animals
- 2 farms in Cambodia :
  - CP breeding farm for laying hens and broilers
  - One 120 000 laying hens farm on the road to Takeo

### Semi-commercial sector :

1) Sector 2:

- Moderate to low bio-security level
- Between 500 to 10 000 animals
- Most of the laying hen's farms and some broilers farms.





- 2) Sector 3
- No biosecurity
- Between 500 to 3000 animals
- Most of the duck "farms".



Village poultry sector (sector 4) :

- Village or backyard production with no biosecurity
- Most of the poultry population in Cambodia







Pick up arriving from province





Middleman on motorbike



Middleman on motorbike



Sellers in an open live birds market (Tcha Ampeul)

Live birds transport means and open live birds market

### C Poultry marketing in Phnom Penh

### 1. Qualitative survey at the market places

In order to choose the appropriate places for live birds sampling in Phnom Penh, I have conducted a brief qualitative survey in collaboration with Nget Kiri, a staff of the epidemiology team.

This preliminary study does not allow quantifying the animal movements from the provinces to Phnom Penh, but gives an idea of the main live bird pattern from the farms or the villages to the markets and identified the four main market places where live birds are sold.

#### **Animal movements**

The figure 1 summarises the animal movements between the different stakeholders involved in the poultry marketing in Phnom Penh and shows how complex it is. The animals are either transported directly by the middleman to the market place where they are sold to sellers, or they can be transported directly to the seller's house if agreement has been made. The unsold animals are either kept at the market in pens or are brought back to the seller's house.

In Phnom Penh, no movement from markets to farms have been identified since the farmers are selling to middlemen.

#### Estimation of the number of animals sold every day at the market places

For Tcha Ampeul market, the biggest live bird market with O-Russey market, the number of animals arriving in a normal day (with good climate conditions) was estimated around 2000 birds. The animals are coming mainly from Prey Veng, Svay Rieng and Takeo provinces.

In O-Russey, 20 sellers were identified. With an approximate average of 70 (between 20 to 100) animals per sellers, the total number of birds can also be estimated around 2000.

### **Middleman practices**

The middlemen normally arrive from 12am to 2pm in Phnom Penh, depending on their origin. They usually stop before to reach the city for drinking and feeding their animals in order to increase their weight. Those places are usually on the main roads coming from provinces.

#### **Transport system**

4 main transport systems can be identified (see pictures on the opposite page) :

- pick-up, transporting around 600 animals in normal conditions,
- motorbike, transporting between 60 to 100 birds depending on their equipment,
- motorbike with trailer, transporting around 100 animals
- bicycle, transporting less than 50 animals.

#### Authorities responsible for the market management in Phnom Penh

The market management is left to a private company after regular bidding organised by the Municipality. The company has to pay an annual fee to the municipality but can collect the fees from the sellers (for the selling place, for equipment...). They contract a chief of market in charge of collecting the fees and solving any problem between the sellers.

The Veterinary office from Phnom Penh municipality is responsible for controlling the meat arriving in the market. They check that the meat is coming from an official slaughterhouse (the sellers have to show the ticket proving they paid their fees at the slaughterhouse).

The Public health office of Municipality is responsible for the hygiene in the market. Their activities are quite limited.



The blood is collected to be cooked and sold for consumption





The chicken are boiled to remove the feathers manually or mechanically



Machine to remove the feather mechanically

Carcass preparation in an open live birds market

### 2. Poultry slaughtering and risk for human health

The qualitative study at the market places was also the occasion to observe the poultry slaughtering process and to evaluate the risk. The different steps of the carcass preparation are illustrated by pictures on the opposite page.

If one considers that the faeces are the main contagious material, the risk of contamination for the sellers may be decreased since they do not eviscerate the chicken before selling. If the animals are kept and slaughtered outdoor, the risk is also decreased.

On the other hand, in the cover markets, the risk of contamination of the sellers or the person practicing the slaughtering is higher. Indeed, the concentration in a close area, of infected birds, may cause a contamination of the environment that may be sufficient for the contamination of the people in place in the area for a long time.

### II General context of the animal disease control in Cambodia

### A. Veterinary services organisation

### 1. Central level

The Department of Animal Health and Production is made of 8 offices, one cattle station and one veterinary clinic for pets.

The offices are :

- Animal Health office,
- Animal Production office,
- Extension office,
- National Animal Health and Production Investigation Centre (NAHPIC),
- Public Health office (approved but not yet set up),
- Legislation and Conflict office (approved but not yet set up),
- Administration office,
- Planning and Accounting office.

The staff at the central level is officially made of 411 persons but, on a daily basis, only 40 or 50 people are working and have direct mission to fulfil. The details of the staff repartition by province as well as their qualification are given in Annex 2. Among the 139 staff with a bachelor degree, 5 are holders of a post-graduate diploma from abroad (Australia, USA, Japan).

### 2. Provincial level

Each province has an Agriculture Department including an animal health and production office. Then, almost each district has one or several district veterinary officers. There is no official veterinary services representation at the commune or village level. This means that the veterinary officers have to interact closely with the village chiefs and the Village Animal Health Workers, VAHW's, in order to extend their network to the field level. This connection is not systematic and often depends on the good personal relationship between the district vets and the VAHW of the area. This means that **the animal disease reporting system cannot work properly if the district vet does not maintain good and regular contacts with the field workers**. Indeed, the district veterinary services have very limited means, and it is impossible for them to cover the whole district.

Among the constraints observed at the district level, the **lack of technical and financial means** and also lack of motivation are probably the main causes of the inactivity of the veterinary services (in general, because there are, of course, some exceptions). The low salaries explain the lack of motivation of the staff, but the **absence of clear duties and control from the central level** is also contributing to this phenomenon

Another constraint for the animal disease control, is the absence of general consensus, among all the stakeholders, that the animal diseases must be reported to the official services. This is linked to the following point, the absence of legal framework for the animal disease control.

### B. Legal framework for the animal disease control

The general regulatory framework for the actions of the veterinary services is very limited. The only regulatory activities of the veterinary services are :

- Sub-degree 16, recently refreshed, that covers the inspection of animal and animal products in the context of international trade and that also allows the declaration of infected zone at the national level.
- Sub-degree 29 that covers the inspection at the slaughterhouses.
- Sub-degree 26 that creates an official status for the people involved in the animal heath and production activities. Basically, this regulation was made to provide the VAHW with an official recognition and status. This sub-degree obliges the VAHW and the veterinarians to be registered

and to have a minimal training. Three "prakas" (regulation under the sub-degree level) detail the way the VAHW can be selected and trained, the way the VAHW trainers can be selected and the process to create provincial board, in charge of issuing the licence for the animal health practitioners (VAHW and veterinarians).

There is no regulation about the animal disease control, this means that there is no disease subjected to notification and no animal disease control program. The only participation of the official services in an annual control disease activity, is related to the vaccination campaign against the hemorrhagic septicemia.

A draft regulation on the animal disease control, presented in Annex 3, was prepared but is still pending.

### C. National animal diseases reporting and investigation system

Within NAHPIC, the epidemiology unit is in charge of collecting and analysing data on animal diseases in Cambodia. A reporting system has been set up for the main diseases : foot and mouth disease, blackleg, classical swine fever, hemorrhagic septicaemia, pasteurellosis on pigs and rabies. The Highly Pathogenic Avian Influenza was introduced after the regional outbreak.

This reporting system has initially been introduced in the four target provinces of the APIP project (Agricultural Productivity Improvement Project) and is now extended, with varied success, to the other provinces. The reporting system consists of a monthly report of disease occurrence by the district vets to the provincial offices where they are gathered and sent to the central level. That information is then entered in the VFS, Veterinary Field Report, national database. The standardised form used for this reporting system is presented in Annex 4. This form is also used by the epidemiology unit in case of outbreak investigations.

The quality of the data reported by the district veterinarians depends on the good network he/she was able to create with the field workers. None of the diseases reported are subjected to compulsory declaration and the system depends totally on the willingness and awareness of the stakeholders.

### III Avian influenza diagnosis

### A. NAHPIC presentation

### History (extracted from NAHPIC leaflet)

The centre has been established in 1982 under the former name of the National Veterinary Diagnostic Laboratory (NVDL). At the beginning, an NGO had provided technical advisors, materials and training to 10 laboratory staffs in parasitology, serology, haematology and pathology fields. The practical training was conducted at the government hospital. At the same time, one old building was repaired and equipped with new laboratory equipments.

From 1984, students graduated from Preak Leap Agricultural College, the Royal Agricultural University and from abroad were appointed to work at the NVDL. Until 1995, the date the NGO stopped to support NVDL, the staff was made of 36 people. During the transition period with the current IFAD loan project, NVDL had limited budget and had lost some of the technical staff.

The APIP project (Agricultural Productivity Improvement Project) started in June 2000. The animal health and component, funded by an IFAD loan, was included in this World Bank project. The new NAHPIC was constructed under this project and has been achieved in September 2001. Most of the new equipment arrived in July 2002.

Since the building has been constructed, NAHPIC staffs received in-house training and were sent abroad for short training courses, mostly in Asia.

NAHPIC has been upgraded to an office level in December 2003. This is the first step to obtain a semiautonomous status in the coming years.

### Organisation

NAHPIC is organised in 6 sections: pathology, bacteriology; serology/virology, parasitology, haematology/biochemistry and epidemiology, for a total of 28 staffs. The repartition of the staff with theirs qualifications is given in Annex 5.

### B. Background of AI diagnosis and activities implemented under TCP/RAS/3004

### 1. Background

Before the outbreak, no diagnosis was made for this disease and embryonated egg inoculation was not a method used in routine. Only HI test was used for Newcastle disease virus.

In January, antigens from OIE reference laboratory in Weybridge (UK) were ordered. In January, the first samples collected were sent to WHO collaborating centre in Pasteur Institute in Paris with the assistance of Pasteur Institute in Cambodia. The first H5N1 outbreak was confirmed on the 22/01/04. The pathogenicity was also assessed by the study of the nucleotides at the portion of the HA gene coding for the cleavage site region of the heamagglutin of H5 subtype. The strains were found highly pathogenic. The strains have been sent to WHO gene bank with the agreement of the Cambodian Authorities to the request of WHO Cambodia.

From February, the Pasteur Institute in Cambodia started analysing the samples by RT-PCR method and helped to introduce the IFA test in NAHPIC with the assistance of Pasteur Institute and WHO expert (Dr JC. Manuguerra). In March, a laboratory expert, Dr. M.Guittet, was recruited for 3 week mission under the French Cooperation to assist in establishing the HI and IFAT techniques in the diagnostic laboratory at the NAHPIC. The expert concluded that further technical support to the laboratory staff is needed.

In April, NAHPIC staff was trained in the virology section of Pasteur Institute in Phnom Penh by Dr Jean-Marc Reynes and Dr Ong Sivuth. The protocols for HI and IFAT tests were finalised. The training also provided an opportunity to improve the general working methods of the staff. In April, positive samples were sent to OIE reference laboratory in Geelong, Australia, for virus isolation and gene sequencing.

### 2. Activities implemented during the mission

### Control of the supply management

All laboratory equipments ordered under the FAO projects have been correctly delivered to the laboratory. It seems that almost everything has been delivered from the general store room to the laboratory. Nevertheless, the general store management system does not work properly, and it is quite difficult to have an accurate information on the remaining items. The support of a logistician was proposed to improve this situation.

The new list of equipments proposed to be ordered under the Japanese funds has also been checked to control its consistency with the real needs of the laboratory. It was noted that many of the items requested are intended for food hygiene analysis. Among those new equipments, a biosafety cabinet as well as 2 additional incubators could be dedicated to the egg inoculation technique planned to be introduced by the end of the year in the laboratory.

### Improvement of the traceability

In order to improve the traceability of samples collected in the frame of the surveillance program, a new form for the samples identification and submission has been introduced. A regular control was set up to check its appropriate use as well as the consistency of the results released.

Staff in sections involved in the reception or manipulation of samples as well as the team bringing samples to the laboratory, have been trained to the use of this form and the procedure for registration of the samples.

The recording and traceability of the samples collected in the frame of HPAI investigations or surveillance activities is now satisfactory.

### Control of the avian flu access data base

The data-base created in February for the avian influenza outbreak follow-up has been checked. Some problems have been fixed after the mission and the corrected version will be transferred during the follow-up mission.

### Follow up of the diagnosis activity

Regular contacts were made with Pasteur Institute in charge of the training of two serology/virology NAHPIC staffs (3 weeks training on HI test and IFAT and continuous follow-up on the interpretation of results). The choice of the best samples to collect for the IFAT was discussed also with the OIE reference laboratory in Geelong. A list of samples to be collected during the post mortem examination of suspected animals was proposed to the pathology section (Annex 6).

It was noted that the IFA test using the cloacal swabs is not validated. It was advised to the field teams to perform the tracheal swabs in stead of the faecal swabs, even if this sampling is more delicate. The virology section has noted that IFAT on faecal swabs needed further validation, and will be able to implement such a validation once a reference technique will be introduced in the laboratory (egg inoculation).

The terms of reference for an additional laboratory expert mission were amended to include the possibility of developing egg inoculation technique in the diagnostic laboratory at the NAHPIC (Annex 7). The necessity f introducing this reference technique was discussed initially with the Thai virologist expert, Dr Arunee Chaising (National Institute of Animal Health, Bangkok, Thailand), sent for one week mission in NAHPIC by JICA and then, at the FAO meeting in Bangkok for the launching of the regional TCP on the harmonisation of the surveillance and diagnosis methods for the HPAI.

If an expert is appointed to carry out training of the laboratory staff in NAHPIC under the national TCP/CMB/3002, it will have to be coordinated with the training activities under the regional FAO TCP/3006 to avoid overlap, and to assure that both trainings are complementary

### Organisation of a proficiency test

Contacts have been made with the OIE reference laboratory in Geelong (Dr. Paul Selleck) in order to organise a proficiency test for the serological techniques (HI test) performed at the NAHPIC. Ten sera with known antibodies titres and 10 others with unknown titres sent by the reference laboratory were tested by the serology team at the NAHPIC with the results sent back to Geelong. The results of this external quality control were satisfactory (annex 8).

The OIE laboratory also provided reference antigens prepared from the Vietnamese strain for HI test and positive controls made from organs smears for IFAT. The use of the local antigen probably increased the sensitivity of the HI test.

### Improvement of the data recording and management

One database on Excel has been developed and transferred to the serology section to improve records keeping and results analysis. A list of samples analysed since April is given in annex 9.

### 3. Current situation

Two laboratories are involved in AI testing: NAHPIC and Pasteur Institute. There is no provincial laboratory able to perform a screening test for AI. The diagnosis strategy for the suspected cases has been prepared since February 2004. The screening test is, at the moment, the IFA test on tracheal swabs with confirmation by RT-PCR (Annex 10)

### NAHPIC:

Human capacity: 4 serology staff (see attached CV on Annex 5) Facilities:

- one room used for serology and virology activities.
- one room for fluorescence microscope.

Equipments already in place:

- one fluorescence microscope in very good condition,
- one incubator, 55 litres,
- two centrifuges
- one freezer,
- two fridges,
- one pH meter
- Elisa equipment (reader, 2 washing machines)
- autoclave
- 1 water bath
- 1 touch mixer
- 1 stirrer
- 1 electronic balance
- 1 computer

Equipments that will be provided under the Japanese funds by the end of September:

- one class 2 safety cabinet
- two incubators (80 litres)
- small equipments (pipettes, glassware...)

### HI test

Protocol: see attached document in Annex 11.

Antigen now used: A/ Chick / Vietnam / 2004 (H5N1) produced by OIE reference laboratory in Geelong. Antigens also available from UK OIE reference laboratory: H5N1 (A/Chick/Scotland/59), H9 and H7.

### IFAT

Protocol : see attached document in Annex 11.

Anti-body : anti-influenza A group purified (ordered from Argene Company).

Specification : murine monoclonal antibody IA52 recognizes a 60 kDa group specific nucleoprotein common to all Influenza A subtypes (H0N1, H1N1, H2N2, H3N2) and variants tested. No cross reactivity with Influenza B and other respiratory viruses. In immunofluorescence antibody shows a both cytoplasmic and nuclear staining.

### Pasteur Institute in Cambodia :

RT-PCR for M segment, H5, N1 and H7 specific gene sequence.

### C. Recommendations

For serology/ virology :

- It is too early to think about the introduction of RT-PCR or RRT-PCR in the national veterinary laboratory. The expertise for gene sequence detection already exists in Cambodia in Pasteur Institute, and it is sufficient for the current needs of the country.

- The sensitivity / specificity of the IFAT implemented in NAHPIC must be controlled with the support of external laboratory (exchange of samples or slides could be organised with Geelong)
- The double check of the slides by Pasteur Institute must continue until the staff are fully confident in reading and interpreting lab results .
- IFAT on faecal swab must be validated by comparison with reference technique (virus isolation when in place, or RT-PCR)
- Additional and regular external quality controls on HI test results must be organised to follow-up the capacity of the team.

- The virus isolation technique could be introduced in NAHPIC with external technical support. See proposed schedule :

- Preparation of the facilities and the waste management system (may be done under the supervision of Pasteur Institute ) (September 2004)
- Regional training (date to de defined) and in-house-training of the staff on the technique and good laboratory practices (1 month mission in October/November 2004)
- 2 weeks mission to follow up on the capacity of the staff to perform the virus isolation technique (December 2004)
- The consistency of the results released must still be systematically controlled by the deputy director in charge of technical matters.

For the regional TCP

- The standard techniques currently performed at the NAHPIC must be strengthened before new techniques are introduced.
- A system for free antigen supply must be organised between the countries involved in the project.
- Pasteur Institute could be contacted to be part of the network because of its close relation with the national veterinary laboratory and its participation in the HPAI diagnosis at the national level.

### NAHPIC diagnosis activities



On the left side, Dr Arunee Chaising, Thai expert; on the right side, Dr Ren Theary, Head of NAHPIC serology/virology section



Post-mortem at NAHPIC



Petechial lesions on a positive HPAI positive chicken (January 2004)

Report to	Outbreak	Province	District	Commune	Village	Sector	Reported total deaths	Subtypes affected (deaths)	Disposal	Sampled	Laboratory	Official
12 01 04	1	Phnom	Pussey Ves	Phnom Penh	Pong Peau	Semi	3300	Lover		4	22.01.04	22 01 04
12.01.04	(asa Tahla 2)	Domb	Russey Reo	Thread	rong reay	Senn-	3300	Layer		4	23.01.04	25.01.04 No. 10
14.01.04	(see Table 5)	Tellin	Det	Inney	Diana Tana a	Com	97	4		2	21.01.04	NO.19
14.01.04	2	Такео	Bati		Phnom Tamao	200	80	Appendix I		3	31.01.04	11.02.04
	(Appendix 1)		<b>D</b>	¥7. (		* ****	24	<b>C1</b> 1 1 (10)				No.67
16.01.04	3	Phnom	Russey Keo	Km 6	Boeung Chouk	Village	34	Chickens (10)	Chickens (7)	2	31.01.04	11.02.04
		Penh				Chicken		Duck (13)				No.67
								Geese (11)	Geese (2)			
25.01.04	4	Kandal	Kien Svay	Kbalkoh	Prekthom	Village	207	Chickens (50)	Chickens (8)	3	05.02.04	26.02.04
						Chicken		Duck (18)	Duck (2)			No.79
								Geese (6)	Geese (6)			
								Turkey (68)	Turkey (2)			
								Guinea fowl (65)	Guinea fowl (35)			
28.01.04	5	Siem Reap	Siem Reap	Slorgram	Boeung Daun Pa	Village	2	Chickens		1	05.02.04	26.02.04
						Chicken						No.79
06.02.04	6	Takeo	Daunkeo	Rokaknong	Snor	Semi-	1690	Layers	1510	4	03.03.04	12.03.04
				U		com						No.102
09.02.04	7	Kandal	Takhmao	Prek Samrong	Prek Samrong	Semi-	1700	Lavers		3	03.03.04	
				5	J	com				-		
11.02.04	8	Siem Reap	Siem Reap	Slorgram	Treang	Zoo	2	Parrots		1	19 02 04	No declaration
11.02.07	0	Stem Reup	Stem neup	Stor gi uni	11 curig	200	-	1 4//015		1	17.02.04	Close to
												outbreak ph 5
11 02 04	9	Siem Rean	Siem Rean	Salakamroeuk	Watho	Semi-	2	Broiler	620	1	02 02 04	05.02.04
11.02.04		Stem Keap	Siem Reap	Salakanii Souk	Watoo	com	2	Diolici	020	1	03.03.04	03.03.04 No.04
13 02 04	10	Kandal	Kiensvav	Prekthmey	Pohos' Angkanh	Semi	167	Proiler	2522	2	02 02 04	NO.94
15.02.04	10	Kanual	Kiciisvay	rickinney	Robos Aligkalili	Semi-	107	Biolier	2333	3	03.03.04	12.03.04
21 02 04	11	Talcas	Translash	Cuananana	Trop con all could	Com	000	D	(00	2	02.02.04	No.102
21.02.04	11	Takeo	Паткак	Steronong	Trapeangiboeuk	Sem-	900	Ducks (880)	600	2	03.03.04	
	10	<b>m</b> 1	0	~		com		chickens (20)				
24.03.04	12	Takeo	Samrong	Chumraspen	Kapnim	Village	324	Chickens		1	26.03.04	09.04.04
						Chicken						No136
27.03.04	13	Kampong	Kg. Siem	Koah	No 6	Village	203	Chickens		1	30.03.04	09.04.04
		Cham		Samrong	number of the second second of the second	Chicken						No136

### Table 2 Laboratory confirmed H5N1 outbreaks in Cambodia (to August 2004)

# IV Retrospective study of the past outbreak : assessment of the spread of the H5N1 virus

The past outbreak has already been described in the report of the FAO consultant Dr. Thomas Rawdon (MAFF New Zealand) following his mission to Cambodia in April 2004. A part of this analysis is given in Annex 12.

