

# **AVIAN INFLUENZA (BIRD FLU)**

## **INTRODUCTION**

Influenza is a disease caused by type A influenza virus, a member of the Orthomyxoviridae family. Influenza A viruses are responsible for major disease problem in birds as well as in humans and lower mammals.

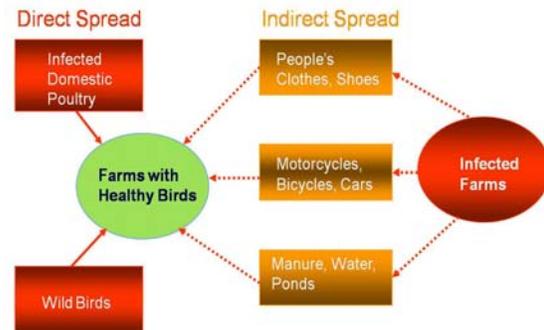
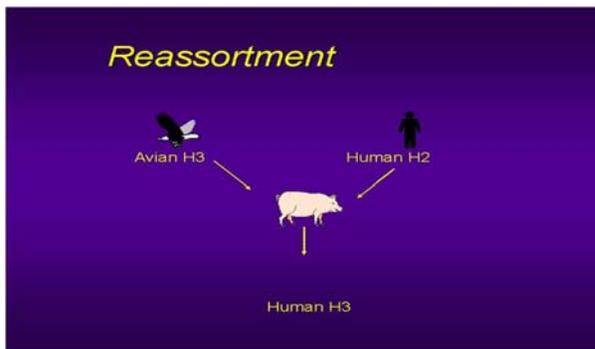
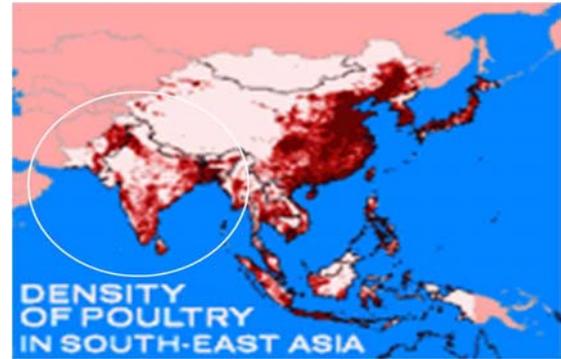
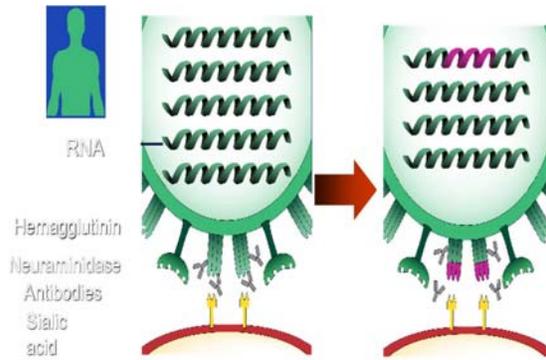
Avian Influenza may play a role in the evolution of new human strains by contributing viral genes via genetic resortment. Antigenic and genetic evidence support the suggestion that the HA gene in a virus responsible for the 1968 pandemic in human originated from a virus circulating in duck's.

Direct transmission of viruses between birds and humans does not generally occur. Further more there is evidence to suggest that the H1N1 Virus present in pigs turkey and ducks may be involved in interspecies transmission. So a swine-avian-human connection could have public health significance. Recent havoc in Hongkong has received the attention of the world.

In contrast to domestic and confined birds free flying birds do not experience significant diseases problems due to influenza viruses but they act as carriers. Influenza viruses are readily recovered from migratory waterfowl particularly ducks and this serve as a source of virus for other species including humans, lower mammals and birds and that such a high rate of infection for the maintenance and emergence of new and potentially higher pathogenic strains through the process of mutation and or genetic resorment.

