VETERINARY COUNCIL OF INDIA

(Statutory body of Government India established under Indian Veterinary Council Act, 1984))

TRAINING MODULE

on

Diagnosis and Control of Bird Flu

A-Wing, 2nd Floor, August Kranti Bhawan,
Bhikaji Cama Place, New Delhi- 110066

Training Module developed with inputs from:

1. Dr. S.C. Dubey, Joint Director, High Security Animal Disease Laboratory, IVRI, Bhopal.
2. Dr. V.M. Bhuktar, I/C Joint Commissioner (AH), Western Regional Disease Diagnostic Laboratory, Disease Investigation Section, Aundh, Pune.
3. Dr. C. Renukaprasad, Director, Institute of Animal Health and Veterinary Biologicals and Southern Regional Disease Diagnostic Laboratory, Bangalore.

Expert Group for finalization of the Module:

1. Dr. R.N. Sreeniwas Gowda, Vice-Chancellor, Karnataka Veterinary, Animal and Fisheries Sciences University, Karnataka.
2. Dr. Lal Krishna, Assistant Director General (AH), Indian Council of Agricultural Research, New Delhi.
3. Dr. R.S. Chauhan, Joint Director, Centre for Animal Disease Research and Diagnosis, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh. 4. Dr. S.C. Dubey, Joint Director, High Security Animal Disease Laboratory, IVRI, Bhopal.
5. Dr. A.B. Negi, Joint Commissioner (LHS), Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Government of India, New Delhi.

Published and printed by: The Secretary, Veterinary Council of India, A-Wing, 2"" Floor, August Kranti Bhavan, Bhikaji Cama Place, New Delhi-110066.

Printed at: Creative Graphics, *88* DSIDC Complex, Okhla Phase-1 New Delhi - *110020.* Phone: *26816824.*

Preface

Consequent upon the decision of the Department *of* Animal Husbandry, Dairying and Fisheries, Ministry *of* Agriculture, Government of India to implement the Continuing Veterinary Education (CVE) programmes, an activity of Professional Efficiency Development Scheme„ through Veterinary Council of India as its nodal agency in the country, it has been decided to implement the skill based training programmes or, diagnosis of livestock and poultry diseases having zoonotic importance at the first instance The primary objective of these trainings is to upgrade the knowledge and skill of the registered Veterinary practitioners aimed at improving quality of Veterinary services through efficient and effective diagnosis of the diseases.

Avian influenza viruses are members of the family *Orthomyxoviridae* and are classified as type A. Further subtyping is based on the antigenicity of two surface glvcoproteins haemagglutinin (HA) and neuraminidase (NA). Based on their pathogenic potential these viruses are classified as highly pathogenic and low pathogenic. The highly pathogenic avian influenza (HPAI) is a highly contagious generalized viral disease generally caused by H5 or H7 subtypes of influenza A virus. The disease, a notifiable disease under OIE guidelines, may cause high mortality m birds, with respiratory, gastrointestinal and/or nervous signs. Wild aquatic birds such as waterfowls and shore birds are recognized as important reservoirs for avian influenza virus but rarely display clinical signs of infection, This disease poses a serious risk of spreading to human beings and thus, is very important from public health point of view and has been a major concern globally.

This Module for a four-day training programme developed and finalized by the experts in the subject emphasizes on the procedures for collection and despatch of laboratory materials, reporting of incidence, different diagnostic techniques and their interpretations, and the bio-security precautions to be followed by the Veterinarians while handling the suspected material/disease outbreak.

The contents of this Module are also available an the website www.vciindia.in.

CONTENTS

Chapter

AVIAN INFLUENZA - AN OVERVIEW

Introduction:

- Avian influenza viruses are members of the family *Orthomyxoviridae* and are classified as A, B and C based on antigenic differences in their nucleoprotein (NP) and matrix (M1) proteins.
- All avian influenza viruses are classified as type A. Further subtyping is based on the antigenicity of two surface glycoproteins haemagglutinin (HA) and neuraminidase (NA). Currently 16 HA and 9 NA subtypes have been identified among influenza A viruses.
- Avian influenza viruses are classified as highly pathogenic (HPAI) and low pathogenic (LPAI) based on their pathogenic potential.
- The highly pathogenic avian influenza (HPAI) is a highly contagious generalized viral disease generally caused by H5 or H7 subtypes of influenza A virus. The disease, a notifiable disease under CIE guidelines, may cause high mortality in birds, with respiratory, gastrointestinal and/or nervous signs.
- Low pathogenic avian influenza (LPAI) can be associated with severe clinical disease in the presence of other infectious agents.
- Wild aquatic birds such as waterfowls and shore birds are recognized as important reservoirs for avian influenza virus but rarely display clinical signs of infection.
- From 2003 to till 2007, more than 75 countries across the world have experienced severe outbreaks of HPAI in poultry leading to culling of millions of birds causing huge economic loss and resulted in social misery.
- Apart from infections in poultry, many countries have reported human cases of H5N1 subtype of HPAI.

Transmission:

- Pig is widely regarded as the potential source of new pandemic strains, although recently quails and humans were also reported as "mixing vessels".
- Birds infected with AI excrete large amounts of virus in faeces and other secretions, which contaminate directly the environment such as dust, soil, water, cages, tools and other fomites.
- It has been reported that one gram of fecal material contaminated with AIV can infect one million birds. AIV may remain infectious in soil, water

or contaminated equipments for weeks to months depending on the temperature and humidity.

- Transmission of the influenza virus between birds occurs directly or indirectly through contact with faecally contaminated aerosols, water, feed and other materials.
- Bird to human transmission also occurs through direct contact with birds or contaminated fomites.
- Contact with contaminated water is regarded as the most important mode of
- transmission between aquatic birds.