The objectives of this retrospective study were :

- to collect more information on the outbreak places in order to make a better analysis of the diffusion of the virus in the country and then in the region. It was proposed by T.Rawdon that the information related to the outbreak places will be transferred to Epicentre New Zealand contracted by FAO to produce a regional analysis of the HPAI outbreak;
- to assess the spread of the virus around the confirmed outbreak places;
- to assess the current situation of the poultry mortality in the former infected places.

### A. Material and Method

The epidemiology units are either the village or the farm. It was decided to limit the survey at the area within 3 km radius from each confirmed outbreak place. The list of the outbreak places is provided in table 2.

### Questionnaire

At the outbreak places, a complete survey has been implemented to try to identify the origin of the contamination by identification of the animals and animal movements during the risk period, and possibly to identify at-risk places using the standardised form prepared by T.Rawdon on the basis of the Epicentre form.

In the villages and the farms selected around the outbreak places, a limited number of information was collected. Those information were related to morbidity and mortality events in 2004 and 2003 with description of symptoms, the vaccination status and possibly, the animal movements in 2004.

For each farm and village, an assessment of the current situation was also performed.

In the villages, the questionnaires were completed by discussion in priority with the village chief or vice-chief and/or the Village Animal Health Worker of the village and, if none of those persons were available, with 2 or 3 families randomly selected in the village. In the farm, the farm owner was interviewed.

### Sampling method and sample size

### Selection of the villages and the farms

The sampling rate for the villages was chosen with a 95% confidence of detecting at least one infected village assuming a between village prevalence of 5%. All the farms identified in the studied areas were visited.

### Selection of the birds to sample

Animal sampling rate in the village or the farm was planned to fit with a 95 % confidence of detecting at least one positive bird assuming a within farm or village prevalence of 25 %.

In the outbreak places, a farm or a village, it was planned to systematically sample the animals.

In the villages around the outbreak place, the animals were sampled in case :

- o of suspected mortality reported for the past 3 weeks,
- the information was not complete
- o each time suspected mortality was reported in 2004

The animal were sampled for both virology (IFAT) and serology (HI test) in order to detect the former infected animals and also animals in incubation.

### Classification of the villages and the farms visited around the outbreak places

According to the outputs of the surveys, the villages and the farms were classified in three categories related to the probability of having faced an outbreak of HPAI from January to May 2004. To classify the villages and the farms, a scoring system has been applied, using criteria chosen to describe an HPAI outbreak on the poultry species. The criteria and their related score are listed in Table 3.

Tuble 5. Chiefin appres to the Honora Minageo and familio for classification									
Criteria	Yes	No							
Outbreak* reported on poultry from January 2004 to May 2004	2	0							
Sudden death reported (health to death within 24 hours without	3	0							
symptoms or with minor symptoms)									
Cumulative mortality during the outbreak over 40% for chicken, 30 %	2	0							
for ducks or daily mortality over 1%									
Case fatality over 60 % for chicken, 20 % for ducks	1	0							
Mortality reported on different species	1	0							
At risk population vaccinated (partly or totally, against NCD and/or	1	0							
fowl cholera									
Mortality on all age categories	1	0							
Total	11								

Tab	le 3.	Criteria	applied :	to the	visited	villages and	l farms	for	classification
1 400		C	appirea		1101000		LOWLING		eraberre ererer

\*Outbreak definition : mortality reported on poultry on a specific period

The villages and the farms were then classified in three categories using the following model :

Score between 0-3 = low probability

Score between 4-7 = moderated probability

Score between 8-11 = high probability

### **B.** Results

In total, 52 villages and 23 farms were visited around 12 outbreak places. The information related to four outbreak places is compiled two by two because the places were situated close to each others. For one outbreak place (outbreak number 6), the information was not translated before the end of my mission. Also, I was not able to use the information collected before my mission around the last outbreak place in Kampong Cham; only the results of the serological analysis are available.

In total 158 animals were sampled in 31 places. A total of 125 IFA tests and 103 serological tests were made in NAHPIC. The number of samples collected in one village or in one farm was always lower than the number expected. Among those places, only two ducks flocks in Siem Reap province were detected sero-positive.

In total, around 40 % of the visited villages are highly suspected of having faced an HPAI outbreak (Table 4) whereas less than 10 % of the farms are in this category (Table 5).

For the only two "farms", which are actually two flocks, the scoring system gave a score of 6, which means that those flocks are classified in the second category, linked to a moderated probability.

									11	12	13	TOTAL
		2		4+10 (Kandal	5 (Siem	6 (Takeo /	7 (Kandal	8+9 (Siem	(Takeo /	(Takeo /	(Kompong	
	1 (PP)	(Takeo/Zoo)	3 (PP)	/ Kien Svay)	Reap)	Daun Keo)	/Takmao)	Reap)	Tramkak)	Samrong)	Cham)	
No Info	33.3% (2/6)					100% (9/9)					100%	21.1% (11)
Low												15,4%(8)
probability		14.3% (1/7)		80% (4/5)	25% (1/4)			20% (1/5)		20% (1/5)		
Moderate												23,1% (12)
probability	33.3%	57.1% (4/7)	100%	20% (1/5)	75% (3/4)		25% (1/4)		20% (1/5)			
High												40,4% (21)
probability	33.3%	28.6% (2/7)					75% (3/4)	80% (4/5)	80% (4/5)	80% (4/5)		
TOTAL	6	7	2	5			4	5	5	5		52

### Table 4. Repartition of the villages and the farms according to the risk of having faced an HPAI outbreak between January and May 2004.

Table 5. Repartition of the farms investigated around the outbreaks, according to their category

				4+10							13	TOTAL
		2	3	(Kandal /	5 (Siem	6 (Takeo /	7 (Kandal	8+9	11 (Takeo /	12 (Takeo /	(Kompong	
	1 (PP)	(Takeo/Zoo)	(PP)	Kien Svay)	Reap)	Daun Keo)	/Takmao)	(Siem Reap)	Tramkak)	Samrong)	Cham)	
No Info			66.7% (2/3)									17,4% (4)
	50% (1/1)					100%		10% (1/10)			100.%	
Low												60,9% (14)
probability	50% (1/1)			100% (3/3)	66.7% (2/3)		100% (2/2)	60% (6/10)				
Moderate												13% (3)
probability								30% (3/10)				
High												8,7% (2)
probability			33.3% (1/3)		33.3% (1/3)							
TOTAL	2	0	3	3	3	1	2	10	0	0	0	23

### C. Discussion

### **Survey parameters**

In one of the duck flock, where 13 birds were sampled, 9 were found seropositive. So, an expected withinfarm prevalence of 20 % would be sufficient for the detection of the seroconversion of a duck flock.

It is not possible to make any conclusion about the chicken population since none of the village or the farm were found sero-positive for this species.

### Scoring system

The number of samples collected is not sufficient to be used as a reference for the validation of the scoring system used in this study.

The only two farms for which serological analysis confirmed the past infection of the animals, the scoring system did not permit to classify them in the category related to the highest probability of an HPAI outbreak. This is probably due to the fact that the same scoring system was applied to the villages and to the farms. Some categories, like the presence of different animal species or different age categories involved in the outbreak do not fit with a farm situation where the animal population is normally homogenous.

### Endemicity of the HPAI virus in Cambodia

The number of samples collected was not sufficient. Indeed, in all the cases, the number of samples collected was much lower than the limit fixed to fit with a 95 % confidence of detecting at least one positive animal if the prevalence for both seroconversion and infection was of 20 %. In this condition, the precision of our study is not sufficient to make any conclusion based on laboratory analysis about the circulation or not of the virus in the visited villages and farms.

Nevertheless, the qualitative study based on the interviews allows identifying that many villages located around the outbreak places probably faced an outbreak of HPAI. Those preliminary results also show that the spread of the virus may be higher in the village poultry population than between the farms.

The spread of the virus in the village population brings the question of a possible remaining pool of virus in the environment or in the surviving animals able to produce new outbreaks.

Some arguments are in favour of the elimination of the virus or the reduction of its circulation and may explain why no new outbreaks were detected since last April :

- Because of the very hot temperature from mid-April to mid-May, the quantity of virus in the environment probably dropt dramatically.
- The case fatality rate on chicken was very high and often reaches 100%, which excludes the possibility for this species to be seen as possible carriers of the virus (none of 79 sera collected on local chicken around the outbreak places where suspected mortality were reported, was found positive).
- Due to the drop of chicken consumption from the end of January until, at least, mid of March, the animal movements in the country dropt dramatically as well as the number of live birds in the markets (almost nil, without official ban of live birds markets!), so the virus transmission was slowed down.
- The human and animal population density is globally low in the country (at the exception of the area around Phnom Penh), and the number of semi-commercial farms is much lower than in the neighbouring countries, this means that the virus did not find sufficient relay for multiplication.

Some other observations are in favour of survival of the virus and its low circulation :

- Stamping out was not implemented in the villages, and disinfection cannot be efficient in this context.
- Not all the outbreak places have been detected and stamping out was not implanted in the villages. The consequences are :

- some affected farms did not implement good cleaning and disinfection measures whereas we know that the virus can survive a long time in the faeces (faeces are often kept for being sold as manure);
- there is a chance that domestic ducks, and domestic waterfowls in general, carried the virus for some weeks after the outbreak and may have helped its transmission at a low level since that (this hypothesis is supported by the detection of two seropositive ducks flocks : see case history in Annex 13). This threat is serious since many ducks here are kept for egg production. This means that animals which faced the outbreak pick in January, February and March, were, for some, still in the rice fields or in the villages in June. Of course, having virus circulating in waterfowls at a low level, does not mean to get a new epizootie. Thankfully, many ducks flocks are raised in the rice fields, quite separated from the local chicken. But if one considers the waterfowl population in the villages, the mixing of species is of serious concerns. At the farm level, the biosecurity improvement will be the only efficient barrier to the introduction of new virus in the farms.
- The close contact between wild birds, known to be carriers, and the domestic poultry population is also of concern, and may contribute to the continuous contamination of this compartment.
- The use of water pond as source of drinking water for some farms (and of course in the villages) is also in favour or the transmission of the virus between either the wild birds population and the domestic poultry population (in villages and in farms), or the local poultry population and the farms population. This is the reason why Yves Froehlich, the technical adviser at the FAO Office in Cambodia, proposed to disinfect the drinking water pumped from the water pond in the farms.
- The live birds markets or collecting places may also contribute to maintain a pool of circulating virus as described in other countries. The conditions are here in favour of such a role since the control of the animal movements (in the country and form the neighbouring countries) as well as the control of sanitary status of the animals arriving in the markets or collected places, are very limited.

The repartition of the villages in the different categories according to the outbreak places does not show big differences (no statistical analysis were done due to the limited number of units per category). This is in favour of a general spread of the virus around every outbreak places.

In conclusion, it is impossible that the virus has been totally eliminated from the environment and it is probably still circulating at a low level without causing outbreak at the present time. This means that it is not possible to exclude the re-emergence of H5N1 virus in the country.

A complementary analysis will be done using the missing information that will be collected during the follow-up mission.

### V. HPAI Surveillance

### A Passive surveillance

### 1. Activities implemented since April 2004

#### 1.1 Under the TF2, the Task Force in charge of the communication

A massive communication campaign has been organised by the TF2 with meetings at the provincial and district levels inviting the provincial and district vets or the farmers and the VAHW's.

A first leaflet has been produced early in the crisis and a second edition with some changes has been financed by the donor trust funds.

Two TV spots are about to be finalised. One will present the good management practices at the farm level to avoid the diffusion of the diseases and another one could be used in case of new outbreak, to remind all the precaution to take to protect the human health and to limit the diffusion of the virus. Those media will be also used to remind to the stakeholders the importance to notify any suspected mortality to the official services. This is probably the most difficult objective to achieve.

Those activities were under the general supervision of the FAO technical adviser, Yves Froehlich and a national consultant in communication.

#### 1.2 Under TF1, the task force in charge of surveillance and investigation

#### Training of the provincial staff

Three sessions of training have been organised by the central team. This training course was intended for the provincial staff of the 24 provinces (4 staffs per province).

The training course was organised in 3 days as follow :

Day 1 : Outbreak investigation

Day 2 :

- Intervention in farm suspected of infectious disease
- Sampling strategy for HPAI suspected case
- Collection of specimen

#### Day 3 : Surveillance strategy

The content of this training course was not directly evaluated since it started before my mission and was organised in Khmer. Nevertheless, it is obvious that a subject as complex as the surveillance methods cannot be taught effectively this way. It is important to have either smaller group and/or to organise follow-up missions in the provinces.

#### Emergency preparedness meeting : information management improvement

On 08/07/04, I organised with the epidemiology team, a meeting with every task forces in order to prepare them to face a possible new outbreak. The main purpose of this meeting was to define the procedure of the information management and traceability within the Department. Indeed, the information flow is not always efficient and some reports of suspected cases were treated with some delay due to a miscommunication between the different offices.

The proposal for information management that results from this meeting is given in Annex 14. Before the end of my mission, the recording book proposed to improve the traceability of information was in place in the Epidemiology unit. The national consultant in communication was in charge to follow-up this matter for the TF2.

### Follow-up program intended for the provincial staff

In order to support the provincial teams in their surveillance activities, it was proposed to organise one or two follow-up missions by the central epidemiology team from mid-July. Indeed, the training courses organised in Phnom Penh in April and May, may not have been sufficient to assure a good comprehension of the principles of the HPAI surveillance. Furthermore, the methodology of surveillance program was not finalised at that time and it was necessary to assure that the provincial teams in charge of its implementation have fully understood their role. It was also an opportunity for the provincial staff to practice sampling collection on chicken.

The schedule of the support missions was prepared in order to visit in priority the provinces involved in the active surveillance programme. For some provinces, it may be necessary to organise two visits before the end of the year, especially if the implementation of the monitoring in the farms or in the markets faced some problems.

The programme of the above missions is given below :

### Support to provincial teams

From July 2004

If after the first visit, the provincial team shows no problem for the different matters related to the surveillance of AI, the second visit will not be necessary.

#### Surveillance

Check if active surveillance programme in the commercial farms in the infected areas is well understood + check the correct use of the forms

For the 7 provinces selected : inform about market monitoring

- how many samples to collect
- the frequency,
- the organisation of the samples shipment.

Provide with the needed equipments : syringes, swabs, transport media, PPE

Inform about the use of the PPE when visiting the farms (even for clinical assessment, the person entering into the building, must wear overcoat). The overcoat can stay in the farm and be reused at the next visit and be replaced when destroyed.

#### Investigation

Assess the capacity of the provincial team to fill the outbreak form.

Explain that they have to report the criteria of the sampling strategy (sudden death / vaccination against NCD and fowl cholera / different categories of age involved / several species affected).

#### Sampling

Check if investigation team knows how to collect samples and possibly organise practice (tracheal swab / cloacal swab / blood samples)

Explain which samples have to be taken in case of investigation of current mortality (tracheal + cloacal swabs) or in case of past mortality (cloacal swab + blood samples).

#### **Farm Census**

Check if referencing is progressing with TF3 Assess if the team have any problem for completing the form or using the GPS equipment.

### 2. Suspected cases reported since May 2004

Since April 2004, only 3 suspected cases were reported and investigated by the central epidemiology team with the support of the FAO/TCP project. It is important to note that they were reported to the DAHP by the Ministry of Health due to human suspicions (none of those human suspicions were confirmed). For each of those cases, a complete survey was done by the central team and samples were collected.

In all the cases, the surveys confirmed an outbreak of poultry mortality which was not reported by the provincial veterinary services, but the link with the human morbidity or mortality was excluded. All the samples collected were negative.

### **B** Study and active surveillance programme

### 1. Proposed strategy and methodology

An active surveillance program has been prepared during the mission. The details of this programme were discussed with the head of NAHPIC and the head of the epidemiology unit.

The programme is divided in three points.

#### - Retrospective study of the past outbreaks

Proposed by the FAO consultant, Thomas Rawdon.

- Active monitoring
  - In the outbreak places, after repopulation, to declare those places free of disease after 6 months.
  - At the market places

This is an important part of the active surveillance program, since this is one of the easiest way to assess the sanitary status of the village poultry population.

In a first phase, it was decided to monitor 2 markets in Phnom Penh and 1 or 2 markets in 7 provinces.

#### - In the parent stock farms

The only parent stock farm providing day-old chicks and pullets to farmers, belongs to CP Company. This farm, based in Kandal province is made of 6 flocks : 4 for broilers and 2 for laying hens.

Because this farm already vaccinated its animals without keeping sentinels, it was proposed to monitor all their dead birds (post-mortem and virology).

For the new coming flocks, another strategy was proposed with or without vaccination .

## - Freedom declaration in the semi-commercial and commercial farms in the whole country.

This monitoring, proposed by the previous consultant was put in third priority. Indeed, the context is not yet to the point of freedom declaration but more to the active monitoring of the different compartment in the country. Since the human resources are very limited, it was decided that this programme could be postponed until the outbreak would be totally under control in the region.

The details of the methodology of this program and the action plan for its implementation are described in annex 15.

### 2. Progress on the active surveillance programme

#### **Retrospective study**

#### See chapter IV.

The study was completed and the information related to the outbreak places sent to FAO/Rome on the standardised forms provided by Epicentre, New Zealand for regional analysis.

#### **Market monitoring**

The methodology has been discussed and explained to the central epidemiology team, for them to be able to inform the provincial teams. I also participated to the first field visits for provincial staff support. The distribution of material has also been prepared, as well as the logistic organisation for samples sending and transport media replacement.

The market monitoring has finally started early August. The provinces, even after receiving the complete explanations and equipments were not very responsive. Only after few weeks, they started to send samples.

Before choosing the sampling places in Phnom Penh, a qualitative survey has been made to identify the main live birds markets in the town and to understand the animals' movements within the city. The average number of birds sold in market was also assessed to fix the number of samples needed. The work on animal movement will be continued by the national consultant under TCP/3002.

This monitoring is planned to continue until the end of the project in December. It could be needed that NAHPIC continue this monitoring, at least in the Phnom Penh markets after this date.

### Active monitoring in the outbreak areas

The methodology for this monitoring has been explained to the central epidemiology team, and tools for its implementation have been prepared (forms, communication system, follow-up).

Every provinces involved in this monitoring received the visit of the central team for explanation of the methodology and material distribution (at the same time as the market monitoring presentation). So far, the monitoring has started in Phnom Penh and Kandal provinces.

The central team must strengthen its follow-up to push the provincial teams to implement this activity on time.

#### Surveillance in parent stock farm

A first meeting has been organised with CP company in June for exchange of information. We organised a second meeting on the 08/07/04 (see Annex 16 for report of the meeting) to discuss the possible methodology for surveillance in the CP breeders farm. At the end of the meeting, the General Manager proposed to submit the surveillance methodology discussed to his hierarchy and to contact the DAHP to start the activities. Since this date, the DAHP did not receive any news from CP.

It is important that the epidemiology team contact again CP to find an arrangement for starting a surveillance program in the breeder flocks.

Note : CP requested the authorisation from the DAHP to import avian influenza vaccines for the second time. At the end of my mission, the DAHP refused to give this authorisation.

A follow-up mission is planned for assisting the epidemiology team to evaluate the activities implemented under this surveillance program and to analyse the data collected.

### VI Data collection and analysis

### A. Current databases

### 1. VFS, Veterinary Field Services report Database

The epidemiology team uses a database for the monthly disease occurrence reports from the districts. This database was built by a consultant under the APIP project, using TAD info model (see Annex 17 for the main forms). This database is linked with a mapping system (Arcview 3.1).

The epidemiology unit enters every month the report of disease occurrence received from the provinces. They use those reports to produce once or twice a year, a report of the disease occurrence per province and per district. The HPAI has been added to the list of the diseases to be entered in the database.

### 2. Serum bank database

This database has been created at the same time as the VFS database. It does not seem that the information related to the samples stored are regularly entered in this database. See Annex 18 for the menu.

An assessment of the appropriate use of this database as well as needed modifications are required,

### 3. HPAI Samples management

During the mission, an Excel database has been prepared for the serology section in order to collect all details samples being analysed in the context of the HPAI surveillance activities. This database will be of use to assist the validation of the techniques used in the laboratory when a reference technique will be introduced (virus isolation).

### B. Database developed for the animal farming census

In the frame of the identification and geo-referencing of commercial and semi-commercial farms in the country, I have asked D.Chavernac, computer scientist from CIRAD to develop an Access database using the questionnaire proposed for the census. The information collected in this database could be used to facilitate the implementation of the active surveillance program by providing lists of farms in specific areas. The information collected will be also used for the estimation of the animal production capacity in the country. This is why, it was proposed to extend this census, in a medium term, to the other animal production, especially the pig production. Generated information should be shared with the Department of Statistic of the Ministry of Agriculture.