Avian Influenza viruses are distributed throughout the world in many domestic birds, including, turkeys, chinkens, guinea fowl, quails, pheasants, geese and ducks. Migratory waterfowl, particularly ducks have yielded more viruses than any other group. While domestic turkeys and chicken have experienced the most substantial diseases problem due to influenza. Influenza viruses have also been isolated from caged birds, including mynas, parakeets, parrots, finches and howks.

Influenza is reported in several other countries including Belgium, Scotland, Italy, Soviet Union, Australia, Hongkong, France Israel and Pakistan.



## EPIDEMIOLOGY:

Avian Influenza viruses with H5 and H7 antigens are more virulent with high pathogenicity while H3 serotypes can cause only mild diseases with respiratory signs in turkey.

The disease can spread by import of birds/chicks from infected countries and can be spread by cull birds/live birds transport of infected eggs/egg trays/infected feed/feed bags etc.

## **RESISTANCE TO CHEMICAL AND PHYSICAL AGENTS:**

Avian influenza Viruses are relatively sensitive to inactivation by lipid solvents such as detergents. Infectivity also rapidly destroyed by formain, beta propiolactone, oxidizing agents, dilute acid etc. They are also inactivated by heat, extremes of pH and dryness.

## **TRANSMISSION AND CARRIERS:**

Infected birds excrete virus from the respirator tract, conjunctiva and faeces. Thus likely modes of transmission include both direct contact between infected and susceptible birds and indirect contact between infected and susceptible birds and indirect contact including aerosols (droplets) or exposure to virus-contaminated with faecal material of Birds and mammals e.g. feed, water, equipment, clothes, vehicles and insects. Thereby viruses are readily transported to other areas by people and equipment shared in production or live bird marketing. There is no evidence of vertical transmission.

## **SYMPTOMS:**

Morbidity and mortality ranges from mild from to 100%. The signs of diseases are extremely variable and depend on the species affected, age, sex, concurrent infection, virus, environment, factors etc. Signs may reflect abnormalities of the respiratory, enteric reproductive or nervous systems. The signs most commonly reported include pronounced depression, decreased feed consumption, increased broodiness and decreased egg production with soft shelled eggs, increased mortality, mild to severe respiratory signs including coughing, sneezing, rales and excessive lacrimation, huddling, ruffled, feathers, oedema of head and face, cyanosis of unfeathered skin in co-ordination and other abnormalities of the nervous system. Hemorrhages on shank skin or vesicles filled with clear fluid or blood are observed on combs and wattles.



**Edema of head , purple discoloration of comb , wattles**



**Respiratory involvement , Excessive lacrimation,**



## Cessation of egg-laying, rubbery eggs , soft shelled eggs



### GROSS LESSION:

- Caseous exudate in the trachea.
- Air-cacs thickned with fibrenous of caseous exudate.
- Catarrhal to fibrinous peritinitis and "egg yolk pentonitis".
- Cedema of the head with swollen sinuses.
- Congesed and hemorrhagic wattles and combs.
- Congestion and hemorrhages on the legs.
- Presence of necrotic foci on the liver, spleen, kidneys and legs.
- Severe swelling of combs and wattles with periorbital oedema.
- Swelling of the feet with ecchymotic discoloration.
- Petechial hemorrhages on various serosal and mucosal surfaces, particularly of the junction between gizzard and proventriculus.

## HPAI : PM gross lesions (H5N1)

Congestion & various degree of hemorrhages in URS  
( larynx & trachea) : trachietis



## Haemorrhages in proventriculus,liver, thigh muscles & splenomegaly



### HISTOPATHOLOGY:

The most consistent and most severely affected tissues are brain, heart lung, pancreas and primary and secondary lymphoid organs. Lymphocytic meningoencephalitis with local gliosis, neuronal necrosis and neuronophagia are common, but adema and hemorrhage may also be seen. Focal degeneration to multifocal-diffuse coagulative necrosis of cardiac myocytes has been reported usually with accompanying lymphohistiocytic inflammation.

## **DIAGNOSIS:**

A diagnosis of influenza A virus infection is conclusively demonstrated by the isolation and identification of the virus, but detection of antibodies to the virus is a very useful indirect diagnostic tool.