3. Pathogenicity:

- HPAI is infectious for all commercial, domestic and wild birds.
- Chicken and turkey are the most susceptible species where most pathogenic symptoms are observed.
- Duck and geese show symptoms only after infection with very virulent virus.
- Normally no significant disease condition observed in wild birds even though HPAI virus has been frequently isolated from a wide range of wild birds.
- Pigs living in close proximity of poultry farms may acquire avian influenza virus and sero-convert without showing clinical signs. However, they could remain carriers of the virus and spread the infection.
- There is a possibility that if low pathogenic H5 and 1i7 subtypes of At infection is allowed to continue in poultry population for long time, it may then mutate to highly pathogenic strain, which depends on the presence of basic amino acid at the cleavage site of the haemaqglutinin protein.
- Pathogenicity of individual Ai viruses vanes arid should be determined in order to develop prevention, control and eradication strategies.

4. Diagnosis:

- Clinical diagnosis
- Post mortem lesions
- Laboratory diagnosis
- Isolation of influenza viruses from tissues, swabs (tracheal pharyngeal and cloacal) and faecal materials
- Serological diagnosis by HI test

- Differential diagnosis from Newcastle disease, infectious laryngo-tracheitis (ILT), Fowl cholera (caused by *Pasteurella multocida*)*)* and other diseases which induce similar clinical signs all times.

5. Prevention and Control

5.1. Biosecurity:

Some of the common biosecurity measures, which can be ensured at the farm level, are enumerated as follows:

- Clean overalls and footwear must be worn when entering the poultry farm.
- The site of poultry farm should have limited access with possibly only one entry point.
- Visitors and vehicles should b8 Kept out of the premises.
- Vehicles entering into the farm should be washed with a pressure hose using brushes and an approved disinfectant.
- Keeping the farm access routes parking areas, storage yards and surroundings clean will avoid attracting free-living birds to the farm and entering the sheds.
- Free-living birds can bring infection to the poultry farm. Preventing the
- Pigs living in close proximity of poultry farms may acquire avian influenza virus and sero-convert without showing clinical signs. However, they could remain carriers of the virus and spread the infection.
- Keep wild birds, dogs, cats, rodents and other livestock away from the farm by implementing active control programme for these animals.
- The birds should be provided with clean fresh drinking water and the drinkers should be periodically cleaned.
- Feeders, hoppers and feed bins should be regularly cleaned. The feed storage bins should be sealed and made rodent and wild bird proof.
- Damaged eggs, dead birds, litter and manure should be promptly and properly disposed off by incineration or deep burial.
- All vehicles used for transportation of birds, eggs, feed and manure should be thoroughly washed, cleaned and disinfected after each use.
- All containers, crates and other equipment should be regularly cleaned and disinfected before and after use. All equipment used for injecting vaccines should also be cleaned and disinfected before and after each use.

- After depopulation cycle, the building and all equipment should be thoroughly cleaned and disinfected. The surplus feed, litter and dead bird, if any, should be removed and properly disposed of.

5.2. List of disinfectants to be used:

The list of disinfectants, which are active against avian influenza virus, their concentration and recommended use, are as follows:

- Rectified spirit or Savlon or Dettol (1% solution) can be used for cleaning of hands, feet of farm workers and visiting officials.
- Two percent (2%) solution of NaOH should be used at the entrance on foot mats to clean the shoes. This solution can also be used to scrub and clean gumboots and other items.
- Sodium hypochlorite: 2% active chlorine solution (disinfection of equipment)
- Quaternary ammonium salts: 4% solution (treatment of walls, floors, ceilings and equipment)
- Calcium Hydroxide: 3% solution (treatment of walls and floors)
- Cresolic acid 2.7_% solution: (treatment of floors)
- Synthetic phenols 2% solution: (treatment of floors)
- Vircon-S® where available
- Formalin and permanganate: for fumigation

6. Status and Preparedness in India

6.1. Surveillance of HPAI virus in India:

- To study the status of HPAI and develop facilities for diagnosis in India, the surveillance work on HPAI was started in the year 2001 after the establishment of High Security Animal Disease Laboratory (HSADL), a Biosafety level - 4 laboratory at Bhopal.
- Until February, 2006, only a non pathogenic H9N2 virus could be isolated from Indian poultry. In February, 2006, the first case of H5N1 was isolated from Maharashtra state. Further investigations led to detection of F15N1 virus in chicken samples collected from Gujarat state. A second outbreak of H5N1 was detected in Maharashtra in March, 2006. In the same month, one case of H5N1 was also detected in Madhya Pradesh Further testing of samples from various parts of the country did not lead to detection of H5N1 indicating that the outbreaks have been contained and controlled.
- In 2007, after a gap of 15 months, , H5N1 virus outbreak was detected at one farm in Manipur.

- In January, 2008, the disease again broke out in West Bengal and affected as many as 13 districts of the total 19 districts in the State.

6.2. Screening of imported birds:

- In order to maintain the status of freedom from HPAI in the country and to avoid introduction through imported birds, all types of birds are screened for the presence of HPAI at the quarantine stations under Government of India policy,

6.3 Control measures adopted during the outbreak of H5N1:

- Following the report of mass deaths of chickens samples are collected for laboratory confirmation. On confirmation, emergency measures are initiated for mass culling and ban on movement of poultry from the suspected areas as per the national policy and international guidelines.
- Other infected materials such as feed, feed ingredients, egg, egg trays and feathers are also need to be destroyed
- The control measures in addition to culling of birds include house to house mopping, spraying burning of bamboo baskets m which the birds are generally kept, flaming and spraying of steel wire cages used for keeping birds and whitewashing of the rooms in which the birds are generally kept.
- Follow up surveillance of the affected areas.
- The human population in contact with the infected poultry including farmers, labourers, their family members are screened for the infection and preventive medicines given.