The NAHPIC staff who has the responsibility of the management of the databases, has very limited skill in computer in general and Excel and Access in particular. For this reason, a training course has been proposed under the FAO TCP project, in order to help the team to get more autonomy on the database management.

### **General conclusion**

Despite the general lack of human and financial resources, the veterinary services of Cambodia worked hard to control the past outbreak of HPAI and to prevent any new epizooty. The capacity of the official services in term of organisation and control, largely improved since January this year. A significant improvement was also achieved at the national veterinary laboratory where the serology/virology team was very responsive to the intensive training they received on diagnosis and testing of avian influenza. The general condition of work in NAHPIC improved due to the increase of activity.

Regarding, the past outbreak, the demonstration that the virus has circulated outside of the outbreak places, brings the question of the endemicity of this virus in the country and more generally in the region. It must be stressed out that the sanitary measures such as the ones used western countries are unimaginable, in the current situation, in a country where a large part of the production is made of village chicken. This means that the circulation of the virus will hardly be stopped if the biosecurity is not improved at least at the farm level. The protection of the scavenging chicken population is of concern and appropriate and realistic solutions are more difficult to find for this sector.

If the HPAI virus becomes a more serious public health concern, the situation will have another extent, since Cambodia is not prepared to subsidise a massive culling of animals. None of this question could have an unique answer and a regional approach for the control measures must be an objective. The regional TCP on the harmonisation of the surveillance and diagnosis methods is a first step in this direction.

Finally, a follow-up mission, supported by CIRAD, is planned just before the end of the project to assist the epidemiology team to evaluate their surveillance activities and to analyse the data they will have collected so far. More precisely, it is proposed, during this mission to:

- Assess the activities under the approved active surveillance programme and provide support to the central team.
- Assess that the animal disease reporting system is working well, using the tools put in place.
- Support the Epidemiology central team for the analysis of the data generated by the active surveillance programme. Assist in the writing of a mid-term progress report.
- Support the serology team for the analysis of the data generated by the sample data base. and issue technical recommendations on the most appropriate samples to be collected for immunofluorescence test.
- Transfer the Access data-base set-up by CIRAD for farm census.
- Adapt the National Databases (database system for disease reports and bird flu database) to the current needs of the epidemiology central team and the regional epidemiological network

### **Summary recommendations**

• The farm referencing must be completed and all information entered in the new access database and linked with a mapping system.

The information collected could be shared with the statistic department of the Ministry of Agriculture.

- For HPAI diagnosis :
  - It is too early to think about the introduction of RT-PCR or RRT-PCR in the national veterinary laboratory. The expertise for gene sequence detection already exists in Cambodia in Pasteur Institute, and it is sufficient for the current needs of the country.

- The sensitivity / specificity of the IFAT implemented in NAHPIC must be controlled with the support of external laboratory (exchange of samples or slides could be organised with Geelong)
- The double check of IFAT results by Pasteur Institute must continue until the staff are fully confident in the reading and interpretation of the results.
- IFAT on faecal swab must be validated by comparison with reference technique (virus isolation when in place, or RT-PCR)
- Additional and regular external quality controls on HI test results must be organised to assess the diagnostic capacity of the staff
- The virus isolation technique could be introduced in NAHPIC with external technical support. See proposed schedule :
  - Preparation of the facilities and the waste management system (may be done under the supervision of Pasteur Institute ) (September/October 2004)
  - Regional training (date to de defined) and in-house-training of the staff on the technique and good laboratory practices (1 month mission in October/November 2004)
  - o 2 weeks mission to follow up on the performance of the staff (December 2004)
- The consistency of the results released must still be systematically controlled by the deputy director in charge of technical matters.

• Continue to support the provincial teams for a good implementation of the active surveillance programme

• The market monitoring could continue after the duration of the project as normal surveillance activities of NAHPIC for the HPAI virus.

• The NAHPIC epidemiology team should contact again the CP company and urge them to start an active surveillance programme in the breeders farm.

• The capacity of the epidemiology team for database management using Excel and Access should be assessed .

• The data collected in the frame of the surveillance and investigation activities should be analysed and shared for scientific purpose.

### Acknowledgements

I would like to thank for their assistance, the staff at the Department of Animal Health and Production in Phnom Penh, the staff at the FAO office and Pasteur Institute in Cambodia.

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# Annex 1. Poultry statistics

	Laying hens Broilers farms		farms Duck		ts farms	Other	
							,
KOMPONG THOM					13	9200	
Staung					11	8500	
Stung Sieu					1	200	
Baray					1	500	
KOMPONG SPEU	8	148 200	41	204 900			
Phnom srourh			1	3000			
Sbar Mou	2	9600	17	74 800			
Samrong Tong	4	14 500	17	52 800			
Kong Pisei	1	100 000	6	42 300			
Ao Dong	1	9 500	1	32 000			
ТАКЕО			40	81 038	303	244 656	
					flocks		
Kirivong					74	46 200	
6					flocks		
Daun Keo			3	59 742			
Tram Kak					1	17 500	
Trang					16	6 500	
Angkor Borei					25	19 300	
Samrong			32	2 000	54	39 339	
Borei Cholsar			5	19 296	5	45 468	
Prev Korbach					125	49 800	
SIEM REAP	6	10 920	18	30 780	98	48 870	
Siem Reap	5	10 420	18	30 780	67	49 270	
Kor Lang					9	3 000	
Puok					16	4 600	
Saut Nik Kum					2	400	
Kompong Sdei	1	500			4	1 600	
SIHANOUKVILLE			261 208	146	64 194		
Stung Hao			624	14	20 200		
Mitapheap			34 354	17	20 830		
Prev Nop			226 230	115	23 164		
KANDAL	46	158 395	18	63 032	11	13 740	
Kien Svav	7	17 000	3	15 000		10 / 10	
Koh Thom	7	9 800					
Muk Kampol	1	4 500				•	
Ang Chrol	7	31 225	9	34 532			
Ponhie Leu	7	24 550	1	4 000			-
Saan	12	38 700	3	5 500			
Takhmao	5	32 620	2	4 000			
Lovir Ai		52 020		1000	11	13 740	
КАМРОТ					6	9 358	
Angkor Chev					6	9 3 5 8	
PHNOM PENH	5	42 955	10		17	27 574	
Russev Ken	2	30 500	8	29 079	14	21 000	
	3	12,455	2	2,017	3	6 574	
KOMPONG		12 100	1	1 000	36	54 125	
CHHNANG			Î	1 000	50	0.140	
Bor Ribau			1	1 000	22	46 500	
Kompong Tralach					1	200	

# List of poultry farms by provinces and by « srok » in February 2004

V Oll					0	4 005	
Kompong Chhnang					9	4 2 2 5	
Rolear Eir					4	3 200	
BANTEY MEANCHEY	1	1 400	2	240	52	84 417	
Preah Net Preah			2	240	20	23 450	
Malay	1	1 400			1	120	
Phnom Srok					4	15 247	
Mondol Borey					20	37 200	
Serei Sor Phaun					7	9 400	
KOMPONG CHAM			4	1 500	177	80 789	
Kompong Sieu			1	1 000			
Cheu Prey			1	350	175	79 989	
Kau Meas			2	550			
Prey Chhor					2	800	
BATTAMBANG	1	6 000	6	18 000	54	203 855	
Battambang			3	9 000	6	25 100	
Banau			2	3 000			
Eak Phnom	1	6 000	1	6 000	6	31 700	
Sangker					1	3 000	
Maung Russei					1	2 000	
Bor Vel					3	5 000	
Tbor Kal					37	137 055	
SVAY RIENG					3	600	
Svay Tip					3	600	
RATANAKIRI					3	230	
Banlong					2	150	
					1	80	
PREY VENG					57	26 134	
Peam Ro					21	8 294	
Preah					2	1900	
Kompong Trabek					4	4 190	
Kompong Lao					5	1 750	
Pear Rang					25	10 000	
KOH KONG	1	900	3				
Smak Meachey		2	2	330			
Kompong Sela	1	900	1				

Total 16 provinces : Laying hens farms : 68 ( 368 700 birds) Broilers farms : 138 (690 303 birds) Ducks : 617 farms + 360 flocks (879 142 birds)

8 provinces did not reported poultry farms

# Poultry statistics (DAHP 2002)

Provinces	Chicken population	Duck population
Banteay Meanchey	341 871	107 805
Battambang	580 399	134 974
Kampong	526 980	122 000
Kampong Spueu	924 603	110 399
Kampot	747 447	397 917
Kandal	1 144 857	114 231
Koh Kong	47 070	4 982
Kompong Cham	244 561	270 910
Kompong Thom	552 337	80 017
Kratie	326 951	25 577
Krong Kep	21 694	8 077
Krong Pailin*	0	0
Krong Preah Sihanouk	253 638	77 239
Mondul Kiri	25 470	4 940
Oddor Mean Chey	0	0
Phnom Penh	153 530	69 543
Preah Vihear	332 639	19 275
Prey Veng	795 585	473 526
Pursat	2 139 662	64 129
Rattana Kiri	0	0
Siem Reap	628 015	141 089
Stung Treng	69 342	15 951
Svay Rieng	0	0
Takeo	1 129 974	492 575

				VAHW		Civil		Servant		
Provinces	Communes	Districts	Villages	trained <sup>1</sup>	Bachelor degree	Diploma <sup>2</sup>	Trained <sup>3</sup>	Unskilled	Total Gov	Total province
Banteay Meanchey	9	64	623	357	3	4	19	0	26	409
Battambang	13	96	733	499	13	15	63	7	98	695
Kampong Chhnang	8	69	553	244	2	4	14	0	20	284
Kampong Speu	8	87	1308	266	4	6	5	29	44	354
Kampot	8	92	478	217	3	8	0	51	62	341
Kandal	11	147	1,087	138	12	22	366	4	74	616
Koh Kong	8	33	124	31	1	1	6	0	8	47
Kompong Cham	16	173	1,758	320	6	15	28	10	59	438
Kompong Thom	8	81	732	211	2	13	26	0	`41	293
Kratie	5.	46	250	229	0	2	9	3	14	257
Krong Kep	2	5	16	0	1	1	0	1	3	6
Krong Pailin	2	8	79	40	1	3	7	0	11	62
Krong Preah Sihanouk	3	22	94	228	1	2	9	3	15	258
Mondul Kiri	5	21	90	10		1	0	4	5	20
Oddor Mean Chey	5	24	231	0	2	1	0	0	3	6
Phnom Penh	7	79	637	0	40	27	10	31	108	216
Preah Vihear	7	49	208	44	1	1	3	8	13	70
Prey Veng	12	116	1137	537	4	10	38	0	52	641
Pursat	5	49	485	236	1	3	43	0	47	330
Rattana Kiri	9	49	240	149	1	3	0	19	23	195
Siem Reap	12	100	906	249	3	8	13	11	35	319
Stung Treng	5	34	128	105	0	2	4	1	7	119
Svay Rieng	7	80	690	422	3	5	34	0	42	506
Takeo	10	100	1,116	468	5	7	31	3	46	560
DAHP					139	134	84	54	411	

# Annex 2. Distribution of the Village Animal Health Workers and the veterinary officers per provinces in 2002

<sup>&</sup>lt;sup>1</sup> Village Animal Health Workers (VAHW) are recruited locally and receive short training by DAHP or other Non Government Organizations such as Vétérinaires Sans Frontières (VSV) or Vétérinaires Ruraux du Cambodge (VRC) following standard programs approved by DAHP <sup>2</sup> Diplomas are one or three year courses <sup>3</sup> Trained staff may only include short courses offered by the Department or up to a year.

#### Annex 3. Draft regulation on animal disease control

(extracted from S.Desvaux final report, July 2004) 25/06/02

# CONTROL AND PREVENTION OF ANIMAL DISEASES

The first draft of this regulation has been made in 2000. This revised version has been prepared by the Technical Adviser in animal health and veterinary public health at the DAHP.

# Introduction

The control of the animal diseases is a matter of collaboration between the different institutions, organizations and private stakeholders dealing with livestock. This means there is no efficient control of animal disease if all the stakeholders and organizations are not involved.

This collaboration presupposes that a clear definition of the duties of each partner has been discussed and explained. In Cambodia, the organization of private veterinary services is changing. The recognition by the Government of the Village Animal Health Workers, brings a new level between the farmers and the official veterinary officers and possibly the private vets.

Those VAHW, because of their close contacts with farmers, have a very important role to play in the control of animal diseases. So, they need to have their duty explained in a regulation.

It is important, as well, to precise the responsibility of the farmers. Indeed, they have to play an active role in the epidemiological surveillance of the herd. But, to allow them to play this role, the Government has the responsibility: first to inform them about the recognition of the main diseases, and second to organize a system of subsidized slaughtering if a stamping out policy is chosen to fight a disease.

This last question is a major one. Indeed, it is totally useless to introduce the idea of control diseases, with obligation of declaration and may be slaughtering of animals, if nothing is clearly foreseen for compensating the losses.

Like for every regulation, the Government, in collaboration with relevant organizations and group of producers, has to be very wise and has to prepare everything to allow the implementation of this regulation. There is nothing worst than a regulation unable to be applied.

# **General comments**

• This regulation will be completed by more detailed procedures.

Indeed, the procedures in case of outbreak can differ from a disease to another one because the conditions of transmission of the disease, the possibility of vaccination or the sensitive animals may differ.

So, once the list of control diseases will be established according to the epidemiological situation of the country and the international regulations, the technical services should be provided with the **procedures in case of suspicion** and in case of outbreak for each of those diseases.

• The list of diseases under list 1 could be defined by a committee led by the director of DAHP, with representatives of private sector (especially veterinarians or experts in animal health), and the director of NAPHIC. This committee could as well propose procedures for the control of the diseases using the international references. Indeed, the choice of list 1 diseases is very critical and needs to be carefully discussed between every partner.

## **Chapter 1, General provisions**

1.1 The purpose of these regulations is to enable the responsible body to control, prevent or restrict the spread of animal disease within a defined geographical area, a single or multiple number of Provinces or districts or any part of, or the whole of, the Kingdom of Cambodia.

1.2 Within the context of these regulations the correctness of any action taken to prevent the spread or occurrence of any disease must be compatible or comparable with the generally accepted veterinary knowledge about the disease in question. The arbitrator in such matters of veterinary knowledge will be the Organization International Epizootic (O.I.E).

1.3 Within the context of these regulations the following means:

Animal means any live animal other than Homo Sapiens of any age or sex and shall include any semen or any egg or zygote.

Animal Quarantine station means a facility under control of the veterinary authority where a group of animals is maintained in isolation, with no direct or indirect contact with other animals in order to undergo observation for a specified length of time, if appropriate, testing and treatment.

**Temporary Quarantine area** means an area of land that is declared quarantine area for purpose of holding animals during the control of a list 1 disease.

A list 1 disease is a disease declared by the responsible Minister under the list 1 to be a notifiable disease subject to compulsory health police measures in case of outbreak or suspicion, in order to eradicate or control that disease.

A list 2 disease is declared by the responsible Minister under list 2 to be a notifiable disease without compulsory health police measures in case of outbreak or suspicious.

A list 3 disease is a disease declared by the responsible Minister under the list 3 to be a disease that may be subject to facultative or compulsory official control programs in a specific area."

**Border post** means any airport, or any port, railway station or road checkpoint open to international trade of commodities, where import veterinary inspections can be performed.

**Carcass** means the dead body or any part of a dead body that has not yet been changed into cooked food or a finally processed product and shall include tusks, horns and hair removed while it is still alive.

Department means the department of the Ministry of Agriculture, in charge of the animal health.

**Destroy** means to completely burn or bury to a depth of 50 centimeters from the surface and to cover with earth 50 centimeters above the surface. If burning or burying is not appropriate then other methods may be used provided it is supervised by an inspector.

Director means the Director of Animal Health and Production.

**Disinfection**: means the application, after thorough cleaning, of procedures intended to destroy the infectious or parasitic agents of animal diseases, including zoonoses; this applies to premises, vehicles and different objects which may have been directly or indirectly contaminated.

Official ear tag means an official ear tag placed in the left ear of bovines or buffalo and inscribed with a series of letters or numbers that have been allocated to signify ownership by a particular owner or family.

Emerging disease is a new disease, a new form of a disease or a disease that occurs in a new area.

Inspector means a public servant appointed to work on for the veterinary authorities.

Licensed person is a person licensed to undertake the treatment of animals according to the praka number 368.

**Official control program** : means a program that is approved and managed or supervised by the veterinary administration for the purpose of controlling a pathogen or disease by specific measures applied throughout the country or within a zone or zones of the country.

**Owner** shall include the person in possession. In the case where the owner is not apparent any person tending the animals or in whose custody the animals are at given time.

**Products of animal origin**, means products of animal origin intended for human consumption (fresh meat, meat products, gelatin, eggs, eggs products, milk, milk products and honey), for pharmaceutical or surgical use (animal organs, tissues and organic fluids), for animal feeding (meat-meal, liver-meal, bone-meal, feather-meal, pork fat and some milk products), and for agricultural or industrial use.

**Property.** Means any harness or equipment or moveable object used in association with animals or any fodder or organic matter.

Responsible person shall mean the Director of Animal Health and Production.

Responsible Minister or Minister shall mean the Minister of Agriculture Forests and Fisheries.

**Social contract**: is the commitment that the state enters into, with the livestock owners of a specified area to control or limit certain diseases that have been placed on list 3 of this regulation.

Treatment measure means any procedures or actions commonly used to limit reduce or prevent the spread of disease.

**Veterinarian** is a person licensed according to the praka number 368 or veterinarians of the Department of the Animal Health and Production.

Village Animal Health Worker is a person licensed according to the praka number 368.

1.4 The Minister shall have power under the decree to appoint inspectors, veterinarians or other persons necessary to enforce these regulations, issue rules for issuing licenses, fixing fees or giving exemptions from fees in specific cases and prescribing other matters to carry out the provisions of this Prakas.

1.5 The Department is responsible for implementing this regulation. The provincial animal health offices have to refer directly to the Department for any matter related to this regulation, and more generally, to any actions related to the animal health.

1.6 Regulations issued by the minister shall come into force from the day they are published in the Government Gazette or a national newspaper.

### Chapter 2, List 1 diseases

As explained in introduction, the farmers must be included in the control of the animal diseases. It means that the regulation must introduce the obligation for them to declare any suspected list 1 or list 2 disease outbreak in their herd. Of course this duty should be implemented progressively, after information campaign and after the official publication of the compensation rate for the loss of animal in case of stamping out policy. The farmer must also be informed about the danger to spread a disease (by transporting sick animals, by selling sick animals...).

2.1 The Minister, on advise from the Director of DAHAP may from time to time declare any disease of animals a list 1 disease by placing its common and scientific names or a symptomatic description of the disease in the case of an emerging disease on list 1 of this decree by praka. The praka will come into effect by placing a notice to this effect in the Government Gazette, a national newspaper and /or posted on the notice board of the OAHP in all province's and district offices.

2.2 The owner of animals or representatives and the veterinary services providers have the obligation to notify, as soon as possible, any suspicion or confirmed case of list 1 disease to the village chief or the animal health and production office. The village chief has the responsibility to transmit in a brief delay this information to the animal health and production office."

The MAFF will be responsible to spread the information relating to this article to the farmers, as well as the technical knowledge useful to recognize the list 1 diseases."

2.3 When a suspicion of list 1 disease is reported to veterinary authorities, chief of animal health concerned must carry out a check without delay, may order to take sample(s) for laboratory confirmation, and must inform the Department.

By waiting the confirmation of the suspicion, he must take any measures to avoid the spread of the disease.

2.4 The following are the procedures that the Director or the provincial chief of animal health office may use or direct an inspector to use to control a confirmed list 1 Disease.

(a) Declaration of a temporary Animal Quarantine Area in order to stop livestock movements into or from a given area;

(b) Collect samples from any animal whether in a declared quarantine area or not for analysis and may require the destruction of any animal or property, or subject them to any treatment or measure, based on the results of any test or tests;

(c) Humanely kill any or all-animals affected or believed to be affected by the disease ; This point should be detailed in a praka or an internal note.

(d) Destroy or suitable treat all carcasses, animal products or any property that may be considered infected or believed to be infected, in such a manner so as to prevent the spread of the disease: This point should be detailed in a praka or an internal note.

(e) For the prescribed fee or fees seek the assistance of any person licensed according to praka number 368 to help carry out any vaccination, treatment measure or other duties they may be directed to perform that is necessary to control any disease outbreak; Point to discussed :

The regulation mentions that licensed person will be paid according to prescribed fees. It should be also defined how those fees will be established. We could mention,

for instance, that a committee, formed by the Government and the representative of licensed persons, will discuss the fees each year at the national level or the provincial level (the discussion could take place at the board set up for the licensing).

(f) Under take any treatment measure that may be necessary involving animals or the environment in order to prevent further spread of the disease;

(g) According to the specific technical prescription for the disease, vaccinate all or some of the animals in a temporary Quarantine area in order to prevent the spread of the disease;

(h) Declare any area a disease free area in order to prevent the movement of animals into the area unless they have been inspected and subjected to a procedure or treatment thought necessary to prevent the spread of a disease

(i) Monitor the importation and /or manufacture and use of any vaccine for that disease.