It is necessary to collect (from necropsied birds) tracheal and cloacal swabs as well as lungs, pancreas and spleen tissue in brain heart infusion broth and use it for inoculation of embryonated eggs (9 to 10 days old) by allantoic route for isolation of the virus. The allantoic fluids from the infected embryos can be used for detection of haemagglutination activity which can be inhibited by use of a battery of specific reference A.I. antisera for detection of H antigens.

Other tests like ELIS, Nucleotide probing, Radial diffusion hemolysis tests and use of monoclonal antibodies can be done at reference laboratories with the help of global organization including FAO/WHO/OIE etc.

## **Differential Diagnosis:**

Most of the symptoms found are similar to New castle disease infectious Laryngotrachitis or infectious bronchitis.

## **Lesions of RD**

In the viscerotropic velogenic form of ND, Oedema of the interstitial tissues of the neck, Haemorrhages and necrosis in the proventriculus, gizzard and on the small intestinal wall. Small haemorrhages are often present in other internal organs.

In the neurotropic velogenic and mesogenic forms of RD, acute laryngitis and tracheitis congestion and catarrhal exudates and air sacs are thickened and cloudy sometimes haemorrhages in the proventriculus but rarely elsewhere are observed Gross lesions may not be present in birds that only show nervous signs.

## **PREVENTION AND CONTROL**

Methods for prevention and control of influenza virus infection center on preventing the initial introduction of the virus and controlling spread, if it is introduced. One critical aspect in reaching and goal of how the viruses are introduced, how they spread and how such events can be prevented in which biosecurity is the first line of defence. Study group formulated by minister of agriculture, Govt. of India on poultry diseases including IBD, IBH and avian Influenza, March 1996 has advised the Govt. of India on vaccination strategies and other measures to control Avian Influenza in India in further they include.

- Physical separation of healthy birds from infected group.
- Preventing contact of recovered birds with healthy birds.
- Minimize the contact with migratory birds especially ducks, waterfowl etc.
- Proper disposal of carcass, ailing birds, litter etc.
- Creation of a disease free zone at International borders by depopulating or reducing the population of birds near International boundaries.
- Ban or import of biologicals, reagents, livestock and the livestock products from countries which have reported the disease in the recent past.
- Periodical screening of poultry and captured visiting migrated birds, ducks, waterfowls to insure absence of antibody or virus excretion. This exercise should be entrusted to a laboratory where high disease security facilities are available.
- Educating the field staff and poultry farmers about the disease, it's devastating effects, clinical signs, collection and transport of clinical samples.

### **Specimen Collection for Diagnosis and Surveillance in Suspected Avian Influenza**

In mammals including humans, pigs and horses, influenza is primarily respiratory tract infection of both the respiratory tract and the large intestinal tracts. Specimens for isolation of respiratory viruses in cell cultures or embryonated chicken eggs and for the direct detection of viral antigen or nucleic acid should generally be taken during the first 3 days after onset of clinical symptoms of influenza.

#### **Type of specimens**

A variety of specimen from animals and birds are suitable for the diagnosis of viral infections of the upper respiratory tract:

Nasal swab, throat swab, tracheal swab

In addition to swabs from the upper respiratory tract, sampling of avian species for influenza infection should include"

cloacal swab, faecal specimen

Whenever possible, cloacal swabs should be collected from live or freshly killed birds. Faecal specimen collected from cages or from the environment are often the only specimen that are available and cannot be assigned with total certainty to the species of origin. If dead animals are found as part of the investigation, highly pathogenic avian influenza virus should be suspected and representative internal organs, including brain, spleen, heart, lung, pancreas, liver

and kidney should be sampled together with sampling of the respiratory and intestinal tracts. Specimen for the laboratory diagnosis of influenza infection should be collected in the following order of priority:

**From live animals**

- Tracheal
- Throat/nasal
- Cloacal
- Faecal (environmental)
- Drinking water

**From dead animals**

- Lung lavage
- Pooled tissue (including trachea and lung)
- Faecal (environmental)
- Cloacal
- Drinking water
- Procedures for specimen collection

**Materials required**

- 1-3 ml screw cap plastic tubes
- Polyester fibre-tipped swabs
- Viral transport medium
- Instruments for post-mortem examination

**Specimen storage**

Specimen in viral transport medium for viral isolation should be kept at 4°C and transported to the laboratory promptly, if specimens are transported to the laboratory within 2 days, they may be kept at 4°C otherwise they should be frozen at or below -70°C until they can be transported to the laboratory. Repeated freezing and thawing must be avoided to prevent loss of infectivity - Sera may be stored at 4°C for approximately one week, but thereafter should be frozen at -20°C.

Specimen should be collected and transported in a suitable transport medium on ice or in liquid nitrogen. Standard precautions should always be followed and barrier protections applied whenever samples are obtained from patients. Specimen for influenza should not be stored or shipped in dry ice (solid carbon dioxide) unless they are sealed in glass or sealed, taped and double plastic

bugged. Carbon dioxide can rapidly inactivate influenza viruses if it gains access to the specimens through shrinkage of tubes during freezing.

### **Specimen Transport**

Transport of specimens should comply with the WHO guidelines for the safe transport of infectious substances and diagnostic specimens (WHO 1997). The receiving laboratory should be notified before shipment of specimens in order to arrange for an import license for the specimens. Transport of specimens within national borders should comply with the procedures detailed within each country's regulations. Primary receptacles shall be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break be punctured or leak their contents into the secondary packaging. Secondary packaging shall be placed in a final outer package with suitable cushioning material. Any leakage of the contents shall not substantially impair the protective properties of the cushioning material or of the outer packaging.

#### **For liquids**

The primary receptacle (s) shall be leak proof and shall not contain more than 500 ml. There shall be absorbent material placed between the primary receptacle and the absorbent packaging, if several fragile primary receptacles are placed in a single secondary packaging. They shall be either individually wrapped or separated so as to prevent contact between them. The absorbent material shall be in sufficient quantity to absorbent of the primary receptacles and there shall be a secondary packaging shall be capable of withstanding without leakage an internal pressure producing a pressure differential of not less than 95 kPa (0.95 bar). The outer packaging shall not contain more than 4 liters.

#### **For Solids**

The primary receptacle (s) shall be sift-proof and shall not contain more than 500g. If several fragile primary receptacles are placed in a single secondary packaging they shall be either individually wrapped or separated so as to prevent contact between them and there shall be a secondary packaging. Which shall be leak proof. The outer packaging shall not contain more than 4 kg. For air transport, the smallest overall external dimension of a completed package must be at least 10 cm. Packaging must conform to certain performance standards. For further information about definitions, packaging requirements, markings and labels, accompanying documentation and refrigerants, please refer to the competent authority, current IATA shipping guidelines, commercial packaging suppliers or available courier companies.

## Virus Transport Medium

### (A) Transport medium 199

1. Tissue culture medium 199 containing 0.5% bovine serum albumin (BSA)
2. To 1 liter of 0.5% BSA add:

Sr. No	Chemicals	Quantity
1.	benzypenicillin	(2x 10 <sup>0</sup> IU /Litre)
2.	streptomycin	(200 mg / litre)
3.	polymyxin B	(2x 10 <sup>0</sup> IU /Litre)
4.	gentamicin	(250 mg / litre)
5.	nystatin	(0.5x 10 <sup>0</sup> IU /Litre)
6.	ofloxacin hydrochloride	(60 mg / litre)
7.	sulfamethoxazole	(0.2 g / litre)

3. Sterilize by filtration and distribute in 1.0-2.0 ml volumes in screwcapped tubes

- OR -

### (B) Glycerol Transport medium

NaCl	8 gm
NaCl	0.2 g
Na <sub>2</sub> HPO <sub>4</sub>	1.15 g
KH <sub>2</sub> PO <sub>4</sub>	0.29