6.4. Immediate and future surveillance plans:

- The process of screening of migratory birds across the country during winter season has been initiated.
- Active surveillance is being carried out within 10-20 Km areas bordering with neighbouring countries. Further, in view of the threat perception, active surveillance system has also been activated in all other states.
- The state forest departments and central diagnostic laboratories have been asked to be vigilant for any signs of abnormal mortality of poultry or migratory birds within their domain. The samples would be sent to HSADL, IVRI, Bhopal for screening of HPAI.

DIAGNOSIS OF AVIAN INFLUENZA

1. Clinical diagnosis:

- The clinical investigation must be performed on all susceptible species present on the farm, and it must begin from the most peripheral units.
- Particular attention must be paid to any vaccinations performed.
- All this information must be reported in the epidemiological inquiry (see Annexure II). All the birds present PER SPECIES must be identified, and for each species identified, a report containing the date of onset of clinical signs, description of clinical signs and reported percentage mortality must be prepared.

1.1 Low pathogenic strains

- Mild to severe respiratory symptoms.
- Decrease in egg production up to 45% (takes 2 - 4 weeks to recover).
- Mortality 3% (layers), 15% (broilers).

1.2 Highly pathogenic strains

- Severe respiratory distress, depression, ruffled feathers. Watery eyes and sinuses.
- Cyanosis of combs, wattles and shanks. Edema of head and sinuses.
- Diarrhea (initially bright green later white).
- Nervous signs as with highly virulent Newcastle (Ranikhet) Disease
- Sudden death (within 24 hrs of first signs) mortality can approach 100°ia

1.3. Differential Diagnosis:

HPAI virus needs to be differentiated from Newcastle disease, infectious laryngo-tracheitis (ILT), fowl cholera (caused by *Pasteurella multocida*) and other disease which h induce similar clinical signs at times.

		HPAI	**Newcastle disease**	**Fowl cholera**
CLINICAL SIGNS				
1.	Swollen head, comb and wattle with cyanosis	Present	Absent	Cyanosia and oedema of comb and wattle
2.	Red colour shank due to haemorrhage	Present	Absent	Absent
3.	Paralysis of legs and wings	Absent	Present	Absent
4.	High mortality	Upto 100%	Velogenic from :	Low mortality

			Upto 100% Mesogenic from : 30-40% Lentogenic from : 5-10%	
GROSS LESIONS				
1.	Haemorrhage in epicardial fat	Present	Usually absent	Absent
2.	Haemorrhages on tip of proventricullus papillae	Absent	Present	Absent
3.	Haemorrhagic ulcer at ileo-caecal function	Absent	Present	Absent
4.	Visceral gout and nephrosis with swollen kidneys	Present	Absent	Absent
5.	Pneumoencephalitis	Absent	Present in mesogenic form	Absent
6.	Small necrotic foci (Pin head size) in liver	Absent	Absent	Present

2. Postmortem lesions:

2.1 Gross lesions:

In per-acute cases (day1 & 2), death occurs without gross lesions. Generally, congestion, hemorrhage and edema are found in internal organs In acute cases (day 3-5), the following lesions are observed :

(day 3 -5), the following lesions are observed:

- Subcutaneous edema of head and neck
- Conjunctivae congested and petechiated.
- Abdominal fat, serosa, peritoneum with petechial haemorrhages
- Diffuse haemorrhages between hock and feet and thigh muscles .
- Petechia! haemorrhage on whole viscera
- Air sacculitis, peritonitis (cattarhal, fibrinous)
- Atrophy of lymphoid organs

2.2 Histopathological lesions:

- Perivascular cuffing (PVC) of lymphocytes in brain, heart, spleen, pancreas.
- Coagulative necrosis in kidneys, lungs, spleen.

- Oedema, hyperaemia, hemorrhage in heart muscles.
- Necrotic epithelial cells and urate casts in kidney tubules.

3. Laboratory diagnosis:

- Highly pathogenic avian influenza (HPA!) is classified as select agent and must be worked with under biosafety level 3 or 4 (BSL 3 or 4) laboratory conditions.

3.1 Collection of pathological samples

In cases of suspected avian influenza the following pathological samples must be collected and sent to the laboratory:

- At least 5 diseased birds (for post mortem examination),
- Pooled tracheal and lung samples from at least 5 diseased birds,
- Pooled intestine samples from at least 5 diseased birds,
- Cloacal, pharyngeal and tracheal swabs from healthy birds,
- Cloacal swabs must be collected from at least 30 birds, this will allow the detection of infection with a confidence level of 95% if the prevalence of faecal excretors is >0.1. Swabs must be collected ensuring that at least one gram of faecal material is actually on the swab, and must be subsequently immersed in virus transport medium.
- At least 10 serum samples.

Samples must be packaged appropriately (in leak proof containers, wrapped in at least two plastic bags), to avoid dissemination of the infectious agent, and transported under refrigeration to the laboratory inside a polystyrene box (ice box) containing ice or icepacks. The polystyrene box must be appropriately disinfected before leaving the premises.

The samples must be accompanied by the appropriate form (see Annexure III).

3.2. Serological diagnosis:

The infection yields positive antibody test as early as four to ten days post . Rising antibody response can be detected by AGID, HI or ELISA.

3.2.1. Agar Gel Immunodiffusion (AGID) test: The method described by OIE 2004) is used.

- To 25 ml of PBS (Avian) 0.25 gm of agarose (Promega, USA) is added, and boiled to dissolve the agarose completely to give 1% (w/v) agarose gel.