(j) Limit the movement of both affected and none affected animals within the temporary quarantine area; Point to discussed :

The limitation of animal movement is one of the most efficient way to avoid the spreading of animal diseases. But in the context of Cambodia, a total ban of animal movement during the field work period would have very bad consequences for farmers. So, by implementing this regulation, the inspectors should take into account those particular situations and propose adaptations.

(k) Cause any vehicle used to transport stock or having travelled in an infected area or a suspected infected area to be disinfected and cleaned to the satisfaction of an inspector;

(1) Apply to any animal a means of identification described under *Chapter 5.5*;

2.5 Records must be kept of every properties or animals destroyed in application of this regulation under the responsibility of the Director, or the provincial chief of animal health, so that:

(a) compensation may be paid.

(b) any compensation paid will be no greater than the fair market price at the time of destruction.

2.6 Where a disagreement arises over the value of any property, animal or carcass, an arbitrator, acceptable to both sides, may be called in to settle the dispute Point to discussed :

The procedure to appeal must be defined more clearly. The possibility to choose an arbitrator acceptable from the both sides, may be difficult to apply and can be another source of disagreement.

The normal procedure would be that the arbitrator is a civil servant assigned by MAFF to execute the control of animal disease. And, in a second time, it can be possible to appeal to the court.

In all cases, when animals are submitted to a procedure of slaughtering and destruction, the farmer must be clearly informed about the way to appeal about the amount of the compensation.

Another solution to avoid trouble would be to have a national rate for compensation. This rate could be modified regularly according to the market price and should take into account the type of animal (age, breed, weigh, pregnancy).

2.7 Where a dispute cannot be settled by arbitration it may be done as a tort in a court of law.

2.8 Compensation may not be paid to any owner who has been convicted in a court of law of any offence under these regulations.

2.9 Forbid the importation or transit of any sensitive animals and potentially infected products of animal origin from areas outside the Kingdom of Cambodia in the event of an outbreak or suspected outbreak of any list 1 disease in any other country.

# Chapter 3, List 2 diseases : notifiable diseases

3.1 The Minister, on the recommendation of the director of DAHP may from time to time declare any diseases of animals a list 2 disease by placing its common name and scientific names or a symptomatic description of the disease in case of an emerging disease on list 2 of this decree by praka.

3.2 The owner of animals or representatives and the veterinary services providers have the obligation to notify, as soon as possible, any suspicion or confirmed case of list 2 disease to the village chief or the animal health and production office. The village chief has the responsibility to transmit in a brief delay this information to the animal health and production office.

The MAFF will be responsible to spread the information relating to this article to the farmers, as well as the technical knowledge useful to recognize the list 2 diseases."

### Chapter 4: List 3 diseases

# **Control program**

4.1 The Minister, on the recommendation of the director of DAHP, may place any diseases affecting animals on the list 3 of this decree by praka, as a disease submitted to an official control program.

The declaration of an area submitted to this official control program will be made by public notification by the Director according to the recommendation of the provincial animal health offices.

4.2 Any diseases placed on list 3 forms part of a commitment entered into by the DAHP, the community in a declared area, and the VAH System, to prevent or limit that disease

Point to discussed :

A good definition of community has to be found.

4.3 The size of any area declared under a control program shall be commensurate with the epidemiology of the disease or diseases in question.

4.4 There is nothing in these regulations that will prevent any licensed person or owner of any animal from vaccinating or treating livestock for any disease listed on list 3 if the animals are resident outside any area declared under official control program.

4.5 The Department can declare any province or area :

under a compulsory official control program for one or several list 3 diseases,

under a facultative official control program for one or several list 3 diseases.

This will authorize the animal health offices to :

(a) Investigate and evaluate any factor that may contribute to or exacerbate any disease state listed on list 3.

(b) Undertake all steps necessary to inform livestock owners in the area as to the objectives and benefits of the program and to manage the measures to be put in place to limit the effects of an animal disease.

(c) Ensure that all licensed persons in the area are adequately trained to undertake the treatment or preventative measures to limit the disease or diseases.

(d) Help negotiate a fee between licensed persons and the community in the area to undertake the planned disease limitation or prevention program.

# Comments :

To have facultative control program with official endorsement give the possibility to private sector to initiate any actions to control animal diseases. The Government, in addition to its endorsement, can decide to give a financial support or not for those facultative control programs.

Relating to the compulsory control program, the Government has to participate to the cost of the program.

4.6 All licensed person, practicing in an area declared under a control program under this Sub Decree may assist in any vaccination or control program and shall be paid by the livestock owners the fee negotiated for each particular procedure (s) for that season.

4.7 It will be the responsibility of the community and community leaders to ensure that animals are presented as agreed in a timely manner for any treatment or disease preventative program.

4.8 When necessary the DAHP may require the provision of land to be set aside for the setting up of either temporary or permanent yarding in which animals may be treated or held for inspection.

4.9 If an area has been declared under an official control program and no licensed person is available, or in the event of an extensive disease outbreak, the Director, may use any licensed person to undertake any treatment or vaccination required.

### Comments :

The list 3 represents the list of diseases with facultative or compulsory control programs. This means that the Authorities will decide with the community to apply some of those programs in specific areas. For this point it should be explained if those programs are totally facultative or can become compulsory. It may be decided that above a certain percentage of participants in a certain area, all the farmers of this area should apply the program. The more the size of the area is big, the more one can have a chance to eradicate the disease. So, provincial offices should push to have a control program at least for the district level. But this should not prevent to organize such programs in areas smaller than a district but coherent in term of epidemiology.

# Chapter 5 Responsibilities of Licensed Persons.

5.1 It is a requirement of these regulations that any licensed person shall:

(a) Report a disease outbreak or a suspected disease outbreak of any disease listed on either list 1 or list 2 as soon as practically possible to the nearest OAHP office or village chief. Point to discussed :

Has the VAHW to inform directly the OAHP or can he inform the village chief only? In term of efficiency, the first option is better, but is it feasible?

(b) Keep records of all animals they have vaccinated on the prescribed form that shall include

i. the type and batch number of vaccine used;

ii. the date of use;

iii. where the procedure was undertaken (name of district, commune and village + name of owner).

(c) Provide the information in 5.1. (b) when required of the number of animals treated and/or vaccinated in any given period.

(d) In the event of an outbreak of any disease listed in list 1 a licensed person may be asked to assist with any treatment, vaccination programs or other measures imposed on stock in the province in which they are licensed.

5.2 Not withstanding the requirements of 5.1 any licensed person may ask for a fee to: (a) regularly report on the disease incidence in a given area,

(b) Collect and submit specimens under the guidance of the OAHP as part of a disease study program for onwards transmission to the NAHPIC.

(c) asked to assist, or undertake, with the identification of animals in a given area.

# **Chapter 6 Animal identification**

6.1 The Director may require any owner or group of owners to identify their animals by applying any one or combination of methods :

(c) Branding with a hot iron in the case of cattle or buffalo on the rump or flank.

(d) Ear marking, ear or body tattooing in the case of pigs and ruminant animals.

(e) Ear tagging, tail tagging or any similar means of identification in the case of ruminant animals and pigs.

(f) Microchip or any other similar electronic means of identification in any animal.

(g) Aluminium leg bands in the case of poultry.

6.2 While the presence of a brand, tattoo, ear, tail or leg tag is prima-facie evidence of ownership the owner must first have registered the identifying marks and the method of identification with the OAHP in the province in which they reside.

Point to discussed :

# It should be decided, if the identification, for official purposes, will be done only by inspectors or as well by licensed persons.

6.3 The identification of animals intended for slaughter or export is compulsory and has to be done according technical conditions that will be given in a further praka. Any animal intended for slaughter or export has to be accompanied with, respectively, a national or international sanitary certificate with mention of the identification. **Comments :** 

The obligation of identification must not be separated from the obligation of sanitary inspection before transport and obligation of presentation of an identification document. Indeed, the identification of the animal is useful for two main reasons :

- identify the origin of the animal in case of detection of disease (it means that the identification mark must be accompanied with an identification document with the mention of the origin of the animal),

- be able to associate the good animal with a sanitary certificate.

6.4 It will be an offence under these regulations to remove, to alter, or make unreadable any means of identification that has been applied or attached to any animal or animals.

6.5 The application of any distinguishing identification for purposes of ownership shall be at the cost of the owner in the following cases:

(a)To establish ownership of any animal.

(b)To allow the free movement of an animal for slaughter.

(c) In all cases where an animal is to be exported

6.6 Where any identification of any animal is required as part of a control program of a disease listed under list 1 the cost of identification of any animal(s) will be the responsibility of GOC

6.7 The minister by notice in the Government Gazette may make further regulations as to the manner of registration of any identification mark, the configuration of any identification mark and any other administration procedures for the purpose of identifying the ownership of animals.

# Chapter 7

# Declaration of a Quarantine Area

7.1 The **Director**, on advice of provincial animal health chief, may declare any area, any district or geographical area a quarantine zone for any particular specie(s) of animals or products of animal origin by placing a description of the boundaries of the area on *a* public announcement.

7.2 Any area declared as a quarantine area must have erected at the major entries into the area a sign to indicate the boundary of the quarantine area.

7.3 Where the **Director** declares an area, a temporary quarantine area, as part of a disease control program for a disease listed under list 1 he may require all animals within the quarantine area to be identified in a particular manner. Any animal in a quarantine area found not to be identified in the approved manner may be seized by an inspector and taken to a secure area until the ownership of the animal (s) is established.

7.5 To be in charge of, or own an animal that is not identified, in a declared quarantine area is an offence under these regulations and is punishable by a fine. In the case of unidentified animal(s) or where the ownership cannot be established the animals may be sold in order to defray the costs of seizure or any treatment as outlined in Chapter 8.

7.6 The Director may, by publication in the Government Gazette, bring into force further regulations controlling:

- i. The setting up and operation of quarantine areas,
- ii. the conditions of entry into and from quarantine areas,
- iii. What animal, animal product and animal by products must enter or leave The Kingdom of Cambodia through particular import or export quarantine area.

7.8 It will be an offence to try to import any animal unless they are identified in the manner required by the DAHP and accompanied by a Zoo sanitary certification from the country of export that the animal (s) have been treated in the manner required by the Government of Cambodia.

# Chapter 9 Appointment and Duties of Inspectors.

8.1 The Minister may by publication in the Government Gazette appoint competent officials to carry out the requirements of these regulations. Any competent official appointed must be: -

- (a) In the case of the police, an officer above the rank of (Captain);
- (b) In the case of the army, an officer above the rank of Captain;
- (c) Any other public official the Minister may wish to appoint.

8.2 To carry out the requirements of these regulations, the Director of Animal health and Production may appoint:

- (a) inspectors who are permanent employee of the DAHP
- (b) a person licensed under praka number 368.

# **Comments** :

This relation between the private sector and the Government needs to be very well prepared to assure the protection of both sides. To carry out their mission the private workers need to get assurance of a defined salary. On the other side, the Government has to impose duties on the private workers in order to assure a good service.

8.3 The DAHP may limit the powers of any licensed person appointed under these regulations to any part or parts of these regulations.

8.4 An appointed inspector may alone, or accompanied by a competent person,

- (a) Enter any property during the hours of daylight;
- (b) Stop and search any vehicle;
- (c) Require any stock to be unloaded from a means of transport for inspection;

(d) Seize any animal(s) and hold them in a secure area while investigating an alleged offence against these regulations;

8.5 An inspector may seize and hold in quarantine any animal if there is reason to suspect the presence of any disease listed under list 1 of these regulations, or if the animals without a means of official identification, are in a declared quarantine area without authority, are being transported without the required authority, or being driven through a declared quarantine area.

# **Chapter 10**

The penalties must be defined in coordination with the ministry of Justice.

# New chapter or new articles to add in general provision if the notion of farm management needs to be introduced in this regulation

#### 1.

The owner of farm with more than (50?) animals intending for consumption must declare its farm to the district or provincial animal health and production office.

### 2

The livestock production must preserve environment and follow the standards relating to the control of animal wastes. Those standards will be taken by the MAFF.

### 3.

The livestock production must follow the standards relating to animal housing, animal welfare and animal feeding.

# Annex 4. Standardised reporting forms used by the epidemiological network

#### MONTHLY REPORT OF ANIMAL DISEASE STATUS IN THE KINGDOM OF CAMBODIA

											Distric	t		
		ţ			ţ		Ļ				Month		. Year.	
Name of disease	District south totol subsect Note 11	Location Jobal Number Vote 2 Note 3 31 1 4	Obo Status no r i stat end of month Note 4 Note 5	Daic of findex call	Kigi Vistem 8	Popula Species	Age grupping	Total In Afforded St-7	Casos Pora	orted month the Sta	ugrilers 1d	Cotel nun	Destra	ully eak Sloughters
							95.8							
	 				i									
									·					
		l							1			1		L

Notes:

(1) District serial number . This indicates the serial number of the outbreak in the district for the current year. A '1' indicates the first reported outbreak, etc.
(2) Location. Enter name of commune
(3) Enter total number of villages affected during this outbreak
(4) Diagnosis. Enter 'C' for laboratory confirmed or 'S' if diagnosis suspected on clinical and epidemiological findings
(5) Status at end of month. Enter 'C' (continuing) if outbreak is still active at the end of the reported month, or 'E' (ended) if all restrictions were lifted during month.

DAH	P - REPORT OF DISEASE	OUTBREAK INVEST	IGATION	1	j			
1	TYPE OF REPORT - 1 = init	tia!, U = update (circle	correct ar	nswor)	I U			
2	DIAGNOSIS 2.1 Name of disease							
2.2	Diagnosis C = Confirmed by Lab, S = Suspected (dircle correct answer) C S							
2.3	Clinical/post mortem specim	ens sent to Ver. Diagnos	stic Labor	atory?	Yes / No			
24	If yes, name of VDL	2.5	Date of	submission				
3	LOCATION (of index case)							
3.1	Province	3.2	District		/			
3.3	Commune	3.4	Village					
3.5	Enter name and address of	uwhor of index case:						
4	TIME	4.1	Date ou	tbreak reported				
4.2	Date of index case	4.3	Date of	1 <sup>st</sup> investigation	-			
44	Date of this report	1.5	End dat	e				
5	HISTORY	<u></u>	And Million Destination					
51	1 is there any relevant vaccination history Yes 7 No							
	if yes, give details							
5.2	2 Any animals recently moved into allected area Yes 7 No							
1	If yes, give details							
53								
0.0	Lif yes nive details		a tesetiny					
6	MAJOR CLINICAL SIGNS							
7	MAJOR POST MORTEM FI	NCINGS						
8	POPULATION AT RISK							
8.1	Number of villages affected	istach man if precared	\					
8.2	i Management system - Lise r	Todes			1			
83	Sex affected F - female. N	= male. C = castrated.	A = a / /	orde answer(s)	MECA			
8,4	Species affected (boy, buf, s	ui avi far elc)						
8.5	Tota: number at risk by species							
9	NUMBERS OF DISEASE RELATED EVENTS BY SPECIES							
9.1	TTAL NUMBER OF DISEASE-KELATED EVEN (5) BY SPECIES							
9.2	Total number of deaths to da	ite, by species		, 1	1			
S.3	Total no. siauphtered to date	, by species		· - <del> </del>	<u>+</u>			
10	PROBABLE SOURCE OF	AGENT			<u> </u>			
-								
11	ACTION(S) TAKEN		Quar S	au Vacch Mov	/t			

Name of investigating officer

· · · · · · · · ·

Signature ....

APIP – Animal Health Component Training in Veterinary Epidemiclogy

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# Annex 5. NAHPIC staff

Position	Name	Qualification	Workshop and Training Courses
Head of NAHPIC	Dr. Sorn San san@forum.o rg.kh	- Bachelor Degree of Veterinary Science (Cuba) - Master of Tropical Veterinary Science (James Cook University, Australia, 1997- 1999)	Training Courses         -The value-Based Project Planning and Management, and Gender Awareness Training Course (24/06/2002 – 30/06/2002, DAHP)         - General Management Course Supervisory Management (Institute of Technology and Management (Phnom Penh), 15/03/2001 – 28/04/2001)         - Certificate of the International Training Course on Veterinary Epidemiology (FAO, Vietnam 12/01/1995 - 27/01/1995)         - Management and organizational development working group (MOD) (05/12/1994 - 16/12/1994, Cambodia)         - Computer Skill Training Course Database Management (Institute of Technology and Management (Phnom Penh), 25/06/2001 – 03/08/2001)         Workshop         - International Workshop on Pasteurellosis in Production Animals (Indonesia, 10/08/1992 - 13/08/1992)         - 6 th Animal Science Congress of the Asian-Australasian Association of Animal Production Societies (AAAP). (23/11/1992 - 28/11/1992, Thailand)         - Gastro-internal parasite in small ruminant (13/03/2001-16/03/2001, Vietnam)         - OIE regional workshop on animal health information systems with emphasis emphasis on animal health economics (6-8/11/2001, Cambodia)         -Identification of Investment priorities to improve the profitability and sustainability of smallholder pig production in SE Asia & Paacific (12-13/07/2001, Vietnam)         - Pig Management CD-ROM Workshop (03-14/12/2001, University of Melbourne Australia)         -Workshop on "Research and Development Strategies for the Livestock Sector in South East Asia Through National and International Patherships" (11-15/03/2002, Thailand)         - First Meeting of the National Coordi
Vice Chief Responsible for administration and finance	Mr. Keo Samnang 012833551	Technician	<ul> <li>Training</li> <li>The Value-Based Project Planning /Management/Gender (24-30/06/2002, Cambodia)</li> <li>Project Management (15March-03 May 2001,ITM Cambodia)</li> <li>ASEAN Specialized Course on the application of Drug &amp; Chemical Residue Testing, (1-26/02/00, Malaysia)</li> <li>Epidemiology Data Collection / Management Information Systems (12-22/09/ 2000, Cambodia)</li> <li>Training on the Application of Drug Residue and Chemical Testing (1-26 Feb 2000, Ipoh Malaysia)</li> <li>Agricultural Extension Principles and Practices (05-16 /10/1998, Cambodia)</li> <li>ACIAR Training Course in Epidemiology (5-6 Aug-1996, Thailand)</li> <li>FLISA Technique on HS, NDV, FMD, Brucellosis Typing (1-2 /03/1994, NDVL Cambodia)</li> </ul>

			<ul> <li>Project Implementation and Management (Sept 1994, Thailand)</li> <li>ELISA Technique on HS, NDV, FMD, Brucellosis Typing (16 Aug-10 Sept 1993, Sri Lanka)</li> <li>General Laboratory Techniques Course (1982-1990, Cambodia)</li> <li>General Laboratory Techniques.(OctDec.1990, France)</li> <li>English Language Advanced Level 12 (May 1996, ACE Cambodia)</li> <li>Computer Advanced Powerpoint (27 Nov 2000-09 Jan 2001,ICS Cambodia)</li> <li>MAFF Intensive English (31 June-27 July 2001 Cambodia)</li> <li>General Computer Basic (Oct-Nov 1991 Cambodia)</li> <li>French Language on DELF Degree 1-2-3-4 (1992-1993 Alliance France, Cambodia)</li> <li>Spanish Language (June 1983, Cambodia)</li> <li>Intensive English Language Study (June-July 1993, CDRI Cambodia)</li> <li>English for Animal Worker Short Training (February 1990, Cambodia)</li> </ul>
			-ACIAR Workshop on Cattle Buffalo Fasciolosis Control in Cambodia (3-6/11/98, Indonesia) -ACIAR Workshop on Fasciolosis Control (2000, Cambodia) -ACIAR Final Workshop on Fasciolosis on Cattle Buffalo in Cambodia (2-7/12/2001, Australia)
Vice Chief Responsible for technical and chief of epidemiology section	Mr.Holl Davun 012833795 012943911	Bachelor's degree in animal health and veterinary science	Training         Training         -Technical Training Course on Diagnosis for Bovine Spongiform Encephalopathy (BSE) (05-21/11/03, Japan)         -FAC/JICA/DLD Regional Workshop / Training on Veterinary Epidemiology and its application (27-31/10/03, Thailand)         -Information and Communication Technology (22/08-21/09/03, Malaysia)         -On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia)         -OF-APHCA Regional IT Training on Use of Available Database (07-09/02/00, Thailand)         -Principle of Veterinary Epidemiology, Data Collection / Management and animal Health and Production Information System (12-22/09/00, Cambodia)         -Epidemiological and Economic Investigation as a Panning Basis for Animal Health Program (02/07-03/08/99, Germany)         -Feed Quality Control and Assurance (20/09-05/10/97, Malaysia)         -Animal Quarantine Management (25-29/11/96, Thailand)         -MSkhop         -Workshop on Animal Health Information Technology, Introduction of GIS to Animal Health. Application to FMD disease (24-28/02/03, Philippine)         -Workshop on Project Management Training (13-17/01/03, Philippine)         -Regional BSE Seminar / Workshop (28/10-1/11/03, Malaysia)         -2 <sup>24</sup> -2 <sup>16</sup> -01E/FAO-AHPCA/DLD Regional Workshop on Bovine Spongiform Encephalopathy (BSE) (09-11/10/03, Thailand)         -Third Annual Progress and Planning Meeting of ACIAR Project on Control of Fasciolosis in Cattle and Buffalos in Cambodia, Philippine and Indonesia (28-30/11/