Distilled water to make 1 litre

2. Autoclave PBS and mix 1:1 with sterile glycerol to make 1 litre
3. To 1 litre PBS/glycerol add:

Sr. No	Chemicals	Quantity
1.	benzypenicillin	(2x 10 <sup>0</sup> IU /Litre)
2.	streptomycin	(200 mg / litre)
3.	polymyxin B	(2x 10 <sup>0</sup> IU /Litre)
4.	gentamicin	(250 mg / litre)
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6.	ofloxacin hydrochloride	(60 mg / litre)
7.	sulfamethoxazole	(0.2 g / litre)

### **Preparing specimen collection vials**

To sterile plastic screw-cap vials dispense 1.0-2.0 ml of transport medium. It is preferable to store these vials at -20 °C until use. However, they can be stored at 40 °C for 48-96 hours (optimally less than 48 hours) or at room temperature for short periods of 1-2 days

### **Preparation for collection of specimens**

Vials should be assigned a number that corresponds to that on the Field Data Collection Sheet. When possible, the following information should be recorded on the Field Data Collection Sheet: type of animal sampled, specimen collection etc. Tissue culture medium (A) is widely used for collection and transport of clinical specimens from all species. The glycerol-based medium (B) provides longer-term stability of specimens where cooling is not immediately possible; it is suitable for egg inoculation but not suited for tissue culture inoculation. Clinical specimens should be collected as described below and added to transport medium. All specimens should be kept on ice or at 4 °C. Standard precautions should always be followed and barrier protections applied whenever samples are obtained from patients.

#### **Nasal Swab**

A dry polyester swab is inserted into the nostril, parallel to the palate, and left in place for a few seconds. It is then slowly withdrawn with a rotating motion, down the inside of the nose. Specimens from both nostrils are obtained with the same swab. The tip of the swab is put into a vial containing 2-3 ml of transport medium and the applicator stick is broken off. Throat swab the posterior pharynx is swabbed vigorously, and the swab is placed in transport medium as described above.

#### **Tracheal Swab**

The trachea of live birds is swabbed by inserting a polyester swab into the trachea and gently swabbing the wall. The swab is then placed in transport medium as described above. Tracheal swabs from dead animals, including pigs at slaughter houses, can be taken after removal of the lungs and trachea from the carcass.

The trachea is held in a gloved hand and the swab inserted to its maximal length with vigorous swabbing of the wall. The swab is then placed in transport medium as above.

### **Cloacal Swab**

A cloacal swab from a live bird is taken by inserting a swab deeply into the vent and vigorously swabbing the wall. The swab should be deeply stained with faecal material. The swab is then placed in transport medium as above.

### **Faecal Specimens**

Faecal specimens from the cages of live poultry in bird markets or from wild birds in the field are collected from freshly deposited wet faeces; the swab should be heavily coated with faeces. The swab is then placed in transport medium as above.

### **Sera Collection or Influenza Diagnosis and Surveillance**

Optimally an acute phase serum specimen (3-5 ml of whole blood) should be taken soon after onset of clinical symptoms but not later than 7 days, and a convalescent phase serum specimen should be collected 2 to 4 weeks later. In serological surveillance studies at slaughter houses or from free flying wild birds that are bled and released only a single sample of serum is available. Serum samples are stored at -20 °C.

### **Tissue specimens**

Tissue specimens should ideally be frozen immediately, without transport medium, for isolation of virus in eggs or tissue culture. For diagnosis, an acute-phase serum specimen (3-5 ml of whole blood) should be taken soon after onset of clinical symptoms and not later than 7 days after onset.

It is advisable, in case of any high mortality observed in a farm or finding wild birds dead near the farm houses, not to handle it without using protective covering. In case of any suspicion of Avian Influenza, it should be put in leak proof polythene bags, double packed, and birds should be put in leak proof polythene messenger/courier to any regional diagnostic laboratory of the country identified for diagnosis of Avian Influenza.