- The gel is cooled to 37°C and 25 pi of 10% sodium azide is added to it. The gel is poured to a thickness of 3 mm in a petri dish (90 mm diameter).
- A set of 7 wells (1 central well + 6 surrounding wells) is made using a template and a cutter. Wells are 5 mm in diameter and 3 mm apart.
- The central well is dispensed with 30 µl of known H7N7 or H9N2 avian influenza virus antigen.
- 30 µl of known positive antiserum is dispensed in alternate wells and known negative serum is dispensed in one well. The field sera are dispensed in the remaining two wells.
- The petri dish is covered with a lid and thereafter placed in a humid chamber at 37°C for 24 h. The precipitin lines are observed in between antigen and serum wells in positive reaction. However, if no precipitin lines are seen then it should be kept in refrigerator at 4°C for 24-48 hrs.
- After 48-72 h the petri dish is observed for precipitation lines.

3.2.2. Haemagglutination (HA) and haemagglutination Inhibition (HI) test: **3.2.2.1. Preparation of 1% (v/v) Chicken RBCs:** The method described by WHO (2002) is used.

- 5.0 ml of chicken blood is collected and diluted with 5 ml of Alsevers solution.
- 0.5 mi of diluted blood is mixed with 10 m! PBS (pH 7.2) in 15 ml tube and centrifuged at 1000 rprn for 10 min.
- Supernatant is discarded and the pellet re-suspended in 10 ml PBS.
- This washing was repeated thrice and the RBC pellet obtained was measured and suspended in PBS, so as to make 1% (v/v) RBCs

3.2.2.2. Haemagglutination (HA) Test: The method as per OIE (2005) is used.

- In to each well of a plastic V bottomed microtitre plate 25 µl PBS is dispensed.
- In the first well 25 µl of amnio-allantoic fluid (virus) is dispensed and serial two fold dilutions are made from 1:2 to 1:4096.
- Then 25 µl PBS is dispensed to each well.
- Then 25 µl of 1 % (v/v) chicken RBCs is dispensed to each well
- The last row of the microtitre plate is kept as RBC control, which is prepared by dispensing 50 µl of PBS and 25 µl of 1% chicken RBCs only. Mixing is done by tapping the plate gently. The plate is then incubated for
- 30 min at the room temperature (20-25°C).
- The HA titer is determined by tilting the plate and observing the presence or absence of tear shaped streaming of the RBGs against the RBC

control. The reciprocal of the highest dilution giving complete HA (no streaming) is taken as HA titer. One HA unit is the amount of Haemagglutinin contained in the end point dilution of the HA titration. The "unit" of Haemagglutinin is not a measure of absolute amount of virus but is an operational definition dependent on the method used for the HA titration. A unit should, therefore, be defined as the amount of the virus in 0.25 µl of AAFs.

3.2.2.3. Haemagglutination Inhibition (HI) Test: The method described by OIE (2005) is used.

- Into each well of a plastic V bottomed microtitre plate, 25 µl PBS is dispensed
- Into the first well 25 µl of test serum sample is dispensed and serial two fold dilutions are made from 1:2 to 1:256.
- Then, 25 µl of 4 HAU of virus is added to each well.
- Mixing is done by tapping the plate gently and then the plate is incubated
- at room temperature for 30 min.
- Then 25 µl of 1 % chicken RBCs is added to each well and after gentle tapping, the plate is incubated at room temperature for 30 min.

Four controls are put viz., RBC control, positive serum control, negative serum control and virus control. For RBC control 50 µl PBS is added to all the rows along with 25 µl of 1 % chicken RBCs. For positive serum control 25 pl PBS, is dispensed in all the wells of a row. Then 25 µl positive serum is placed in first well and serial two fold dilutions are made across the row. 25 µl of 1% chicken RBCs is added to each well of that row. For negative serum control, 25 µl PBS is dispensed in the last well of the row and then 25 µl of test serum followed by 25 µl of 1 % chicken RBCs is dispensed. For virus control 25 µl of PBS is added to all the wells of any selected row. Then 25 µl of 4 HAU virus is dispensed in first well and its two fold dilution is made across the row. Further 25 µl of PBS is added to each well. Then 25 µl of 1 % chicken RBCs is added to all the wells of the row. The haemagglutination Inhibition titer is recorded as the highest dilution of serum causing inhibition of 4 HAU of antigen. The agglutination is assessed by tilting the plates. Only those wells in which the RBCs streamed at the same rate as that of RBC

control wells are considered to show inhibition. The validity of the results is assessed against a negative control serum which should not give a titer > 4 and a positive control serum for which the titer should be within one dilution of the known titer.

3.3. Isolation and characterization of AIV:

Due to the high risk to humans, HPAI virus can only be handled in biocontainment facility i.e. BSL-3 or BSL-4. No other laboratory is allowed to handle the virus.

BIOSAFETY PRINCIPLES AND PRACTICES IN LAB DIAGNOSIS

1. Containment

- Primary containment protects the laboratory workers and the immediate laboratory environment from exposure to biological agents Primary containment is achieved through good microbiological technique and the use of safety equipment and personal protective equipment.
- Secondary containment protects the environment outside the laboratory, and is provided by facility design and operational procedures.

2. Basic Laboratory Practices

	Bio-safety Practice	Routes of Exposure Blocked
1.	Do not mouth pipette	Inhalation, ingestion, skin and mucous membrane contact
2.	Manipulate infectious fluids carefully to avoid spills and the production of aerosols	Inhalation, skin and mucous membrane contact
3.	Restrict use of needles, syringes, and other sharps to those procedures for which there are no alternativfes; dispose of sharps in leak-and puncture-poor containers	Percutaneous, inhalation
4.	Use lab coats, gloves, safety eye wear, and other personal protective equipment	Skin and mucous membrane contact
5.	Wash hands after all laboratory activities, following the removal of gloves, and immediately following contact with infectious agents	Skin and mucous membrane contact
6.	Decontaminated work surfaces before and after use, and immediately after spills.	Skin and mucous membrane contact
7.	Do not eat, drink, store food, or smoke in the laboratory	Ingestion, skin and mucous membrane contact.