Epidemiology section Vice-chief	Mr. Nget Kiri 021925672	Bachelor's degree in animal health and veterinary science	<ul> <li>Training</li> <li>-On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia)</li> <li>-Training in principles of Veterinary Epidemiology, Data collection/ Management, Animal Health and Production Information System (12-22/09/00, Cambodia)</li> <li>- Disease Surveillance and research (23/04-12/05/01, Thailand)</li> <li>- The Application of Molecular Biology in the Diagnosis of Poultry Diseases (01-27/02/99, Malaysia)</li> <li>Wokshop</li> <li>-Seminar on "Gene Technology-New words in animal nutrition". (24-25/10/02, Cambodia)</li> <li>- Third Annual Progress and Planning Meeting of ACIAR Project AS1/96/160 on Control of Fasciolosis in Cattle and Buffeloes in Cambodia Indonesia and Phillipine (28-30/09/00, Cambodia)</li> <li>- FMD Surveillance in Cambodia (19-23/03/01, Cambodia)</li> </ul>
Epidemiology staff	Mr.Chhim Vutha	Medium level, Prek Leap Agricultural College	<ul> <li>Training</li> <li>-FAO/JICA/DLD Regional Workshop / Training on Veterinary Epidemiology and its application (27-31/10/03, Thailand)</li> <li>-On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia)</li> <li>- Training in Principles of Veterinary Epidemiology, Data Collection/ Management, and Animal Health and Production Information System (APIP TA) (12-22/09/00, DAHP, Cambodia)</li> <li>- ASEAN Specialized Course on the application of Drug &amp; Chemical Residue Testing (1-26/02/00, Malaysia)</li> <li>- Training on Parasitology, Chuala Lumkorn University (04-22/09/94, Thailand)</li> <li><i>Wokshop</i></li> <li>- Workshop on FMD Surveillance in Cambodia, Department of Animal Health and Production, Phnom Penh. (19-23/03/01, Cambodia)</li> <li>-FAO/JICA/DLD Regional Workshop/ Training on Veterinary Epidemiology. (27-31/10/03, Thailand)</li> <li>- Third Annual Progress and Planning Meeting of ACIAR Project AS1/ 96/ 160 on Control of Fasciolosis in Cattle and Buffaloe in Cambodia, Indonesia and Philippines Cambodia, Malaysia. (28-10/11/00, Cambodia)</li> </ul>
Epidemiology staff	Mr.Nou Kimsay 012933884	Medium level, Prek Leap Agricultural College	<ul> <li>Wokshop</li> <li>-On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia)</li> <li>-Workshop on FMD surveillance in Cambodia (19-23/03/01, Cambodia)</li> <li>-Third Annual Progress and Planning Meeting of ACIAR Project (AS1/96/160) on control of Fasciolosis in Cattle and buffaloes in Cambodia, Indonesia, and Phillipine (28-30/11/00, Cambodia)</li> <li>-Training in principles of Veterinary Epidemiology, Data Collection/ Management, Animal Health and Production Information System (APIP, TA) (12-22/09/00, Cambodia)</li> </ul>
Serology Section Chief	Mrs. Ren Theary 011932896	Bachelor's degree in animal health and veterinary science	<ul> <li>Training</li> <li>-Hog Cholera Disease diagnosis (27/10-29/11/03, National Institute of Animal Health, Thailand)</li> <li>-Serology Technique for CSF diagnosis (including cell culture) (13/01-13/03/03, Tsukuba National Institute for Animal Health, JAPAN)</li> <li>-On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia)</li> <li>-General Livestock Farming for Cambodia (04/07-19/09/99, JAPAN)</li> <li>- English</li> <li>Wokshop</li> <li>-Regional Workshop on control of classical Swine Fever (CSF) in South-East Asia (25/09-26/09/03, Vietnam)</li> </ul>
Serology staff	Ms. Neak	Medium level, Prek Leap	Training

	Sotheary 012875512	Agricultural College	-Brucellosis Diagnosis (27/10-05/12/03, National Institute for Animal Health, Thailand) -Elisa for FMD (2 months, 2003, Reference Regional Laboratory for FMD, Thailand) -On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia) -Quality Control Vaccine HS (6 weeks, 2001, Veterinary Biologics Center inPakchong, Thailand) -Serology analysis for brucellosis (1 month, 2000, Malaysia) -Newcastle Diseases Vaccine Production (2 weeks, 1999, Cambodia) -Poultry Diseases diagnosis (1 month, 1998, Veterinary Research Institute, Malaysia) -Livestock Diseases and Zoonoses (3 weeks, 1997, Chiang Mai Faculty of Medicine, Thailand) -Elisa FMD (2 weeks, 1994, Thailand) -English, Computer courses
Serology starr	Chanvisal 012833795 012943911	Agricultural College	-On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia) -Feed Quality Control and Assurance (20/09-05/10/97, Institute Haiwarn, Malaysia) -English Language Training Stream 1
Bacteriology Section Vice-chief	Mrs. Sok Koam 012925672	Medium level, Prek Leap Agricultural College	Training -Surveillance and Epidemiology of Salmonellosis and other Food borne Diseases (24-31/08/03, Thailand) -On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia) -Technical Training of Bacteriology (09/06/99-17/03/00, JAPAN) - Survey on Bacteria in the Food (19/05-24/06/97, Cambodia) -Technical Training of Bacteriology (1994, Thailand)
Bacteriology Staff	Mrs. Pol Reakheta 012511104	Bachelor's degree in animal health and veterinary science	Training         - Meat analysis in Pasteur (1 week per month during 9 months, 2003-2004)         -Labo Analysis of Feed Toxicology (25/08-05/09/03, Malaysia)         -On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia)         -Surveillance and Epidemiology of Salmonellosis and other foodborne diseases. (28/01-02/02/02, Cambodia )         -Diagnostic Technology and Control Measures for Major Livestock Diseases. (11/01-05/02/99, Thailand)         Wokshop         - Workshop on Food Contaminant Monitoring (03/01/01, Cambodia)
Bacteriology Staff	Mrs. So Pheany 012890308	Medium level, Prek Leap Agricultural College	Training -Labo Analysis of Feed Toxicology (25/08-05/09/03, Malaysia) -On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia)
Haematology Chief	Mrs. Im Oudompanha 012927203	Bachelor's degree in animal health and veterinary science	Training         -Diagnosis on Newcastle Disease (27/10-29/11/03, National Institute for Animal Health, Thailand)         -On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia)         -Bacteriology technique (09/08-08/09/00, Veterinary Research Institute, Malaysia)         -Chemical analysis on Food (21/07-02/09/97, Institut Technologique du Cambodge, Cambodia)         -Computer MS-Access 2000 Level 1         -English level of Headway         Workshop         -Workshop (BSE) ( 28/10-01/11/02, Malaysia)
Haematology staff	Mrs. Seng Bunnary 016867121	Medium level, Prek Leap Agricultural College	<b>Training</b> - General courses in Parasitology (02-27/06/03, National Institute of Animal Health, Thailand) -On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia) - Basic English for science

	N. N.	Cartificante of antoning	
Haematology staff	Mr. Yit	science	Training
	Chichg	science	-On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia)
	011942529		-Course on Bacteriology (1982, Cambodia)
			-Couse on Haematology (1984, Cambodia)
			-Course on Histopathotology (1985, Cambodia)
			-Course on Serology (1986, Cambodia)
Biochemistry	Ms.Thach	Bachelor's degree in	Training
Chief	Phalla	animal health and	On job Training of Laboratory Technics with Pay Halliwell TA (06/01-11/02 Cambodia)
		veterinary science	-Biochemical Analysis (23/08-23/09/01)
Parasitology	Mr. Bun	Bachelor's degree in	Training
Chief	Chan	animal health and veterinary science	-Training course on advanced studies on protozoan diseases (10 months, 2002-2003, Japan)
	012825186		Animal health,Thailand)
			-On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia)
			-Principle of Veterinary Epidemiology, Data Collection / Management and animal Health and Production Information System (12- 22/09/00 Cambodia)
			-Scientific Communication (2 weeks, 2001, CARDI, Cambodia)
			-English Language
Parasitology	Mrs. Ngin	Bachelor's degree in	Workshop
Staff	Nounpisey	animal health and	-Workshop on Control of Fasciolosis in Cattle and Buffalo in the Kingdom of Cambodia (1997, viet Nam)
	023881131	veterinary science	Training
			-Training course on Diagnosis and disease control (07/02-03/03/00, Thailand)
			-Special Training course in Advanced Studies on Protozoan diseases (24/10/01-06/09/02 Japan)
			-English Course 1 year
Parasitology	Mr. Seak	Bachelor's degree in	
Staff	Sophary	animal health and	Discovers Technology and Control Measures for Maior livesteak Discovers Theiland
		veterinary science	-Diagnosis recimology and control measures for major restock Diseases manand
	011953209		workshop
			-3 <sup>th</sup> International Conference on Control of Cambodia Fasciolosis in Cattle and Buffaloes. Cambodia
Parasitology	Mrs. Chhoy	Medium level, Prek Leap	Training
Staff	Mom	Agricultural College	-On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia)
	012692595		-Training Vaccine Production and Quality Control (15/08-15/10/00, Thailand)
	012085585		-Training Veterinary Parasitology analysis research Institute (23/08-23/09/01, Malaysia) -Computer ICS
Parasitology	Mr. Sem	Medium level, Prek Leap	Training
Staff	Tharin	Agricultural College	-Diagnostic Parasite Disease (12/05-12/07/03 Thailand)
	012925197		-On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia)
	01202318/		-Lab Technology Course in Veterinary Parasitology (12/08-08/09/00, Malaysia)
L			

			-Parasitology (04/09-18/09/94, Thailand) -English languages (Phnom-Penh University) -Computer (Word 7.0, Excel) -Administrative affair and Secretary
			Worshop Third Annual Progress and Planning Meeting of ACIAP
Pathology Chief	Mr. Chhin Manov 012837672	Bachelor's degree in animal health and veterinary science	Training         -Training Workshop in applied Epidemiologist Skill (1 996 Thailand)         -Veterinary Biochemical Analysis.         (02/08-08/09/00 Malaysia)         -Technical Training of Pathology and Histopathology (09/05/02-05/03/03 JAPAN)         -Basis English for Science         -Computer Training         -On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia)
Pathology Staff	Mrs. Tith Sothearos 012500941	Medium level, Prek Leap Agricultural College	<b>Training</b> -On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia) -Asean Specialized Course on the application of Molecular Biology in the Diagnosis of Poultry Diseases (01/02-27/02/99 Malaysia) -Computer -English Language
Pathology Staff	Mr. Lim Sopheak 092818880	Certificate of veterinary science	Training -On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia) - Course on General Animal Diseases (1982-1983 Cambodia) - Course on Technical Animal Control and Diseases Research. (1984-1985 Cambodia) -Course on Certificate Pathology. (1986-1988 Cambodia)
Pathology Staff	Mr. Lim Sorphea 012692112	Medium level, Prek Leap Agricultural College	Training -On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia) -Training Course on Agricultural Project Preparation and Appraisal Health (1996, Cambodia) -Course in MAFF English
Administration Section chief	Mrs. Heng Morany 011950572	Bachelor's degree in animal health and veterinary science	Training -Veterinary Biochemical Analysis (05/08-05/09/03 Malaysia) -Training course on Diagnostic Technology and Control measures for Major livestock Diseases (02/06-27/06/03 Thailand) -On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia) -General Livestock Farming for Cambodia (06/07-19/09/98 Japan) -General Computer (MS-Widows, MS-Word, Quickbooks Accounting ) -English - Financial Accounting
Administration Staff	Mrs. Chou Vanna 092805143	Medium level, Prek Leap Agricultural College	Training -On job Training of Laboratory Technics with Roy Halliwell, TA (06/01-11/02, Cambodia) -Computer Course (P.T.S.) (1996,Cambodia) -English Course (1997,Cambodia) -Computer Course (1997,Cambodia) -Library Course (1997,Cambodia)

Administration Staff	Mr. Chhun Sophean	Medium level, Prek Leap	Training
Stall	Sopheap	Agricultural College	-On job Training of Laboratory Technical with Dr. Roy Halliwell (27/06/01-05/11/02, Cambodia)
	016856686		- Computer (2000, Cambodia)
			-General Accounting (2000, Cambodia)
			- English (1994, Cambodia) -Thai (1994, Cambodia)

APIP = Agricultural Productivity Improvement Project (IFAD loan)

# SAMPLES TO COLLECT DURING POST-MORTEM FOR HPAI DIAGNOSIS BY IFAT AND RT/PCR

# Samples to collect

	Pasteur (RT-PCR)	NAHPIC
Swab from trachea in transport media*	1(-28°C)	
Swab from cloaca in transport media*	1(-28°C)	
Dry swab from trachea for deposit on slide and		2 slides(4°C)
fix in acetone		
Dry swab from cloaca for deposit on slide and		2 slides (4°C)
fix in acetone		
Direct brain smear to be fixed in acetone		2 slides (4°C)
Direct pancreas smear to be fixed in acetone		2 slides(4°C)
Direct spleen smear to be fixed in acetone		2 slides(4°C)
Swab from cloaca in PBS for rapid test		1(4°C)
Swab from trachea in PBS for rapid test		1(4°C)

\*Possibility to pool swabs from 5 animals must be discussed with Pasteur

If organs are collected for RT-PCR : the following organs have to be sampled and placed individually in cryotubes (possibility to pool organs must be discussed with Pasteur) :

- brain
- lung
- liver
- spleen
- pancreas

# Presentation of the slides

Direct smear from organs



Dry swab from Trachea and Cloaca



B = Brain, P = Pancreas, T = Trachea swab, S = Spleen, F = Cloacal swab

# Annex 7. Terms of reference for Laboratory Expert for the National Veterinary Laboratory in Phnom Penh

**Objectives of the mission:** support the national veterinary laboratory in the good implementation of the techniques for avian influenza virus diagnosis.

<b>Duty Station:</b>	Capital at the National Animal Health and Production Investigation
	Centre, NAHPIC.

**Duration:** 4 working weeks.

# Activities

Under the general supervision of TCEO and the FAO Representative, and in close collaboration with the Chief of the Laboratory, the Director of the Veterinary Services, the Laboratory Expert will undertake the following activities:

- Supervise the good management of the samples coming into the laboratory
- Assess the procedure implemented by the serology/virology section for avian influenza diagnosis (immunofluorescence test for antigen and hemaglutination inhibition test)
- Assess the bio-safety conditions for introducing egg inoculation technique in serology/virology section (if not yet done). A particular attention must be brought to the waste management
- If every conditions are met, train the serology/virology section on the egg inoculation technique
- Supervise the pathology section for appropriate sampling method.
- Prepare a brief technical report (in English) at the end of the mission

The Laboratory Expert will work in collaboration with the other technical adviser and international consultants.

# Qualifications

Senior laboratory expert, well experienced in the diagnosis of animal diseases with:

- practical skills in the above mentioned tests for avian flu diagnosis,
- practical skills in medical microbiology,
- He/she will have level C proficiency in English.

# Annex 8. Results of the proficiency test

# HI test quality control

Phnom Penh, 16/07/2004

Proficiency test organised between : National Animal Health and Production Investigation Centre, Phnom Penh, Cambodia Under the responsibility of Dr. Sorn San, Head of NAHPIC and CSIRO Australian Animal Health Laboratory, AAHL Geelong, Australia Under the responsibility of Dr. Paul Selleck, Virologist.

Serum	NAHPIC results with 8 HAU	Australian Animal Health Laboratory
Nb		results with <4 HAU
11	Neg	<2 (neg)
12	8	4
13	128	128
14	NEG	<2 (neg)
15	32	16
16	256	256
17	Neg	<2 (neg)
18	64	64
19	32	16
20	Neg	<2 (neg)

NAHPIC used 8 HAU of the Vietnamese antigen provided by the AAHL (for the HA test, they found Ag titre 1/64)

# Annex 9. List of samples collected since April 2004

Province	District	Commune	Village	Market	NAHPIC	Date	Context of sampling		Produ	uction syste	em	GPS
					code	sampling						coordinate
							Surveillance	Suspected case	Village P	Semi-Com	Com	
Kompong Cham			Village1				1		1			
Kompong Cham			Village 2				1		1			
Kompong Cham			Village 6				1		1			
Phnom Penh	Russey Keo	PP thmei Quarter	Rong Chak		024	20-mai-2004	1		1			
Phnom Penh	Russey Keo	PP thmei Quarter	Chres		023	20-mai-2004	1		1			
Phnom Penh	Russey Keo	Km 6 quarter	Ben Tchouk		028	25-mai-2004	1		1			
Phnom Penh	Russey Keo	Km 6 quarter	Spien Kou		027	25-mai-2004	1		1			
Phnom Penh	Russey Keo	Km 6 quarter	Ben Tchouk		026	25-mai-2004	1			1		
Takeo	Tramkak	Pong Pean	Prey Mien		025	20-mai-2004	1					
							1					
Takeo	Samrong				029	27-mai-2004	1		1			
Takeo	Samrong		Pon Leu		030	27-mai-2004	1		1			
Takeo	Samrong		Prey Snoul		031	27-mai-2004	1		1			
Takeo	Samrong				032	28-May-04	1		1			
Prey Veng	Kam Chy Mear	Sieng Kvieng	Chouk		033	28/ 05/2004		1	1			
Takeo	Tramkak	Srey Ronoung	Tropaing Robaoum		034	6-Juin 2004		1	1			
Takeo	Bati				035	3-juin-2004	1		1			
Takeo	Bati				036	3-juin-2004	1		1			
Takeo	Bati				037	3-juin-2004	1		1			
Takeo	Bati				038	3-juin-2004	1		1			
Takeo	Bati				039	3-juin-2004	1		1			

# Samples collected since April 2004 in the context of HPAI surveillance and investigation activities

Phnom Penh	Russey Keo	PP Thmei Quarter	Khmougn	040	4-juin-2004	1			1	
Phnom Penh	Russey Keo	Svay Pak	Lo Kam Ba	041	4-juin-2004	1			1	
Phnom Penh	Russey Keo	Km6 quarter	Cham Chamrey	042	4-juin-2004	1			1	
Takeo	Daun Keo			043	7-juin-2004	1		1		
Takeo	Daun Keo			044	7-juin-2004	1		1		
Takeo	Daun Keo			045	7-juin-2004	1			1	
Takeo	Tramkak			046	7-juin-2004	1		1		
Takeo	Tramkak	Sral Ronougn	Vihear Kpov	047	8-juin-2004	1		1		
Takeo	Tramkak	Sral Ronougn	Sra Cheunign	048	8-juin-2004	1		1		
Kandal	Thakmao	Thakmao	Prey Samrong	049	14-juin-2004	1			1	
Kandal	Thakmao	Thakmao	Kompong Samnegn	050	14-juin-2004	1		1		
Siem Reap				051	16-juin-2004	1			1	
Siem Reap				052	16-juin-2004	1		1		
Siem Reap	Siem Reap	Siem Reap		053	17-juin-2004	1			1	
Siem Reap	Siem Reap	Siem Reap	same farm as 053	054	24-juin-2004	1			1	
Rattanakiri	Loum Path	Seda	Pa Tat	055	23/07/04		1	1		
Kompong Cham		Vil Vong		056	30/07/04		1			
Koh Kong	Sre Ambel	Boung Priev	Tek Poung	057	31/07/2004		1	1		
					Total	39	5			

			Nun	nber of a	nimals /	species			Total animals sampled	Total sera	Total viro	Result							F	RT/PCI	R	
LC	Fighting Cok	В	LH	Villade D	Suckling D	Laying D	Turkeys	Others	sampica			Viro -	Sero-	Viro+	Sero +	sero- /viro-	Sero+/viro+	Sera+/viro-	sera- /viro+	Not done	neg	pos
4									4	4	0		4									
14				2					16	16	0		16									
12									12	12	0		12									
1									1	1			1									
4									4	4	4					4						
1									1	1	1					1	19					
3									3	1	3	2				1						
					14				14	0	14	14										
1									1	1	1					1						
												<u> </u>										
5		-		1					6	2	6	4				2						
4		-							4	3	4	1				3						
5									5	1	5	4				1						
9				4					13	7	13	6				7						
				2					2		2	2										
4									. 4	4	4					4						
2									2	1	2	1				1					1	
1				2					3	1	3	2				1						
3									3	3	3					3						
5							2		7	7	7					7						

			T	1	1	1	T	1	T	1	Г	1	T	1	1	1	[		1		
					6				6	0	6	6									
					8				8	0	8	8									
			6						6	0	6	6									
4				2					6	4	6	2				4					
3				3					6	3	6	3				3					
1			3					2 GF	6	6	6					6					
1									1	1	1					1					
2									2	2	2					2					
3									3	2	3	1				1			1	1	
			4						4	4	4					4					
1									1	1	1					1					
						3			3	3	3							3		1	
1			1						1	1	1					1					
						3			3	3	3							3		3	
						10			10	10	10					4		6		10	
8				4					12	10	12	2				10				3	
2									2		2										
7	(	6							13	13	13										
	1. mar																				
113	(	6 0	13	20	28	16	2	2	200	133	167	65	33			74	0	12	1	 19	0
LC	FC	В	LH	Village D	Meat D	Laying D	Turkey	Other species	Animal sampled	Sera	Viro	Viro -	Sero -	Viro +	Sero+	Sero- /viro-	Sero+/viro+	Sero+ /viro-	Sero- /viro+	PCR-	PCR +