Postmortem of suspected birds should not be conducted in the farm, it should be done by the trained veterinarian in laboratory at least having P3 facilities.

The success of virus diagnosis depends largely on the quality of the specimens and the conditions under which the specimen is transported and stored before it is processed in the laboratory.

## **GENERAL GUIDELINES & CARE TO BE TAKEN DURING HANDLING OF SUSPECTED AVIAN INFLUENZA MATERIAL**

### **(i). Disposal of culled poultry, accumulated litter and other contaminated materials**

The prompt and effective disposal of culled birds and contaminated materials that cannot be effectively disinfected (e.g. feed, litter and eggs) is essential. Depending on local circumstances, burial may be the preferred method of disposal. Covering the buried carcasses with lime is a requirement since it protects the carcasses from being uncovered by animals and earthworms. Lime should not be placed directly on carcasses because in wet conditions it slows decomposition. It is best bury poultry and contaminated materials at the affected area or farm. This may not always be possible because of the local well-water table level or other environment conditions. Therefore, a burial place away from the infected farm may be the best option, especially in situations where a number of farms are infected in a given area. In this case a common and centrally located burial site may be most practical. The transportation of culled birds and other contaminated materials must be done in covered leak-proof container vehicles should never leave the contaminated area without first being thoroughly disinfected. The disposal of litter can pose special problems as virus may be spread in dust. Therefore, it is necessary to moisten the surface of the litter with detergent or disinfectant solution before disturbing the pile prior to disposal. Compositing (thermal deactivation of the virus) of litter and other contaminated organic wastes is a recommended option that can best be undertaken in closed and vermin proof sheds on the affected farm. If sheds are not available, after spraying the material to be composed with detergent or disinfectant solution, push it into mounds away from any potential source of surface water and cover securely with black or dark plastic traps. Composting should be done in a secure area not accessible by other animals such as rodents, cats, dogs or birds. To ensure inactivation of virus, the mounds should not be disturbed for at least 3 months. In general, open-air-burning of carcasses and contaminated materials is not recommended. Open air burning should not be practiced because of the extended time it takes to achieve complete combustion of the carcasses and waste, and the inability to easily verify that all infective pathogens are destroyed in the resulting incomplete combustion process. If burning must be attempted as the only practical method of disposing of dead birds and

contaminated materials, closed incineration should be practiced. Incinerators should be well maintained by knowledgeable operators. Before disposal work starts, personnel should be fully briefed. The nature of the disease and hygiene requirements associated with zoonotic diseases should be explained on site. It is crucial to select a site that is well protected from People and scavenging animals. On some occasions it may be necessary to mount a guard at the site for the first few days.

## **(ii) Decontamination and Disinfection**

- Strict adherence to decontamination and disinfection procedures is essential to the control of HPAI infection in affected areas-
- Decontamination involves thorough cleaning and disinfection of the infected site to remove all contaminated material and source of virus.
- Individuals should be trained to conduct the procedures.
- Initial cleaning of organic matter from sheds, equipment and vehicle by brushing and washing with detergent and water is an essential step before chemical disinfection.
- It is very important to recognize that influenza virus can be spread on clothing, footwear.
- Poultry crates, feed sacks and egg filters and these items must be disinfected or destroyed after each use.
- Paper or wood items cannot be effectively decontaminated so they should be destroyed and buried.
- All farm materials (e.g. feed containers, water buckets), tools, equipment, vehicle and the structures that are physically or functionally connected to the areas where infected poultry are located should be properly cleaned and disinfected.
- Following disinfectants/antiseptic solution can be used for disinfection of poultry house.
- Waterers feeders which have been rendered free of organic matter.
- Povidoneiodine
- Glutaraldehyde
- Phenyl 0.5% solution for 30 min.
- Potassium permanganate 0.1% or min.
- Benzalkonium - chloride
- Paraoxides

### **(iii) Precautionary measures for General Public**

Proper handling of poultry that are ill, suspected of having bird flu or dead bird suspected of having bird flu or dead should be handled by trained veterinarian only. Bird should not be necropsied in the field. Only a veterinarian, wearing protective clothing, should handle it. Dead bird should be put in a leak proof plastic bag, double packed and sealed. It should be transported under chilled conditions immediately to designated laboratory authorized to handle bird flu suspected materials.