3. Prevention of Aerosols and Droplets

- Handling of liquids or dry powders is likely to generate aerosols or droplets.
- Procedures such as centrifuging, mixing, and pipetting that involve high energy tend to produce respirable aerosols that stay airborne for extended periods and are small enough to be inhaled.
- Opening containers and streaking plates produce droplets that settle quickly on surfaces, skin, and mucous membranes.
- Procedures involving infectious material should re performed inside a biological safety cabinet (BSC) whenever possible. A properly operating and properly used BSC will contain any aerosols and droplets generated during handling of infectious agents.

4. Personal Protective Equipment

- Personal protective equipment (PPE) is considered a primary barrier to infectious agents and proper use will reduce the likelihood of infection.
- Personal protective equipment includes safety eyewear, lab coats, and gloves, and is used to supplement the containment provided by laboratory practices and safety equipment.
- PPE is most effective when used to supplement primary control methods such as biological safety cabinets, safety centrifuge cups, and other containment devices.

Laboratory coats

- Laboratory coats protect street clothes against chemical and biological spills, and provide additional body protection
- Laboratory coats made of 100% cotton are flame resistant and nonreactive to many chemicals. Generally. a 100% cotton lab coat is recommended over polyester-cotton blends
- It is good laboratory practice to remove lab coats or gowns before leaving the laboratory to minimize the spread of contamination outside the laboratory.
- Lab coats should be left in the laboratory and must not be taken home for washing.

Gloves

- Gloves are available that provide protection against a variety of hazards, including infectious agents, chemicals, and radioactive material.
- Standard latex examination type gloves provide protection against microbiological hazards, including human blood and body fluids.

- Contamination control requires that gloves be removed prior to exiting a BSC or touching noncontaminated laboratory areas and equipment (such as clean areas, phones, computers, door knobs, etc.).
- Always check gloves for pinholes prior to use and wash hands after removing gloves.

Eye and Face Protection

- Safety glasses, goggles, and face shields provide protection against chemical reagents and disinfectants.
- Additionally, they also prevent infection that can result from the splashing of pathogenic organisms in the eye.
- Normal prescription eyeglasses are not safety glasses and do not provide adequate eye protection for laboratory operations.
- Microbial infection can occur as a result of splashes to the eye. Goggles with indirect venting provide a good barrier against such splashes.
- A face shield can be worn in addition to goggles (face shields do not provide adequate eye protection by themselves) to provide protection against splashes to the face and mouth.

Respiratory Protection

- N95 masks should be worn during culling or collection of samples or laboratory work involving suspected cases of AIV.
- If N95 masks are not available, then surgical masks can be used to provide respiratory protection.

FOOD SAFETY AND PUBLIC HEALTH ISSUES

Recent episodes of HPAI virus (H5N1) infecting human and causing mortality particularly in the SE Asian countries and China, is a major issue for public health concerns. To evolve a public health policy in respect of influenza in human due to involvement of HPAI virus, will be beyond the scope of this document. However, the health of farm hands, attending veterinary officer, and other staff engaged in culling and disinfection of infected premises should be under strict observations for a period of at least three weeks All persons entering a suspected farm should wear protective clothing. Sero-conversion ? in these in-contact persons should be monitored.

WHO interim recommendations for the protection of persons involved in the mass slaughtering of birds potentially infected with highly pathogenic avian influenza viruses

Avian influenza is a highly contagious disease of birds which is currently epidemic amongst poultry in Asia. Exposure to infected poultry and their faeces or dust/s01! contaminated with faces can result in human infection. These

recommendations have been developed because human infections have been identified in association with the current poultry epidemic.

i, Culiers and transporters should be provided with appropriate personal protective equipment: Protective clothing, preferably coveralls plus an impermeable apron or surgical gowns with long cuffed sleeves plus an impermeable apron; disposable examination gloves; masks: the minimum requirement is well-fitted surgical masks, where N95 masks are available their use is recommended; 2 goggles; boots or protective foot covers that can be disinfected.

ii. All persons who have beer in close contact with the infected birds should wash their hands frequently. Cuiiers and transporters should disinfect their hands after the operation.

iii. All persons exposed to infected chickens or to farms under suspicion should be under close monitoring by local health authorities. It is recommended that persons at specific risk of inhaling possible infected material (e.g. cullers and farmers involved in mass culling at commercial farms) receive prophyiaxis with antivirals. They should also be vaccinated with the current WHO recommended influenza vaccine to avoid simultaneous infection by human influenza and avian influenza and to minimize the poss3bi!ity of a re-assortment of the virus's genes. Additional health monitoring of chicken cullers, others involved in the process and their family member, should be carried out These individuals should report any relevant health problems (respiratory complaints, flu-like illnesses or eye infections) to a health care facility, Persons at high risk for severe complications of influenza (e.g immuno-compromised, over 60 years old, or with known chronic heart or lung disease) should avoid working with affected chickens.

Serological surveillance of exposed animal workers and veterinarians should be done In liaison with designated laboratories, full blood and post mortem specimens (intestinal contents, anal and oro-nasal swabs, trachea, lung, intestine, spleen, kidney, brain, liver and heart) of animals (including pigs) should be collected for investigation of new viral isolates.

4. WHO advice for people living in an area affected by HPAI virus

4.1. Advice about contact with chickens, ducks or other poultry in an area with HPAI

- People should avoid contact with chickens, ducks or other poultry as much as possible. Children should not have contact with poultry or any other affected birds.

- Do not bring (live or dead) chickens, ducks or any other poultry with you when you are visiting friends or family, even if you think your birds are healthy.
- Avoid contact with chicken farms, duck farms or any farm where birds have been sick, killed or are though to have bird flu.
- If yon do come into contact with an environment that has had sick/dead chickens, ducks and other poultry - wash your hands well and monitor your temperature for 4 days, If you develop a high temperature consult your doctor to see whether treatment is needed.