# Annex 10. Animal diagnosis strategy for HPAI suspected case



TIME LINE for laboratory results :

2 WD in NAHPIC ⇒ 4 days maximum for 80 % of the samples for complete NAHPIC results 3 WD in Pasteur ⇒ 5 days maximum for 80 % of the samples for complete results

\* It is possible to pool the positive samples by farms and by species

# DETECTION OF ANTIBODIES AGAINST AVIAN INFLUENZA VIRUS SUBTYPES BY HAEGGLUTINATION INHIBITION TEST

(according to WHO manual on animal Influenza diagnosis and surveillance WHO/CDS/CSR/NCS/2002.5 and Newcastle handbook, NAHPIC 1999)

# **I**-Equipments :

- 37°C waterbath
- 56°C waterbath
- Centrifuge
- Autoclave
- Refrigerator and freezer
- Weighing balance: accuracy 0.1mg
- pH meter and control solution
- Pipettes and pipettor
- Multichannel pipettors (8 and 12 channels)
- Racks

### **II - Supplies :**

- Centrifuge tubes (graduated conical 15 ml)
- V- shaped 96- well microtiter plate(Costar 2897, packaging 100 per box)
- Coverplate [Costar.3931, 25/pack, 50/case sterile]
- Pipette Tips Plastibrand® 1000 st/pcs 200 µl (Z-015-4)

### **III - Buffers and reagents :**

- Red blood cell in Alsever's solution (chicken)
- Distilled water or deionized water
- Phosphate buffer saline (PBS) pH 7.2
- Antigen H5N1 A/Chick/Scotland/59 (Veterinary Laboratories Agency, PA4626, 1 ml) and antigen H7N1 A/African/983/79 (Veterinary Laboratories Agency Ref.PA4379, 1 ml]
- Positive control sera. Store at +4°C for 5 to 10 years. Once reconstituted about a month but will last for about 6 months if a preservative is added (methyolate or sodium azide for example). (Veterinary Laboratories Agency, anti H5N1 A/chicken/Scotland/59,PA44140.5ml and anti H7N1 A/africanstarling/Eng/983/79, PA4415 0.5 ml)
- Negative control serum (Veterinary Laboratories Agency, PA0631, 0.5 ml). Store at +4°C for 5 to 10 years. Once reconstituted, last about a month but will last for about 6 months if a preservative is added (methyolate or sodium azide for example)

# **IV - Preparation of reagents and solutions :**

### 1- Phosphate buffer saline (PBS) 10 X stock Ca, Mg free

- Take 800 ml of distilled water into the baker and weigh :

- Na <sub>2</sub> HPO <sub>4</sub> (dibasic sodium phosphate)	BDH GPR <sup>TM</sup> prod 301584L (500g)	=	9.2 g
- KH <sub>2</sub> PO <sub>4</sub> (monobasic potassium phosphat	te)BDH GPR <sup>™</sup> prod 296084J (500g)	=	2.0 g
- NaCl (Sodium chloride)	BDH GPR <sup>TM</sup> prod 102414J (500g)	=	80.0 g
- KCl (Potassium chloride)	BDH GPR <sup>TM</sup> prod 295944B (500g)	=	2.0 g

- Pour into a new bottle, wash 2 times and fill until 11. Adjust pH 7.3-7.4
- Sterilize by autoclave at 105 kPa (15 lb) or 121°C for 15 min.
- Storage: 1 year at room temperature and at +4°C when opened. PBS 1X: stored 3 weeks at +4°C.

# 2- Alsever's

- Weigh out, dissolve in distilled water, and q.s. to 500 ml:

$-C_6H_{12}O_6$ (D(+) Glucose)	BDH GPR <sup>TM</sup> prod 101174Y (500 g)	=	10.25 g
- Na <sub>3</sub> C <sub>6</sub> H <sub>5</sub> O <sub>7</sub> , 2H <sub>2</sub> O (Sodium citrate dihydrat	te) BDH Lab. reag. prod 30128 (500 g)	=	4.00 g
- NaCl (Sodium Chloride)	BDH GPR <sup>™</sup> prod 102414J (500 g)	=	2.10 g
$-C_6H_8O_7$ (Citric acid)	BDH GPR <sup>TM</sup> prod 277804L (500 g)	=	0.28 g

- After thorough mixing, check pH 6.1

- Adjust pH with 1 N NaOH or 1 N HCl

- Sterilize by filtration with 0.22 µm or sterilize by autoclave at 110°C for 10 min. and store at +4°C.

#### 3- Standardized RBCs 0.5% in PBS pH 7.4

Collect blood from three or more adult chickens into Alsever's solution (1 part to 1 part blood). Chickens have to be healthy and if possible, free from antibodies to avian influenza. Store at +4°C for 2 weeks.
After sedimentation, aspirate under the supernatant the volume of red blood needed for the day and transfer this volume in a 15 ml tube.

- Add PBS 1X qsp 15 ml. Mix gently.

- Centrifuge at 1600 rpm (500 g) for 10 min. Aspirate supernatant.

- Repeat PBS wash two times.

- Prepare a 1: 100 dilution of the suspension in a new 50 ml tube by adding for example 0.5 ml of RBC suspension to 49.5 ml PBS pH 7.2.

- Store for 1 day at +4°C.

### V- Procedure for antigen titration :

This procedure will be used for every new batch of antigen

	Concentration	Microtiter plate	Incubation time25°C	Appearance of control cells
Chicken	0.5%	V	30 min	Button*

\* = flows when tilted

- Add 50 µl of PBS 1 X in each well of the plate except the wells E1, F1, G1, H1 where 66 µl of PBS are added.
- Add 50  $\mu$ l of the control antigen to the wells A1 to D1 and 33  $\mu$ l to the wells E1 to H1.
- Make serial twofold dilutions by transferring 50 µl from the wells of the first numbered column to successive column (stop at column # 11). Discard the final 50 µl (dilutions of rows A to D start at 1:2 (column # 1) and stop at 1:2048 (column # 11) and dilution of rows E to H start at 1:3 (column # 1) and stop at 1:3072 (column # 11).

- After homogenization of the RBC suspension, add 50 µl of RBC suspension to each well on the plate.

- Mix by trapping the plate gently. Cover the plate.

- Incubate the plates at room temperature (22°C to 25°C). Check cell control for complete settling of RBCs (column #12).
- Record the result for each row and calculate the geometric mean to get the dilution giving 1 HA unit (per  $50 \mu l$ ).

# Interpretation

In the absence of hemagglutination, chicken RBCs form a compact button on the button of the wells. A " - " symbol is used to record the absence of hemagglutination. Hemagglutination can be determined by tilting the plates and noting the absence of tear-shaped streaming of erythrocytes which flow at the same rate as RBC controls.

The highest dilution of virus that cause complete hemagglutination is considered the HA titration end point. The HA titer is the reciprocal of the dilution of virus in the last well with complete hemagglutination.

Hemagglutination is not a measure of an absolute amount of virus, but is an operational unit dependent on the method used for HA titration. An HA unit is defined as the amount of virus needed to agglutinate an equal volume of a standardized red blood cell suspension

# VI- Preparation of standardized antigen for the HAI test and "Back titration"

# This procedure will be performed within the same day before any HI run.

- 1- The standard for the HAI test is 4 HA units of virus/ antigen added to twofold dilutions of antisera. Since we are adding 25 μl of antigen in the test, we need a virus dilution that contains 4 HA units/ 25 μl or 8 HA units/ 50 μl. Calculate the antigen dilution by dividing the HA titer (which is based on 50 μl) by 8 because you wish to have 8 HA units/50μl. For example, an HA titer of 160 divided by 8 is 20. Mix 1 part of antigen with 19 parts PBS 1X to obtain the desired volume of standardized antigen (ex: add 0.1 ml antigen to 1.9 ml of PBS). Calculate and prepare dilution. Keep a record of the dilution prepared.
- 2- Perform a "back titration" to verify units by performing a second HA test using the standardized antigen dilution preparation. Store the diluted antigen at 4°C and use within the same day.
   Add 50 μl of PBS 1 X in each well G –A (column #1-4)
  - Add 100 µl of the control antigen to the wells H1-H4
  - Make serial twofold dilutions by transferring 50 µl from the wells of the first numbered column to successive column (stop at column # 7). Discard the final 50 µl (dilutions start at 8 units).
  - After homogenization of the RBC suspension, add 50 µl of RBC suspension to each well on the plate.
  - Mix by trapping the plate gently. Cover the plate.
  - Incubate the plates at room temperature (22°C to 25°C). Check cell control for complete settling of RBCs (column #8).
- 3- Record results.

# Interpretation

Standardized antigens must have an HA titer of 4 HA units / 25  $\mu$ l. This titer will hemagglutinate the first four wells of the back titration plate. If an antigen does not have an HA titer of 8, it must be adjusted accordingly by adding more antigen to increase units or by diluting to decrease units. For example, if complete hemagglutination is present in the fifth well, the virus now has a titer of 16 and the test antigen should be diluted twofold. Conversely, if hemagglutination is only present to the third dilution, the antigen has a titer of 4 and an equal volume of virus must be added to the test antigen as was used when the antigen was initially diluted. This will double the concentration of virus in the test antigen to give a titer of 8. Continue adjusting the concentration of antigen until 4 HA units/ 25  $\mu$ l (8 units / 50  $\mu$ l) is obtained.

### **VII-** Procedure for hemaggglutination inhibition

1- The coagulated blood sample has to be centrifuged 3000 rpm during 10 min. Collect the serum in the new tube and store at +4°C or -20°C (*the serum to test must be clear*).

- 2- Remove non specific inhibitors from the tested sera: heat inactivate the sera by exposure to 56°C for 30 min (sera from poultry particularly from chickens and quail have low or undetectable levels of non-specific inhibitors of hemagglutination).
- 3- Remove non specific agglutinins from the tested sera:
  - to one volume of packed RBCs (20  $\mu$ l) and 20 vol. of serum (200  $\mu$ l)
  - mix thoroughly and incubate at 4°C for 1 hr, mixing by hand every 10 min to resuspend the cells.
  - centrifuge at 1600 rpm (500g) for 10 min.
  - carefully remove the adsorbed serum without disturbing the packed cells.
- 3- Label appropriate microtiter plates to test the sera.
- 4- Determine the volume of standardized antigen needed for the HAI test. Per serum:  $25 \ \mu l \ x \ 10 \ wells = 250 \ \mu l$ . Prepare an additional 1.0 ml additional volume for " back titration "
- 5- Add 25 µl of PBS to all wells.
- 6- Add 25 μl of each serum to the appropriate first well A1-H1.
- 7- Prepare serial twofold dilutions of the sera by transferring 25 μl form the first well of the numbered columns 1-11 to successive wells. Discard the final 25 μl after column 11.
- 8- Add 25 µl of standardized antigen to wells (A2 to H11).
- 9- Add 25 μl of PBS instead of antigen to the set of sera for serum controls (column 11-H1) and for RBCs controls (column 12)
- 10- Mix by trapping the plate gently.
- 11- Cover the plates and incubate at room temperature (22 to 25°C) for 30 min
- 12- Add 50 µl of standardized RBCs to all wells. Mix as before.
- 13- Cover the plates and allow the RBCs to settle at room temperature (22 to 25°C) for 30 min.
- 14- Verify the RBC control (column 12), the positive control and negative control sera titers, the sera controls (column 1). Record theses verifications.
- 15- Record the HAI titres.

### Interpretation

Hemagglutination and inhibition of hemagglutination are read as previously described.

The positive control antigens and corresponding antisera should give consistent results when compared with previous tests.

HI titres may be regarded as being positive if there is inhibition at a serum dilution of 1/16 (2<sup>4</sup> or  $\log_2 4$  when expressed as the reciprocal as the reciprocal) or more against 4 HAU of antigen. Some laboratories prefer to use 8 HAU in HI test. While this is permissible, it affects the interpretation of results so that a positive titre is 1/8 (2<sup>3</sup> or  $\log_2 3$ ) or more.

# DETECTION OF INFLUENZA A ANTIGEN BY INDIRECT IMMUNOFLUORESCENCE

# 1-Reagents and consumables :

- Viral Transport Medium (see S.O.P)
- PBS Ca, Mg free 1X pH 7.4 (see HI test)
- Acetone (Ref.11-36-66-67, BDH Analar® prod 100033p, 1 liter)
- Monoclonal antibody anti Influenza A type: clone IA52.9 (Argene 18-030, 2.5 ml vial for 80 tests)
- Influenza A Positive and negative control slides (Argene 40-071, 5 slides)
- Goat anti-mouse IgG + IgM immunoglobulins FITC conjugated "human absorbed" (Argene 50-010, 0.5 ml vial)
- Evans blue 1% (Argene 33-030, 2 ml vial)
- Mounting medium Fluokeep (Argene 33-040, 15 ml vial) or buffered glycerine (see S.O.P.)
- Cotton or rayon swab (wooden stick,cotton,individually packed,sterile)
- Cover slip (BDH 100pcs, 24x50 mm).
- Slide (*Teflon coated immufluorescence slide with 6 mm diameter wells*) Ref. 31-010 parking 100 slides 10 wells/slide .Store at +2-8°C.
- Pipette tip
- Centrifuge 5 ml tube with cap

### 2- Equipment :

- Florescence microscope
- Staining jar
- Pipette
- Freezer
- Refrigerator
- Centrifuge

### **3- Sample collection:**

- Cells from trachea or cloaca are collected by swabbing with a cotton wool tipped stick. Then the swab is transferred in VTM vial.

- Brain or pancreas impressions could be also performed. In that case, impression must be dried and fixed in acetone as soon as possible for 15-30 min at -20°C. Allow it to dry, wrap the slide (identification number and date) and store at -20°C till use.

# 4- Transport and storage of samples:

- The samples in VTM should be transported to the laboratory preferably at  $+2^{\circ}$ C to  $+8^{\circ}$ C and stored at the lab. at  $+4^{\circ}$ C. They must be fixed within 24 hours.

# 5- Assay procedure:

- Specimens treatment and slides preparation:
  - i. Pour the VTM containing the sample into a 5 ml centrifuge tube and squeeze the swab into the centrifuge tube too.
  - ii. Rinse the VTM vial with 1 ml PBS, transfer in the centrifuge tube, and add qsp 5 ml of PBS to the centrifuge tube.
- iii. Agitate or pipette gently to liberate the cell until a homogenous milky suspension is obtained.
- iv. Centrifuge at 1200 rpm for 10 min (at +2/+8 °C if possible)
- v. Discard the supernatant by pipetting.
- vi. Resuspend the pellet (cells) qsp 5 ml of PBS and centrifuge at 1200 rpm for 10 min.
- vii. Discard supernatant and perform a second and a third resuspension and centrifugation.
- viii. After the third centrifuge discard the supernatant and resuspend the cells pellet in PBS (usually 0.2- 0.5ml but the volume must be adjusted according to the size of the pellet) until homogeneous suspension is obtained.
- ix. Deposit 30  $\mu$ l of the suspension in 1 well of an immunofluorescence slide (*one 10 wells-slide could contain 10 samples*) and duplicate the slide.
- x. Let the slide dry in the safety cabinet. Drying must be continued until the preparation is completely dry.
- xi. Fix the slide in pure acetone for at least 15 min preferably at -20°C (renew the acetone frequently, since false positives may occur if it becomes rehydrated).
- xii. Allow the residual acetone over the slides to evaporate at room temperature.
- xiii. Fixed preparations may be stored for several months at -20 °C or -80 °C.
- Indirect Immunofluorescence:
  - i. Frozen slides must be warmed up for 15 min before use.
- ii. Distribute 30 µl of the ready to use monoclonal antibody (Anti- Influenza A type) in each well to be tested. Include in each run a positive and negative control slide.
- iii. Ensure that the entire area of each well is covered. Return the reagent to refrigeration immediately after use.
- iv. Incubate at +37 °C for 30 min in incubator or in a well-humidified dark chamber.
- v. Wash 3 x 5min in PBS on a gyratory shaker with slowly movement. (change PBS between each bath. Do not allow the slide to dry between each bath).
- vi. Wipe the excess of liquid on the unpolished part of the slides with blotting paper avoiding drying the wells since immunofluorescence always give the best results when drying does not occur.
- vii. Dilute the fluorescein conjugated antibody 1/100 in PBS + Evans blue 1% 1/100 diluted (1:10 000 final).
- viii. Deposit 30 µl in each well.
- ix. Incubate at +37 °C for 30 min in incubator or in a well-humidified dark chamber.
- x. Wash 3 x 5 min in PBS as before.
- xi. Quickly immerse the slide in distilled water (increase the quality of image obtained by eliminating crystals from PBS)
- xii. Wipe the excess of liquid on the unpolished part of the slides with blotting paper avoiding drying the wells since immunofluorescence always give the best results when drying does not occur.
- xiii. Deposit 2 drops of Fluokeep on the slide, then 1 cover slip. Eliminate air bubbles by pressing on the cover slip and examine under the Fluorescence microscope using a x 40 or a x 25 lens

#### **Reading and interpretation of results**

The positive staining is cytoplasmic slightly granulous, underlined at the level of membrane

One negative result could be assessed if more than 100 cells are visible and all cells are negative (if less than 100 cells are visible and negative, the test is inconclusive).

#### **Preparation of Viral Transport medium**

<b>Tryptose phosphate Broth 2.95%</b>	
Tryptose phosphate Broth (Sigma T-9157 100 g) Distilled water Autoclave 10 min at 110°C. Store at +4°C	29.5 g 1 liter
NaCl solution 0.85%	
NaCl (BDH GPR <sup>™</sup> Prod 102414J 500 g) Distilled water Autoclave 10 min at 110°C . Store at +4°C	8.5 g 1 liter
Sodium Bicarbonate NaHCO3	
NaHCO <sub>3</sub> (Sigma Aldrich® S6014 500 g) Distilled water Autoclave 10 min at 110°C. Store at +4°C	19.25 g 1 liter
Gelatine 5% with NaCl solution 0.85%	
Gelatine (Sigma® G 9391 100 g) NaCl 0.85% Dissolve by heat. Leave until cool before used	5 g 100 ml
Viral Transport Medium	
Gelatine 5% with eau physiologique Fungizone or AmphotericinB (Sigma® A-24ll 250 mg) Peni/Strepto (1 10 <sup>6</sup> u/1 g) Gentamycine (Sigma® G 1397 10 ml) Tryptose Phosphate Broth 2.95%	50 ml 25 mg 0.5 10 <sup>6</sup> u/500 mg 0.8 ml qsp 500 ml

Adjust pH 7.2-7.4 with sodium bicarbonate (NaHCO<sub>3</sub>)

Dispensed 1.2 ml of the medium into the vial 1.8 ml (100-200 vials). Keep in the freezer and one part keep at +4°C for used.

## Annex 12. Description of the outbreak Thomas Rawdon, FAO consultant, June 2004

## PART B: Epidemiological assessment of HPAI H5N1 outbreak data

### 1. BACKGROUND

The Kingdom of Cambodia reported the first case of Avian Influenza type H5N1 within its borders on the 24 January 2004. The first case in poultry occurred in a semi-commercial layer flock of 3000 birds in Phnom Penh. The Department of Animal Health and Production (DAHP) was notified on the 12 January with investigation by the National Health & Production Investigation Centre (NAHPIC) taking place on that and the following day. This case resulted in the first report to the OIE on the 24 January. Later it was determined that first clinical signs on the property began in a group of 7-week old layer replacements on the 20 December 2003. Subsequent assessment of findings has identified the first mortality event associated with H5N1 within Cambodia, as that occurring on the 15 December at the Tamao Wildlife Rescue Centre (45km south of Phnom Penh). This outbreak began with the death of a crested serpent eagle that underwent post-mortem at a private veterinarian in Phnom Penh where initial attempts at diagnosis centred on assessing the possible involvement of West Nile Virus.

Over the last four months there have been a total of 92 reports of avian mortality to the DAHP: 45 in January, 34 in February, 11 in March and 2 in April. 58 of these reports were stood down, while 34 underwent investigation and sample collection. Presently 12 confirmed Avian Influenza H5N1 positive premises have been identified (tables 1, 2). Eight of the confirmed positive sites are in the south of the country (Takao, Kandal and Phnom Penh provinces), 3 are in the north-West (Siem Riep) and 1 in the east (Kampong Cham).

No human cases have been confirmed, although three suspect cases have been investigated by the Ministry of Health (MoH) in collaboration with World Health Organisation (WHO).

#### (...)

#### 3.2 Temporal distribution

The temporality of mortality reports to the DAHP by provincial veterinary authorities is summarised by sector (table 7 and figure 1), region (figure 2 and 3) and outcome (figure 4).