Make sure to keep children away from dead or sick poultry.

- If you need to handle dead or sick poultry, make sure you are protected. Wear protective clothing such as a mask, goggles, gown, rubber boots and gloves, If these are not available, cover your mouth with piece of cloth, wear glasses, use plastic bags to cover hands and shoes and fix these tightly around wrists and ankles with a rubber band or string. Wear overalls that can be washed.
- If you encounter sick and dead poultry for the first time are unsure of the situation, inform the authorities immediately and leave the handling of the poultry to experienced personnel (cullers, clean-up personnel etc.) Decontamination of the yard or chicken pen will help control the spread of the disease.
- If possible ask experienced personnel to help you decontaminate the yard or chicken pen.
- If this is not possible and you have to do it yourself, wear protective gear to protect your eyes, hands, feet and other exposed parts of your body as described above.
- Dead birds should be buried.
- Effective cleaning results in no visible feathers or faeces remaining in the shed.
- Influenza viruses can survive for some time in organic materials so through cleaning with detergents is an important step in decontamination. All organic matter must be removed from poultry houses as much as possible.

- As outdoor areas used by poultry can be difficult to clean or disinfect, poultry should be excluded from these areas for a minimum of 42 days to allow natural ultraviolet radiation to destroy any residual virus. The period of exclusion should be longer in cold weather.
- Spraying of disinfectants on vegetated outdoor areas or soil is of limited value due to the inactivation of these chemicals by organic material. Removal of surface soil is not normally recommended unless it is heavily contaminated with faeces. Dead birds and their faeces should be buried.
- As such as possible seek assistance from your local agriculture authority on how to bury dead birds safely.
- When burying dead birds or their faeces, try to avoid generating dust. Spraying or sprinkle water to dampen the area first. Bury bird carcass and faeces at a depth of at least 1 meter.
- When the dead birds and their faeces have been properly disposed, clean all areas very well with detergent and water.
- Influenza viruses are relatively susceptible to a variety of detergents and disinfectants.
- Contaminated protective clothing should be properly handled or disposed.
- After the area has been cleaned, remove all the protective materials and wash your hands with soap and water.
- Wash clothes in hot or warm soapy water, Hang them in the sun to dry.
- Put used gloves and any other disposable materials in a plastic bag for safe disposal.
- Clean all reusable items such as rubber boots and glasses/goggles with water and detergent, but always remember to wash your hands after handling these items.
- Items that cannot be cleaned properly should be destroyed.
- Shower/wash body using soap and water. Wash your hair.

- Take care not to re-contaminate yourself or the cleaned area by avoiding contact with dirty contaminated clothes and items.
- Most importantly wash your hands every time after handling any contaminated items, Footwear should also be decontaminated.
- After walking around areas that may be contaminated (such as farms, markets or backyards with poultry), clean your shoes as carefully as possible with soap and water.
- When cleaning shoes, make sure that you do not flick any particles into your face or on your or on your clothes. Wear a plastic bag over your hands, shield your eyes by wearing glasses or goggles and cover your mouth and nose with a cloth.

Additionally during an epidemic avoid going to poultry farms and markets where birds are sold. This infection affects predominantly the respiratory tract (breathing passages) in humans and it is acquired by inhalation of the virus. There is a greater risk of getting bird flu while handling the infected bird or infected eggs than eating these foods. If the food is well cooked there is hardly any risk of getting bird flu. Frequent and thorough hand washing is to be observed to avoid getting the disease during processing and cooking of possibly infected poultry. Remember that this virus can survive for long periods of time when the temperature is cold. It can survive longer at freezing temperatures. Therefore, frozen food may have the virus on them. Thorough and frequent hand washing especially by food handlers at home and in restaurants should become a routine practice to help wash out the virus from the hands.