If you have had contact with any dead birds that have died from avian flu or if you have had contact with the droppings of these birds - consult Your doctor to see whether treatment is needed

4.2. Raising poultry at home – in an area affected with HPAI

- If you have any chickens, ducks or any other poultry at home, it is important that you know what to do if and when they are killed or die. You should know how to dispose off them and clean up your yard/pen, etc.
- Whenever you have contact with poultry, the chicken shed/pen or anything with faeces on it -- make sure you are protected by a mask, goggles, gown, rubber boots and gloves.
- If you do not have these items, try to improvise as much as possible; for example use a cloth around the mouth and nose, plastic bags to cover the hands and shoes, overalls that can be washed etc
- Wear this protective apparel to: slaughter the poultry, dispose of the bodies, and clean up the area (see below for advice on how to clean up the area). Make sure that children are not involved.
- After the area has been cleaned, remove all the protective apparel and wash your hands, clothes and if possible your body. A shower is the best alternative.
- If possible wash clothes in hot or warm soapy water, hang them in the sun to dry.
- Discard gloves, plastic bags and any other disposable materials
- Clean all reusable items such as rubber boots and glasses / goggles
- Always wash hands after handling these items

4.3. Advice on how to decontaminate the yard/chicken pen

- After the culling of the poultry. the area must be cleaned.
- Wear all the protective apparel outlined above before starting the cleaning process

- Collect any feces scattered around the yard into a pile to be buried. The feces should be buried at a depth of at least 1 meter
- Try to remove droppings without raising too much di.ist --- causing dried droppings to possibly blow into your face/eyes/mouth.
- Remove as many of the droppings as possible from the chicken coup/shed and bury as above.
- Clean all areas very well with detergent and water
- Discard all disposable items used to protect your-self such as gloves. plastic bags, masks, etc. Place reusable items into a bowl with detergent and water to be washed
- Wash hands very well in soap and water
- Shower/wash body using soap and water and wash hair.
- Take care not to re-contaminate yourself, wash clothes worn during the cull/clean up - use detergent and hot or warm water.
- Dry clothes in the sun
- Any item that may he used again - such as rubber gloves or boots - should be washed very well in soap/detergent and water To ensue the items are clean - wash twice
- Always wash hands after handling contaminated items

4.4. Advice about contaminated shoes and footwear

- After walking around contaminated areas such as farms with chickens, backyards with chickens, or markets, you should clean your shoes as carefully as possible.
- Take care when cleaning shoes not to flick any particles into your face. Wear a plastic bag over your hands and shield your eyes and mouth when cleaning dirty or muddy shoes.
- Leave dirty boots and shoes outside the home until they have been thoroughly cleaned.

4.5. Advice about visiting friends or relatives in health care facilities

- Avoid contact with patients known to have HPAI, especially during active phase of the infection
- If you visit a patient who has HPAI - follow the advice from the hospital staff to wear protective clothing, a mask, gloves, etc.
- You will need to wear special protective clothing when you have direct contact with me patient or the patient's environment.

- The personal protective equipment you will need to wear will include mask gown, gloves and goggles.
- You should receive advice on the proper way to put on protective clothing, especially on how to fit the mask to your face.
- When you leave the HPAI patient's room you must remove these items and wash your hands very well for at least 90 seconds with soap and water.

4.6 Advice about respiratory illness

- Anyone with flu-like illness should be careful with secretions from the nose and mouth
- Children are especially prone to touch their face, eyes and mouth with unwashed hands (e.g. when they have a cold, after playing). Teach children the importance of hand washing after coughing or sneezing and playing.
- Cover the nose, and mouth when coughing or sneezing - use a tissue paper and throw it in the waste once used - teach children to do this as well
- Always wash hands after any contact with respiratory secretions as these can spread disease
- Be careful with respiratory secretions (e.g. coughing and sneezing) when around other people, especially small children. It may be best to avoid contact with individuals who are more at risk of becoming ill (small children or people. with other illness) until flu like symptoms have resolved
- Consult your doctor if the illness is severe.
- Avoid contact with secretions of other people who have flu-like illness.
- Ask for people, especially children, to cover their nose and mouth when coughing or sneezing and to use a tissue paper or handkerchief.

ACTION PLAN OF GOVERNMENT OF INDIA STEPS TO BE TAKEN WHEN OUTBREAK OF AVIAN INFLUENZA IS 'SUSPECTED'

5.1 Legal frame-work of AI control and containment operations

- Notification of outbreak by Government of India s
- Notification under State Act dealing with Control of contagious Animal Disease
- Permission under Wild-Life Protection Act
- Prohibitory Orders

- Action under Cr. P. C.- S 133

5.2 Composition of Rapid Response for Animal Husbandry

5.3 Preparatory steps :

- Use of Maps for Determining Operational Area
- Demarcation of Operational Area on Ground
- Assessment of work load
- Assessment of Infrastructure Availability
- Assessment of spread of infection
- Control of spread of infection

5.4 Assessment of requirement of manpower, material, logistics and fund:

- Assessment of man-power of various ranks and skill
- Assessment of stores requirement:
  - Sodium pheno-barbital or alternative sedative drug
  - Gunny bags- 40 birds per bag
  - Slaked lime- 5- 6 bags of slake-lime per pit of 2m x2m top dimension
  - 2% sodium hypochlorite solution ..... per 100 sq m surface area
  - 4% formalin solution..... per 100 sq m surface area
  - One LPG cylinder (19Kg) for flaming ....100 sq m surface area
  - PPE kits- 2 per member of RRT (including casual labourers) per day + 10% for supervisory staff
  - Spare goggles, nose-masks, hand gloves
  - Gum boots, Dettol, carbolic soap, bottled water
- Assessment of logistics required:
  - Control Room- dispersal centre, telephone. fax, e-mail connectivity,
- emergency power supply,
- Boarding & lodging of members of RRTs
- Assessment of fund requirement