	United and a second			
Sector	Investigation type	Number	Begin	End
Layer	All mortality reports	5	04-01-04	09-02-04
(semi-commercial)	Laboratory Investigation	4	12-01-04	06-02-04
	Confirmed H5N1 +ve	2	12-01-04	06-02-04
Broiler	All mortality reports	6	25-01-04	13-02-04
(semi-commercial)	Laboratory Investigation	3	11-02-04	13-02-04
	Confirmed H5N1 +ve	2	11-02-04	13-02-04
Duck	All mortality reports	9	10-01-04	26-02-04
(semi-commercial)	Laboratory Investigation	4	10-01-04	26-02-04
	Confirmed H5N1 +ve	1	21-02-04	-
Village Chicken	All mortality reports	69	01-01-04	07-04-03
-	Laboratory Investigation	20	02-01-04	27-03-04
	Confirmed H5N1 +ve	5	16-01-04	27-03-04
Zoo	All mortality reports	2	14-01-04	11-02-04
	Laboratory Investigation	2	14-01-04	11-02-04
	Confirmed H5N1 +ve	2	14-01-04	11-02-04
All sectors	All mortality reports	91	01-01-04	07-04-04
	Laboratory Investigation	33	02-01-04	27-03-04
	Confirmed H5N1 +ve	12	12-01-04	27-03-04

Table 7 Date range of	f mortality reports	field/lab	investigations and	nositive cases
Table / Date Tallge U	i mortanty reports,	neiu/iau	investigations and	positive cases

Last reports of H5N1 confirmed mortality in the commercial sector occurred on the 6<sup>th</sup> (layers), 13<sup>th</sup> (broilers) and 21<sup>st</sup> (Ducks) February. These times are likely to reflect overwhelming voluntary closures of such enterprises as a result of

economic pressures. Reports, investigations and confirmations have continued in the village (non-commercial) sector indicating continued circulation (or re-introduction) of virus (see 3.4 Elucidating Source).

Although numbers are small, figure 2 follows a fairly typical epidemic curve after an initial mass of reports which are likely to indicate a catch-up period as notification to report was promoted and public concern arose. The epidemic curve and catch-up period is also evident in figure 3, although the numbers are even smaller. This pattern may however represent a reporting bias and have some of its origins through knowledge dissemination and farmer/public concern. If the pattern is however seen in the context of the likely period of high virus circulation determined through tracing, it would appear not to be artefactual (see 3.4 Elucidating Source). Voluntary depopulation in the commercial sector, with the majority of birds going for human consumption, is likely to have suppressed the peak of the epidemic.



#### Figure 2 Mortality events reported to DAHP (n=92) by region and week of outbreak



South 🗏 East 🖾 North-West





Week of outbreak

#### 3.3 Spatial distribution

The following maps summarise the spatial distribution of investigated sites (confirmed positive and negative) and the distribution of all mortalities reported between 1<sup>st</sup> January and 29<sup>th</sup> April 2004. The pattern of distribution of mortalities in figures 2 and 3 can be appreciated visually in figure 5 and 6 with reported mortality events and H5N1 positive outcomes predominating in the south. There is no statistical significance by province or region (south, east, north-west) regarding a positive outcome. The large number of H5N1 positive sites in the south are countered by the large number of cases stood down. Cluster analysis is likely to have confirmed the visual pattern, but unfortunately GIS data was not made available. According to the 2002 statistics, the southern provinces accommodated around 5 of the 12 million chickens recorded in Cambodia. Proximity of Phnom Penh, with over 2 million inhabitants, to the Vietnamese border results in intense trade in this region. Both these factors would be important contributors to the pattern of mortality reports and confirmed cases (although a reporting bias also as a result of border proximity may have had some effect).



Map 2 Cambodia provinces



Map 3 Communes reporting mortality to DAHP, Jan-April, 2004

Map 4 Sites Investigated and confirmed positive (red) and negative (blue), Jan-April, 2004



## Annex 13. Case report : sero-positive ducks in a surveillance zone

This farm is located within a radius of 10 km from two confirmed H5N1 outbreak places (laboratory results on 05/02/2004 on local chickens and 03/03/2004 on broilers)

It was investigated during the retrospective investigation of the outbreak places.

Date of the visit : 17/06/2004

## Farm background:

Duck farm (egg production) in a flooding area close to the Tonle Sap (with high concentration of wild birds) Free range animals kept in rice fields during the days and in a pen during the night.

Vaccination against pest at 3 months old.

Start to raise a new flock of 1300 animals in 11/2003.

300 males sold in April 2004

Left : 500 animals at the visit time.

#### **Baseline mortality**

No mortality reported in the previous years (activity started 10 years ago)

#### Outbreak event in 2004

500 animals died at the end of February

Symptoms reported by farmers: green diarrhoea / coughing / white eyes (also observed on the live animals, and probably related with vitamins deficiency).

Duration of the symptoms on a single animal : 3-4 days

Duration of the outbreak in the flock : 4-5 days

Treatment used : antibiotic (sulfamine), no more detail.

#### Laboratory results :

13 animals sampled, all were 8 months old.

First, 3 animals sampled on site : 3 were sero-positive

Then, 10 animals were bought for post-mortem and additional sampling.

HI test and IFAT were performed at the National Animal Health and Production Centre, whereas the RT-PCR was done in Pasteur Institute.

Animal	HI test	IFAT	RT-PCR on cloacal	RT-PCR on
	(8 HAU used, H5N1 A/	(on direct pancreas and brain smears	swabs (M segment,	pancreas (M
	Chick/Scotland / 59)	+ on tracheal and cloacal swab	H5 and N1)	segment, H5 and N1)
1.	1/16	Neg (only on cloacal swab)	Neg	Neg
2	1/64	Neg (only on cloacal swab)	Neg	Neg
3	1/32	Neg(only on cloacal swab)	Neg	Neg
4	Neg	Neg	Neg	Neg
5	1/128	Neg	Neg	Neg
6	1/32	Neg	Neg	Neg
7	1/8	Neg	Neg	Neg
8	Neg	Neg	Neg	Neg
9	Neg	Neg	Neg	Neg
10	1/32	Neg	Neg	Neg
11	Neg	Neg	Neg	Neg
12	1/64	Neg	Neg	Neg
13	1/64	Neg	Neg	Neg
Total Pos	9/13 = 69.2 %	0	0	0

## **Comments**:

- Is our virology method sensitive enough for virus detection ? (we will try to send samples to OIE reference laboratory in Geelong for virus isolation)
- Did we collect enough animals to detect possible shedders?
- It seems that seroconversion was quite high in the flock. So our expected prevalence of 20% for detecting at least one positive case (we should have taken 15 animals) was sufficient.

# HPAI reporting system Information to be collected by TF2 and to transmit to TF1

## 1. Information received by phone:

### ▶ Information to collect and to report on the recording book

#### Date received call and time

Name of the person who called + position

Name of the person who received the call

## Report :

- localisation (exact), ask how many places are involved
- species affected : B / LC / LH / G / village D / LD / D for meat
- starting date of the event reported (morbidity or mortality), if no precise information available, try to assess : few days, 1 week, 1 month...
- number of animals affected
- try to assess if this is an acute disease or not (there is sudden death or not)
- 1 or 2 telephone number where Epidemiology team can contact them.
- ▶ if report from provincial officers, ask them :
- to start to complete the outbreak form (Epidemiology form)
- remind them which samples they can collect (minimum 5-10 sick and non sick animals; 2 cloacal and 2 tracheal swabs + blood samples if disease started for more than 1 week) or send **dead** animals
- ► Transmit immediately, by writing, the complete report to TF1 (+Director)
  - ▶ TF1 has to enter this report in its own recording book

#### 2. If report received by fax :

- ▶ enter the information in the recording book
- ► transmit immediately a copy of the fax to TF1 (+ Director)
- ► TF1 must enter the information in its recording book



**Transmission** (the transmission must not be only oral, but always written on paper, even written by hands) Information room to NAHPIS = IMMEDIATE NAHPIS to the information room = 2 times a day NAHPIC diagnosis team to NAHPIS = IMMEDIATE NAHPIS to NAHPIC Bird flu database = each time Follow-up table is updated

Task Force	Responsibilities	Person
TF2 - DAHP-	Collect the suspicions, the rumours, the reports of provinces	To be completed by TE?
Information room	I ransmit immediately the suspected case information to the task force I	10 be completed by 1F2
	Transmit the investigation reports to Ministry of Health	
1	Collect all the information about the control measures taken	
	Compile all the information and write report	
	Send reports to MAFF, International organization and Media	
TF1 - NAHPIS	Train the provincial investigation team	Sorn San ; Keo Samnang, Holl Davun ; Nget Kiri ; Chhim Vutha ;
	Support the provincial investigation team to carry out the investigation	Nou Kimsay, Bun Chan, Sem Tharin, Chhun Sopheap
1	Compile all the epidemiological data's	
TFI – NAHPIC	Analysis the samples	Pathology section : Chhin Manov ; Lim Sopheak ; Lim Sophea
Diagnosis team	Assure a good traceability of the samples	Serology section : Ken Theary; Neak Someary; Sammin Chamvisal;
	conv of the submission form and the case history form	Ngili Noulipisey
	Transmit the positive samples to Pasteur Institute for sub-typing	Bacteriology : Sok Koam, So Pheany
TF3 - Outbreak	Support the provincial team to carry out the control measures	Sen Sovann, Nap Sokhim, Lim Pak, Mao Davuth, Sok Daro, Khi
control team	Transmit the information of control measures to TF2.	Yukeng, Ngem Samoeun, Chhum Chan Dara, Hun Sarat, Phal
		Vannary, Sim Sotheavuth, Yet Cheata, Ni Muoy Ry, Khat Sokhon
Ministry of Health	Transmit every suspicion to DAHP-information room	CDC department : Sok Touch ; Ly Sovann
	Inform	

Abbreviations AS : Active surveillance AMM : animal movement management SO : Stamping Out D : Disinfections OD : Outbreak declaration

# **AVIAN INFLUENZA** SURVEILLANCE PROGRAM **IN CAMBODIA**

## June 2004 (updated in August 2004) Version 1.2

National Animal Health and Production Investigation Centre **Kingdom of Cambodia** 

By:

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## Study and Surveillance of Avian Influenza in Cambodia

This document presents the active surveillance program to be implemented in Cambodia for the next 6 months and possibly, for some part, regularly.

Even if not exposed in detail in this document, the passive surveillance will continue to play a very important role in the detection of HPAI cases in the country. The passive surveillance system was already strengthened by public awareness activities, training of the provincial veterinarians and strengthening of the capacity of the epidemiology unit at the central level. The efforts to get a good reporting system will remain.

## 1. Presentation of the three steps approach

#### 1.1 Retrospective study

- Study in the outbreak areas (3 km radius from the outbreak places) to obtain a clear picture of the spread of the disease and to assess the absence of any new suspected case in the outbreak areas in order to release the outbreak declarations of the 12 outbreak places.

Note : according to OIE Terrestrial Animal Health Code, 2003, a zone shall be considered as infected with HPAI until :

- at least 21 days have elapsed after confirmation of the past case and the completion of a <u>stamping-out</u> policy and disinfection procedures or
- 6 months have elapsed after the clinical recovery or death of the last affected animal if a <u>stamping-out policy was not practiced</u>

In the case of Cambodia, since stamping-out policy was not fully implemented, the outbreak zones should still be considered as infected. This means that even after completion of this active surveillance plan, we are not able to declare this zone free. That is why it is very important to follow carefully the repopulation of those places by an adapted active surveillance plan (point 1.2).

### 1.2 Active monitoring

- Active surveillance in the outbreak areas (3km radius from the outbreak places) after repopulation of the farms to declare their freedom after 6 months.

Note: it is suggested that the outbreak area, in the case of Phnom Penh, is extended to the whole municipality in order to include every commercial and semi- commercial farms of Phnom Penh in this active surveillance program. Indeed, in this area where density of population and density of farms is higher than anywhere in Cambodia, all the precautions must be taken in order to detect immediately any new HPAI case. In Thailand, the outbreak area, called control zone, is 50 km radius from the outbreak place.

#### - Market monitoring

Note: market monitoring is an indirect tool to assess the sanitary status of the village poultry, as well as to detect possible illegal movement of sick animal from the neighbouring countries.

The at-risk places are chosen according to the number of live birds collected everyday and according to their proximity with the borders.

- Surveillance in parent stocks farm

#### 1.3 Freedom status for commercial and semi-commercial farms

- Active serological surveillance of a selection of farms, in order to declare the freedom of infection in the related compartments after 6 months

Note: based on a compartmentalisation approach, it is proposed to assess the freedom status of the commercial and semicommercial farms in the country by doing targeted serological surveillance at a minimum interval of 6 months designed to provide at least 95% level confidence of detecting a prevalence of Notifiable Avian Influenza (HPAI and LPAI of subtype H5 and H7) infected farms of 5% (using EC guideline). Then, each farm should be sampled to ensure the identification of at least one positive bird if the within-farm prevalence is 25% (using OIE Code, draft chapter on Avian Influenza)(T.Rawdon's report) Important Notice 1: the surveillance program in the commercial and semi-commercial farms will allow detecting Highly Pathogenic Avian Influenza virus as well as Low Pathogenic Avian Influenza virus. The interest of identification of LPAI strains is purely scientific since, for the moment, there is no control measures planned for those virus. When positive results will be released, caution will have to be taken to identify the pathogenicity of the strain involved, since the consequences are not the same in term of veterinary measures.

Important notice 2 : After a first phases during which all the efforts were dedicated, on purpose, to the poultry population, the veterinary services could consider the possibility of an active surveillance program on the pig population. This surveillance program could be organized at the slaughterhouse level. The detail of such a program could be discussed within the regional epidemiological network under the regional TCP/3006.

## 2. Assumptions

#### Compartmentalisation

Demonstration of freedom is proposed on a compartmental basis (report of T.Rawdon and S.San, May 2004).

Compartmentalisation of the population at risk follows the definition of the FAO/OIE meeting (5) and FAO expert meeting network (3). To better fit with the Cambodian situation, the animal population of the compartments has been changed as follow<sup>7</sup>:

1.Commercial chicken farm : over 10 000 birds (sector 1)

2.Semi-commercial chicken farms : from 500 to 10 000 birds (sector 2 or 3)

3.Semi-commercial duck farms : from 500 to 10 000 birds (sector 3)

4. Village (backyard) chicken (sector 4)

5. Village (backyard) ducks (sector 4)

Detailed definition of the sector follows the FAO definition with little changes regarding the marketing and description that does not fit totally with the Cambodian situation. Indeed, the definitions given at the meeting differentiate the farms according to their biosafety level and also according to their marketing system (whether or not there are animals movements from the farm to the market). In Cambodia, the big majority of the poultry and poultry products end at the market places (a part is also going directly to the restaurants and a small part to the supermarkets). And most of the farmers sell their animals to middlemen, only few are selling directly to the market. This means that the differentiation between farms that "market commercially" and farms that "sell to live birds markets" is quite artificial here.

Definitions of the sectors adapted to the Cambodian situation

Sector 2 : semi-commercial production system with moderate to low biosecurity level

Sector 3 : semi-commercial production system with low to minimal biosecurity level

Sector 4 : village or backyard poultry with no biosecurity.

#### Village poultry population

To assess the freedom in the village poultry populations would be very difficult to achieve with the current human and technical resources and also because of the organization of this production. (see Thomas Rawdon's report)

Nevertheless, it is crucial to get information on this sector and not only to rely on the passive surveillance system. To do so, it is proposed to start with active surveillance at the market places.

#### **Survey parameters**

The parameters to be applied for appropriate sampling, must be adapted to the epidemiological context and, possibly, to the species concerned.

- Between-farm prevalence : 5 %, following the European Commission Guideline<sup>8</sup>

<sup>&</sup>lt;sup>7</sup> compartments as defined at the joint FAO/OIE meeting, Bangkok : commercial ( 5000 animals) ; semi-commercial (500 -5000 animals) ; village population.

#### Within farm or market prevalence :

• For surveillance purpose in the farms: 20 % expected prevalence for both seroconversion and virus detection. This means that each epidemiological unit should be sampled to provide a 95 % level of confidence of detecting at least one positive animal if the prevalence of AI is 20%.

The seroconversion on affected waterfowl seems to be higher than for the chicken since the case fatality is not so high for this species. So a high expected prevalence can be afforded. This parameter is also advised by the FAO expert group (3)

For the chicken, this limit is probably only valid for LPAI virus, since the current H5N1 strains seem to have a high case fatality for this species. A better understanding of the virulence of the H5N1 current strain is needed to be able to define the parameter of a serosurveillance program for this species or even to decide if the serosurveillance is relevant on chicken in the current context. In any cases, the detection of LPAI is also of interest, and is one of the objectives of our surveillance program in the farms, so the expected prevalence of 20% will be kept for this species too.

• For surveillance purpose in market: expected prevalence between 10 and 15 % for virus detection is proposed.

For market monitoring, the two main objectives are to detect animals in incubation or at the early stage of the disease and to detect animals still excreting virus after infection. It is very difficult to study the bias (especially bias of selection) for this kind of sampling. It is difficult to know how many different batches from different origins are present in one market, and they are probably very numerous. In this condition, it would be necessary to increase the number of animals sampled to limit the effects of those bias. Nevertheless, one has to take into consideration the limited capacity of the laboratory (especially in the impossibility for the moment to pool the samples) and try to find a balance. It is proposed to use an expected prevalence between 10 and 15 %. 2 % prevalence was proposed by the FAO expert group for market sampling, but this prevalence increases considerably the number of animals to be sampled, which is not achievable at the present time.

• For investigation of suspected case with current mortality: expected prevalence of 25 %.

Those parameters have been chosen according to the existing guidelines related to surveillance of HPAI:

- proposed new chapter 2.1.14 for the OIE terrestrial animal health code and,
- European Commission Guideline<sup>5</sup>
- Expert guideline distributed in the frame of the regional FAO project on harmonization of the surveillance and diagnosis for HPAI).

Adaptation has been made to fit with the capacity of both the field teams and the laboratory. For instance, the expected prevalence of 2% for market monitoring, is not achievable at the present time.

- screening test sensitivity (HI and IFAT) : 90 % (need to be better assessed)
- screening test specificity : 100 % (see T.Rawdon's report)

## 3. Diagnosis method and strategy

Three diagnosis methods are currently available in Cambodia for Avian Influenza detection:

- Immunofluorescence test for antigen detection (influenza virus A diagnosis),
- Hemagglutination inhibition test for antibodies detection (against avian influenza sub-type H5, H7 or H9),
- RT-PCR test for RNA detection (H5N1 or H7 virus diagnosis).

The **pathogenicity assessment or the gene sequencing for following the gene derivation** of the strains cannot be performed in Cambodia. If new positive virology results are found during the surveillance, some of the positive samples will have to be sent to OIE reference laboratory. Another option would be to send the samples to a regional reference laboratory in the frame of the regional laboratory network.

• For surveillance purpose in the farm: serological test is used except if there is history of vaccination. It is proposed to also collect swab for double check in case of positive results. If animals in a farm are found sero-positive without virus detection, a complete investigation has also to be done as well as another sampling 2 weeks later.

• For surveillance purpose in market, virology is proposed in order to detect animal in incubation period, at the early stage of the disease or still excreting some virus after infection. Serological test on animal without history would be of minor interest.

• For investigation of suspected case with current mortality: virology test is used systematically, alone or in addition to serological test (see Animal diagnosis strategy for HPAI attached to this plan). The virology test used at the moment is the immunofluorescence test.

## 4. Detail methodology and schedule for those programs

## 4.1. Retrospective study

#### **Objectives:**

- assess the absence of any new HPAI outbreak suspicion for at least 3 weeks in the outbreak zone.
- complete the retrospective epidemiological study of the past outbreak.

#### **Procedure:**

- Visit to the district and the provincial veterinarians to perform the complete epidemiological investigation related to the past outbreak in order to clearly determine the date of the first symptoms and possibly to identify at-risk places outside of the outbreak zone. Assess the current situation of the poultry mortality in the district and in the province.
- Visit the outbreak place: a village or a commercial farm and complete the epidemiological investigation by discussing with the owner of the farm and the chief of the village and/or the Village Animal Health Workers and/or and some families.
- In case the outbreak place is a village, collect samples to assess if the virus is still circulating (virology) or if it has largely circulated (serology). Number of samples to collect : 12 to 15 per species (see table 2 in sample size calculation attached to this plan, using 20 to 25% within-farm prevalence)
- Visit a random selection of villages in the protection zone<sup>9</sup> (3 km around the outbreak zone) to perform :
  - a brief investigation on morbidity and mortality in 2004 and 2003 + vaccination status + animal movement in 2004
  - o clinical assessment (poultry and ducks)
  - o collect samples in the village in case :
    - of suspected mortality reported for the past 3 weeks,
    - the information is not complete and
    - possibly, each time high suspected mortality is reported in 2004.

Number of samples to be collected in case of suspicion: 12 per species (using a 25 % within-farm prevalence)

For surveillance purpose, the animals sampled must have lived in the village at the time the mortality was reported.

- Visit every commercial farm within the protection zone and sample animals<sup>10</sup> to assess if the virus has circulated or is still circulating. Number of samples to take: 12 to 15 samples.
- Visit the at-risk places if any were identified and collect samples<sup>7</sup> to assess the possible circulation of the virus. Number of samples: 12 to 15 per species.

Schedule: this assessment has been finalized on the 18/06.

Number of investigations: 12 in the 12 outbreak places.

<sup>&</sup>lt;sup>9</sup>95 % confidence of absence of disease if no diseased elements identified. Between-farm prevalence : 5%

<sup>&</sup>lt;sup>10</sup> 95 % confidence of absence of disease if no diseased elements identified. Within farm or village prevalence : 25 %.

#### 4.2 Active surveillance

4.2.1 Surveillance and freedom declaration in the former infected farms and in the farms located 3-4 km around an infected place

Objective: assess the freedom of infection for the commercial and semi-commercial farms that were contaminated or that were in a contaminated area (3-4 km around the outbreak places).

It is suggested that every commercial and semi-commercial farms in Phnom Penh are considered within a former outbreak zone.

It is also suggested that the farms involved in this surveillance program had their bio-security level improved beforehand.

## **Procedure:**

- in case of use of vaccination, identifiable sentinel animals have to be kept.
- clinical observation of the animals every week during 30 days (see Follow-up form attached to this plan)
- every dead animal must be sent to the laboratory for post-mortem examination and virus detection (it is proposed to buy the dead birds to encourage the farmer to send them, instead of selling them to the market).
- at day 30 : samples<sup>11</sup> for serology and virology, or virology alone if vaccination history reported,
- clinical observation every two weeks during 5 months,
- at 6 months : samples<sup>12</sup> for serology and virology, or virology alone if vaccination history reported

Schedule: this restocking surveillance was planned to start in June. It finally started progressively from the end of July.

#### Number of farms to be followed:

- former infected farms : 6 (1 in Phnom Penh, 2 in Takeo, 2 in Kandal, 1 in Siem Reap)
- semi-commercial and commercial farm in Phnom Penh: so far, 17 farms have been identified by the Phnom Penh municipality veterinary services (there are more farms but some are under contract with CP group, and it seems to be difficult for the veterinary officer to be able to enter into those farms and some are still empty).
- farm in protection zones of former outbreak zones : 15 (10 in Siem Reap, 3 in Kandal, 2 in Takeo)

#### 4.2.2 Market monitoring

#### **Objectives:**

- early detection of new outbreaks in the provinces,
- detect animal still excreting virus after infection,
- monitor the circulation of H5 and H7 virus -

#### **Methodology:**

#### Selection of the markets:

Two or three markets in Phnom Penh must be monitored (selected among the four main live birds markets of the city).

Market in provinces must be selected according to their localization (close the Thai and Vietnamese borders) and to their poultry population.

The number of markets must also be limited to avoid overloading the laboratory. So far, 7 provinces have been selected: Kandal, Takeo, Kompong Speu, Svay Rieng, Kompong Cham, Battambang and Siem Reap.

#### Number of samples collected

The expected prevalence has been fixed between 10 and 15 % (95 % confidence of absence of disease if no diseased elements identified). The number of animals sold in market is estimated between 1000 to 3000 for Phnom Penh main live birds markets and few hundred for smaller provincial markets. Between 20 and 32 animals should be samples.

It is proposed to sample 25 animals and to collect 10 fecal swabs from the environment.

<sup>&</sup>lt;sup>11</sup> 95 % confidence of absence of disease if no diseased elements identified. Within farm prevalence : 20 %.

<sup>&</sup>lt;sup>12</sup> 95 % confidence of absence of disease if no diseased elements identified. Within farm or village prevalence : 20 %.

#### Frequency:

For provinces, it is proposed to start with one sampling every two weeks in one market place.

For Phnom Penh: one sampling every week in 2 market places.

The proposed sampling frequency must be adapted to the epidemiological context related to the H5N1 circulation in the region. In period where risk of re-introduction or re-emergence is considered high (good climate condition, outbreaks in the neighbouring countries or in the national territory), the frequency can be increased.

#### Schedule:

The market monitoring has started in Phnom Penh in early August. It should have started in Province since mid-July. Some delay has occurred.

#### 4.2.3 Surveillance in the parent stock farms

Objective: Assess the freedom of disease of the only chicks and poulet provider of the country (CP Company)

#### Methodology:

On vaccinated animals without sentinels (current situation):

- post-mortem and virology test on every dead birds,

On vaccinated flocks with sentinels (possibly for the new flocks):

- keep at least 10 sentinel animals per flocks (preferably 30),
- sample those animals every 30-45 days for serological test,
- post-mortem and virus detection on every death in sentinel animals.

On non vaccinated animals:

- report of mortality to the veterinary services if exceed 1% every two days.
- Post-mortem and virus detection on every dead birds.

Schedule: has already been discussed with CP, but not yet started.

#### 4.3 Freedom declaration in the semi-commercial and the commercial farms in the whole country

Objective: declare the freedom of infection in semi-commercial and commercial compartments of the at-risk population.

#### **Procedure:**

Stratified (by province and by compartment), 2-stage design with simple random sampling within units of interest : selection of farms within each compartment and selection of animals within each farms according to the OIE guideline and the European Commission guidelines (Confidence of detection of 95 %; 5 % between-farm prevalence; 20 % within-farm prevalence)

The animals will be tested by serology (if no history of vaccination reported).

This first assessment must be follow-up 6 month later of a further selection of randomly identified farms.

#### Schedule:

First assessment planned to start in July but delayed because the other activities (monitoring in markets and farms in and around the outbreak places) were of first priority.

This program could also be discussed within the epidemiology network and be adapted according to the improvement of the knowledge on this particular strain.

#### Number of farm to be sampled:

Sampling among every commercial and semi-commercial farm in Cambodia could be easily done after completion of poultry farm census.

#### 5. Evaluation and analysis

The central epidemiology team is in charge of collecting the data of those surveillance activities.

A follow-up mission of an epidemiology consultant is planned before the end of the project to assist the team to evaluate the program and to analyse the data collected.

#### 6. References

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3. Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand (2004). Emergency Response for Avian Influenza Outbreak.

4.European Commission (2003). Revised guidelines for surveys in poultry and wild birds to be carried out in 2004, SANCO/10046/2004-Rev.1.

5.Expert meeting recommendations. July 2004. FAO/TCP/3006, Harmonisation of the surveillance and diagnosis methods.

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7. N.Y. Kung et al. The impact of a monthly rest day on avian influenza virus isolation rated on retail live poultry markets in Hong Kong. Avian Diseases 47:1037-1041, 2003.

8.OIE terrestrial animal health code. Proposed new chapter for Avian Influenza.

9.T.Rawdon, S.San (2004). Surveillance Technical Plan : outbreak and post outbreak surveillance for Modifiable Avian Influenza viruses in poultry populations in Kingdom of Cambodia.

## Sample size calculation <sup>13</sup>

#### Extract from T.Rawdon report

The number of units that require sampling in strata 1 (farms/villages) depends on the level of between-farm prevalence of Avian Influenza. Sample sizes below provide 95% confidence of absence of disease if no diseased elements are identified. Herd-level sensitivity is calculated as 1-Type I error = 95%. Herd-level specificity of 100% assumed. Target type I and II errors are 5%.

	3	0	0 /	0	_		
			No of farm	ns in strata 1	1		
Between-farm	10	20	30	40	50	60	
prevalence							
1%	$ALL^2$	ALL	ALL	ALL	50	60	
5%	ALL	ALL	25	33	41	40	
10%	10	16	20	22	23	24	
15%	8	13	16	16	16	17	
20%	8	11	13	13	13	13	

## Table 1 Guide to number of units (farms/villages) in stage 1

1 - From DAHP record or a synthesis of DAHP and private vet data

2 - 'ALL" implies that required confidence (95%) cannot be achieved by sampling all units in strata 1

The number of units that require sampling in stage 2 (birds) or in market depends on the level of within-farm prevalence of Avian Influenza. Sample sizes below provide 95% confidence of absence of disease if no diseased elements identified. An assumption of 90% sensitivity of the testing protocol is assumed. Test specificity of 100% assumed. Target type I and II errors are 5%.

## Table 2 Guide to number of birds in stage 2 or in markets

2		No of birds in	unit <sup>1</sup>
Within-farm	500	1000	>2000
prevalence			
5%	62	64	65
10%	31	32	32
15%	21	21	21
20%	15	15	16
25%	12	12	12
30%	10	10	10

1 - Obtained from farmer when planning surveillance visit or on day of sampling

<sup>&</sup>lt;sup>13</sup> Determined using 'FreeCalc' program

#### ANIMAL DIAGNOSIS STRATEGY FOR HPAI SUSPECTED CASE

**Time line** 



#### TIME LINE for laboratory results :

2 WD in NAHPIC ⇔ 4 days maximum for 80 % of the samples for complete NAHPIC results 3 WD in Pasteur ⇔ 5 days maximum for 80 % of the samples for complete results \* It is possible to pool the positive samples by farms and by species

## Follow-up table for active surveillance in the commercial farms

Province	
District	
Commune	
Village	
Name owner/phone	
Production	Laying hens / Broiler / Ducks for egg / Ducks for meat / Other :
Farm ID (to be filled by NAHPIC)	

Date	Name if the veterinarian	Nb of animals	Action *	<b>Observation</b> (vaccination, treatment, animal movements)	Signature of the owner
			×		
		-			

\*CO = Clinical observation, S = sampling + write the number of samples , PM : collection of dead animals for post-mortem + write the number of corpses.

\*\* If more than 1% daily mortality is observed or reported, samples must be collected or dead birds sent to laboratory (around 12 animals, 2 tracheal and 2 cloacal swabs / animals)

## MONITORING OF THE LIVE BIRDS IN THE MARKETS PLACES **ACTION PLAN**

## List of markets to be monitored :

Phnom Penh : Tchba Ampeul and O Russey

In the province, the market must be chosen by the provincial team. It should be the market with the most important number of live birds sold.

The following provinces will do market monitoring :

- Takeo \_
- Kandal
- Battambang
- Kompong Cham
- Svay Rieng -
- Kompong Speu
- Siem Reap

If there are two main markets in the city or in the province, one time one market is sampled, another time the second one is sampled.

## **Methodology**:

## Number of samples

25 animals sampled per visit (at least 20). One sampling every two weeks.

The animals must be selected from different sellers (if there are 10 sellers : 2 or 3 animals per seller). If there are sick or dead animals, take samples in priority from those animals.

2 tracheal swabs per animal (tracheal swab is better, if really not possible, take cloacal swab). (when virus isolation will be implemented in NAHPIC, only cloacal swab could be sufficient)

+ 5-10 fecal swabs taken on the floor (at the place where the animals are slaughtered for example)

## Identification of the samples

The swab must be labelled as follow :

- the date \_
- the species : LC = Local chicken / B = Broiler / LH = Laying Hens / D = Duck / G = Goose / FF : fecal swab on the floor.
- one number reported on the form (swab ID): 1 for the first animal, 2 for the second...

## Form to complete

The sample submission form must be filled

For the localisation, if the seller knows where the animals are from, it is possible to fill this question, otherwise it is not possible.

## **Transport of the samples**

The sampled must be sent the same day to NAHPIC in an icebox by taxi. The taxi will be paid by NAHPIC and the icebox will be sent back with new transport media.

## Reception and processing of the samples in NAHPIC

Each sample must get a code number (see Manov's book) + administration must make 2 copies of the submission form (one for Epidemiology, one for serology)

All the results of analysis must be sent to Epidemiology.

## SURVEILLANCE IN THE COMMERCIAL AND SEMI-COMMERCIAL FARMS ACTION PLAN

## **Objectives** :

- Control the absence of virus circulation in the former infected areas (both highly and low pathogenic)
- Proof the freedom in the commercial and semi-commercial farms in Cambodia in a delay of x months after repopulation.

## Action plan

Point 1 does not have to be fully completed before starting with the following points.

## 1. Referencing of the commercial farms

Identify the commercial farms in the country and perform their geo-referencing.

Start with the commercial farm in and around Phnom Penh, and then continue by the infected provinces and the non-infected provinces.

The identification of the pig farm has to be done in a second time for a medium-term use.

- Criteria for the farm to be referenced :
  - o for the laying hens and broilers farms : over 500 animals
  - o for the ducks farm : when the animals are kept in premises
  - o for the pig farms : more than 15 sows or more than 50 fattening pigs
- Perform the geo-referencing using GSP equipment

## 2. Active surveillance in the parent stock farms

Discuss the terms of an active surveillance program with CP Company considering they have used vaccination on their animals.

#### 3. General methodology for the provincial teams :

## • For every farm, the dead birds must be collected and sent to NAHPIC.

At the first visit, it must be explained to the farmer, that he should submit his dead animals for analysis. The animals would be bought according to the following rate :

- broilers and laying hens: 2500 riels / kg
- laying ducks : 3000 riels / head
- duck : 4000 riels / head

When dead birds are sent to NAHPIC, the case history form must be completed. 10 animals maximum can be bought by visit.

- for each visit the follow-up form must be completed (see follow up table for active surveillance in the commercial farms attached to this document)
- If the surveillance program starts with the repopulation of the farm :
  - clinical assessment every week during the first month
  - sampling at 30 days (and after 1 month for each new broiler batch)
  - clinical assessment every 2 weeks during 5 months
  - sampling after 6 months

#### *First example* : a laying hens farm has been repopulated on the 09/07/04.

First visit must happen before the 16/07 (one week after repopulation). During this visit, only clinical surveillance will be done. This means that the number of animals in the farm will be estimated (the percentage of used cages will be estimated).

One week, two weeks and three weeks after the first visit, clinical observation must be done. At the fourth visit (means one month after repopulation) samples have to be collected. Then clinical visits must be organised every two weeks during 5 months.

## Second Example : a broiler farm has been repopulated on the 09/07/04

Same procedure as for the laying hens farm for the first month. But after 40 days about, the broilers will be sold to the market. So new animals will be introduced in the farm. If the animals are sold on the 23/08/04 and new chicks are introduced on the 06/09/04, then clinical surveillance must start again, at the frequency of one visit every two weeks, but animals must be sampled after one month (means around the 04/10/04). This has to be repeated for any new batch during 6 months.

- o if the animals have been introduced before may 2004 :
  - clinical assessment every week during 30 days
  - sampling at the first visit
  - clinical assessment every 2 weeks during 5 months
  - sampling at 6 months
- > animals in the farms must be randomly sampled (blood and cloacal swab) :
  - if less than 2000 animals in one flock : 15 animals sampled per flock
  - if more than 2000 animals in one flock : 16 animals sampled per flock
- Samples to take : sera + 2 cloacal swabs in transport media. When samples are collected, the attached form attached to this document (Samples for HPAI surveillance) must be completed.
- Submission of the report to NAHPIC : every week the follow-up table must be faxed to NAHPIC.
- Submission of the samples : the samples must be sent in ice-box by taxi. The ice-box will be sent back to the province with new transport media.

## Logistic

Each province must receive the necessary equipment to implement this active surveillance programme. The quantity of equipment must be estimated according to the number of commercial farms in the province. The equipment must be provided for a 6 months period.

1. List of equipment needed.

- PPE for two teams of 2 persons
- Swab : around 70 per flock for the 6 months (for broilers, it depends on the number of batches)
- Syringes : need around 32 per flock (add 20 extra = 52)
- Transport media (must be refurnished by sending by taxi)

## 2. Use of PPE :

- if no mortality is reported in the farm, normal protection must be used : boots, and overcoat.

- if suspected mortality is reported, complete PPE must be used (+mask, goggle and gloves)

The boots must be properly disinfected after each visit by immersion in TH4 solution (1 for 10 liter of water)

## List of the farms to be visited

## Takeo :

*Tramkak district* : 1 duck farm (1 team of 2 people : 14 perdiem per staff + 28 transport costs within 6 months) *Daun Keo district* : 1 laying hens farm (1 team of 2 people : 14 perdiem per staff + 28 transport costs within 6 months)

*Bati district* : one laying hens farm (1 team of 2 people : 14 perdiem per staff + 28 transport costs within 6 months)

### Kandal:

*Kien Svay district* : 3 broilers farms, 1 laying hens farm (1team of 2 people : 14 perdiem per staff + 28 transport costs within 6 months)

Takmao: 3 laying hens farms (1 team of 2 people: 14 perdiem per staff + 28 transport costs within 6 months)

## Siem Reap :

Siem Reap district : 11 farms (3 teams of 2 peoples : 14 per diem per staff per team + 42 transport costs within 6 months)

## **Phnom Penh**:

32 farms : 5 teams of two people (between 6 and 7 farms per day per team) ;14 perdiem per staff + 14 transport cost per team within 6 months

Per diem = 5 USD Transport cost = 4 USD.

# Samples for HPAI surveillance

Name of the submitter :

Date of s	submission to S	erology or to P	asteur :								
Total nu	mber of animation	als: Lo	С	B	LH		D				
Localisation	Name owner	NAHPIC code number (to be filled at NAHPIC)	Date sampling	Species : LC / B / LH / D + AGE	Serum ID	Swab ID	Analysis requeste filled at i	d (to be NAHPIC	C)	Observat Su: surveilla or Sp:Susp	tion : nce pected
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## Annex 16. CP meeting report on the 08/07/2004

In the objective to define a procedure for active surveillance for the parent stock farm in Cambodia, we met with CP group General Manager, Mr Sawai Tangtanaporn and two of his colleagues.

## **Background** :

There is one CP parent stock farm in Kandal province including 4 broilers breeder flocks and 2 laying hens breeders flocks.

CP has contracted farmers in : Battanbang, Kompong Speu and Kandal provinces.

CP provided the list of the farms where they sold vaccinated animals.

## The outputs of the meeting are;

- Every breeders have been vaccinated and there is no possibility to have sentinel animals for those 6 flocks.

 $\Rightarrow$  We wait for the agreement of CP, to receive all their dead birds for post-mortem and virology detection in order to detect if there is circulation of avian influenza virus (even if normally the virus excretion is very low on vaccinated animals)

 $\Rightarrow$  We proposed them to make an assessment of the immunity of the breeders in order to assess the efficiency of the vaccination they undergone.

- When new breeders will be introduced, and if they still use vaccination, they agreed to have sentinels animals to allow the active surveillance.

#### Monitoring protocol to be approved if vaccination is authorised on the new flocks :

To have an appropriate monitoring, at least 10 sentinel animals per flocks must be sampled every 30-45 days. This means, they should keep at least 15 non vaccinated identifiable animal per flocks (there are 6 flocks, 4 for broilers and 2 for laying hens). Serological test will be done on those animals and dead animals (from the sentinels) will have to be sent for virus detection.

- At the beginning of the vaccination this year, every broilers and laying hens sold to the contracted farmers were vaccinated. Since more than 1 month only the laying hens are vaccinated because the company run out of vaccines.

The protocol f vaccination is :

- o vaccination of the day-old broilers (now stopped)
- o vaccination of the poulettes at 12 and 16 weeks before selling at 17-18 weeks.

 $\Rightarrow$  The vaccination protocol for broilers was not correct since a vaccination on day old chicks does not provide with a good immunity.

⇒ To see the recommended vaccination protocol from FAO (Yves Froehlich 's document)

#### Condition to authorise the vaccination on laying hens poulettes sold by CP :

The DAHP could authorise the vaccination for the laying hens but with the obligation when they sell to the farmers to always have 15 non vaccinated and marked animals. CP should also provide to DAHP regularly the list of the farms where they sold the vaccinated animals (with the quantity of animals sold).

## Annex 17 VSF Database



NATIONAL ANIMAL HEALTH AND PRODUCTION INVESTIGATION CENTRE NATIONAL ANIMAL HEALTH AND PRODUCTION INFORMATION SYSTEM - NAHPIS WELCOME TO 'VFS' THE DATABASE MANAGEMENT SYSTEM FOR THE VETERINARY FIELD SERVICE REPORTS



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NATIO	NAL ANIMAL HEALTH AND F	PRODUCTION INFORMATI	ION SYSTEM
Entry into da	atabase of data in r	monthly disease c	occurrence report.
	Step 1: Enterin	g basic parameters	3
Date report received:			
Province Name:	•	District Name:	
Month:		Year:	
Disease Code:		Outbreak serial no:	
Commune Name:			
	Re-enter C	ontinue C	ancel



Annex 18 Serum bank database



# Annex 19 Database for commercial animal farming in Cambodia Developed by CIRAD

N/	AHPIC dB	Commercia Farming in	al animal Cambodia
Par	Provinces	Census	
	Districts	Farm Visit	
	Communes	Queries	
	Villages		
		CIRAD	Station K. 19

Trorm_Farm		the second second		×
Farms - Farms Production				
Code	Owner	Phone Number	GPS X (Decimal Fo	umat)
RESN				
Province			GPS Y (Decimal Fo	rmat)
002		Production		
District	Districts of the Selected Province	Production	Other Production BREED	Year* DESTINATION
00102	All the Districts	Bovins		
Commune	Communes of the Selected District			
	All the Communes			
Village	Villages of the Selected Commune			
	All the Villages			
Farm Visits	and a survey of the second		en el Regerate de Linad	Fishers of Achieve
Date Agent				
Free Range Ar	imats 🔟 Waterpond	🕅 Cages 🕅 Open Shed	Other Protection	Capacity of the farm
Wall around the farm Wire Fencing around the Farm				
Urigin of the dunking water Perimeter around the premises				Brigin of the animals
Add New Saye Delete				
<				