5.5 Reporting by Members of Rapid Response Team for Duty

- RRTs:
- Reporting formalities- record keeping
- Recording health status of each member
- Briefing -

    - Formation of teams
    - Task to be performed
    - Work-site condition
    - Reporting channel Means of Communication
    - How to communicate with poultry farmer
    - How to address to ethical issues
- Action to be taken at dispersal centre:
    - Administration of Tami flu-dose, timings
    - Observation of side-effects of prophylaxis
    - Issue of copies of report formats
    - Issue of cash advance
    - Allotment of vehicle and equipment
    - Issue of PPE Kits, stores and water bottle
    - Transportation to work site
    - Daily attendance
    - Records for demobilization
- Protocol for demobilization for RRT and subsequent precautions:
        - Minimum of 7 days' Quarantine for members of RRT
        - Disinfection of personal belongings
        - Disinfection of vehicles
        - Bath with carbolic soap
        - Dettol wash of clothes used
        - Disinfection of bags, spectacles, cap, wallet etc.
        - Self-surveillance

Annexure-I

Kit for the Veterinary Officer

1) Paper pad or writing pad and pens
2) Epidemiological inquiry form
3) Equipment necessary for the clinical visit and sampling procedures:

a. 2 disposable suits
b. 5 pairs of disposable shoe-covers
c. 2 pairs of rubber gloves and 5 pairs of latex gloves
d. disposable caps and face masks
e. paper tissues
f. 5 leak proof containers
g. 5 leak proof and water resistant plastic bags
h. torch
i. active disinfectant solution
j. 2 pens and a notepad
k. 100 syringes 2.5 ml with needle
l. 100 thin, small plastic bags
m. 2 pairs of surgical scissors
n. 2 pairs of forceps
o. tape
p. 2 felt tip pens
q. 1 thermic container (ice box)
r. 5 frozen icepacks

- At least two of these kits should be prepared and available at the District headquarters at all
- times.
- Number of each item in the kit may vary depending upon the number of farms to be inspected and their size.
- • Scissors and forceps should be sterilized by hot sterilization.

KIT for the Disease Investigation Officer

- 1 thermlc container (ice box)
- 4 pairs of forceps
- 2 pairs of surgical scissors
- 1 knife
- Tape
- labels and pens
- 100 2.5 ml syringes with needle
- sterile swabs
- 50 test tubes containing virus
- transport media
- 10 leak proof containers
- 2 disposable suits
- 5 pairs of disposable shoe-covers
- 5 pairs of latex gloves
- disposable caps and face masks
- 10 black waste-bags
- 50 rubber bands
- disinfectant solution
- cardboard container

The samples should be placed in isotonic phosphate buffered saline (PBS), pH 7.0-7.4, containing antibiotics. The antibiotics can be varied according to local conditions, but could be, for example, penicillin (2000 units/ml), streptomycin (2 mg/ml), gentamicin (50Ng/ml) and mycostatin (1000 units/ml) for tissues and tracheal swabs, but at five-fold higher concentrations for faeces and cloacal swabs. It is important to readjust the pH of the solution to pH 7.0-7.4 following the addition of the antibiotics.

Annexure-II

AVIAN INFLUENZA EPIDEMIOLOGICAL INQUIRY FORM (AS PER OIE REFERENCE LABORATORY)

date _____

Dr_____

Phone number_____

Name and Address of farm

_____

Phone_____

District _____State _____

Farm code or Identification number _____

Owner_____

Address of the owner _____

Phone _____

Information provided by

_____

Farm Veterinarian Dr. _____

Present NO_____YES_____

INFORMATION CONCERNING THE FARM TYPE OF ESTABLISHMENT :

_____Industrial _____Rural _____Dealer _____Retailer

CATEGORY/ PRODUCTION LINE:

Table-egg layers_____ Meat birds_____

Type:

Grandparents _____

Parents _____

Pullets_____

Meat-type (broiler) _____

Layers _____

NUMBER OF BIRDS AND SPECIES PRESENT

Chickens _____ Meat _____ No. _____ Breeders _____ No_____

Layers _____ No._____

Other_____

Date of placing _____ Sex : _____ Age _____

Hatchery of origin :

Company Hatchery : No. _____ Yes _____

Company : _____

Address _____

District _____ State _____

Phone _____ Fax _____

Debeaking operations: Date_____

Performed by: _____Family members _____ Employed staff _____ External staff _____ Other _____Remarks

HOUSING SYSTEM

Deep Litter: YES_____NO_____

Cage system Yes _____ NO_____

Type of ventilation system:

_ Natural_____

_ Natural with fans _____

_Artificial_____

_Bird proof nets NO _____YES_____

Possibility of contact with wild birds: NO_____YES_____

Species _____

Other birds present on site (captive or free)

NO _____YES _____Species_____

Presence of ponds or lakes : NO_____YES_____

Other water reservoirs NO _____YES_____(specify)

Presence of pigs NO _____YES_____ NO_____

Other animals NO_____YES _____ (specify) _____Remarks

OTHER INFORMATION REQUIRED

Data on introduction/spread of infection information necessary for the points a), b), c) etc., must be collected for all movements of animals/people and therefore may have to be repeated if necessary

2. Movements of birds information required

a) Introduction of birds from other establishments/hatcheries/farms NO__YES__ (Twenty days before the onset of the first clinical signs)

Date_____Species_____Farm _____Hatchery _____

Name of Farm_____

Address_____District_____

b) Introduction of birds from exhibitions/markets/fairs NO_____YES_____
(Twenty days before the onset of the first clinical signs)

Date **NUMBER OF BIRDS AND SPECIES PRESENT**

Chickens _____ Meat _____ No. _____ Breeders _____ No_____

Layers _____ No._____

Other_____

Date of placing _____ Sex : _____ Age _____

Hatchery of origin :

Company Hatchery : No. _____ Yes _____

Company : _____

Address _____

District _____ State _____

Phone _____ Fax _____

Date : _____ No. _____

Species _____

Origin : Fair _____ Market _____ Exhibition _____District _____

c) Exit of birds/eggs to other farms/establishments/hatcheries/abattoirs

(In the time span between 20 days before the onset of the first clinical signs and the date the farm was put under restriction)

No_____ Yes _____

Date _____ No _____

Destination: Other farm _____ Hatchery _____ Abattoir _____ Other _____

Name of establishment

Address _____

District _____ State _____

d) Exit of birds/eggs to other fairs/markets/exhibitions NO_____YES_____

(In the time span between 20 days before the onset of the first clinical signs and the date the farm was put under restriction)

Date _____ No. _____

Destination: Fair_____ Market_____ Exhibition_____ Other_____

Address_____ District_____

3. MOVEMENT OF PEOPLE: POSSIBLE MEANS OF INTRODUCTION OR OF SPREAD OF INFECTION (In the time span between 20 days before the onset of the first clinical signs and the date the farm was put under restriction)

NO _____ YES_____

Date _____

_____Veterinarian _____Technician Vaccinating crew_____ Debeaker_____

Other farmer_____ Dealer_____ Other (specify) _____

Address _____

District _____ State _____ Phone No. _____

Previously visited farm: Name _____ District _____

(Farm put under restriction)

MOVEMENT OF VEHICLES:

Number of dead birds

(A) Transport of animals, (B) Transport of feed. (C) Transport of eggs, (D) Collection of dead animals, (E) Fuel/Gas. (Other) Specify

(In the time span between 20 days before the onset of the first clinical signs and the date the farm was put under restriction)

Date of entry Vehicle (A / B / C / D / E / Other)

Name of company Fax/Phone number

INDIRECT CONTACTS WITH OTHER POULTRY ESTABLISHMENTS

NO _____ YES_____

Date of contact _____

(Sharing of equipment, vehicles, feed, staff, etc. in the time span between 20 days before the onset of the first clinical signs and the date the farm was put under restriction)

Date of contact_____

Name of farm or establishment_____District _____

____shared ____vehicle ____shared feed ____shared equipment ____shared staff ____collection/recycle of litter other (specify)

b) OTHER FARMS OWNED BY THE SAME OWNER NO _ YES _

Name of farm or establishment_____

Address _____

District_____

Species farmed_____ number_____

Empty_____ Full _____

c) POULTRY FARMS LOCATED NEAR THE OUTBREAK

NO _____ YES_____

Name of farm or establishment_____

Address _____

District_____ Distance in meters _____

Species farmed_____number_____

Empty_____ Full _____

ANAMNESTIC DATA

WEEKLY MORTALITY

NB: data concerning mortality rates recorded in the 6 weeks prior to the onset of clinical signs WEEK FROM TO

NUMBER ANIMALS DEAD

Remarks:

_____

_____

_____

Date of onset of AI clinical signs _____

Clinical signs observed by the farmer_____

Total Number of birds

Farm put under restriction

(dead or alive)

Number of ill birds

(Farm put under restriction)

Number of dead birds

(Farm put under restriction)

Number of birds depopulated

NB: This information must refer to the data collected when the farm has been put under restriction with mortality and morbidity referring to the suspicion of HPAI

VACCINATION of birds

Vaccination of birds is practiced : No_____ Yes _____

Date of vaccination Type of vaccine (1) Commercial name Administration route

(1) Live or inactivated

VACCINATING STAFF: _____Family _____Employees _____External staff _____Other_____

Remarks_____

ADMINISTRATION OF DRUGS/MEDICAMENTS

In the last 15 days: NO _____ YES _____ (specify):

STAFF WHO ADMINISTERED THE MEDICAMENT: _____Family _____Employees _____External staff _____Other_____

Remarks_____

CLINICAL INVESTIGATION PER SPECIES

Species_____

Depression _____

Respiratory signs: mild _____ severe _____

Drop or cessation of egg laying _____

Oedema, cyanosis or cutaneous haemorrhages _____

Diarrhoea _____ Nervous signs _____ Other_____

GROSS FINDINGS

Rhinitis and sinusitis_____

Tracheitis catarrhal _____

haemorrhagic _____

Aersacculitis _____

Haemorrhages epicardium _____

*endocardium*_____

proventriculus _____

ovarian follicles _____

Enteritis catarrhal _____

haemorrhagic _____

Pancreatitis _____

Other:_____

Remarks_____

Signature

_____

Date: _____

Annexure III

AVIAN INFLUENZA Sample submission form (*As per OIE Reference Laboratory)*

State_____ District_____

Veterinarian_____

Phone_____Fax_____

Date_____ Accession number_____

Farm

District _____ State _____

Code of Identification number

Owner _____

Complete address

SPECIES AND CATEGORY_____Broiler breeders No_____

Layer breeders No. _____Layers No. _____Broilers No. _____

Other species (specify) No. _____

COLLECTION OF SAMPLE FROM / FOR

__SUSPECT OUTBREAK___ date of notification _____

__Confirmed outbreak

__Farm epidemiologically connected with outbreak _____

__Name and farm code of outbreak _____

Farm located in protection zone__Name and farm code of outbreak

Farm located in surveillance zone__ Name and farm code of outbreak

_____

_____

__Testing for the movement of animals

__Monitoring programme

__Other _____

ANAMNESTIC DATA

Species and category

Onset of clinical signs

Symptoms % mortality Form/to

_____

_____

Species Samples collected

No. samples for detection of Antibodies

No. samples for detection of Virus

SAMPLE IDENTIFICATION

Signature_____ Date: _____