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Office of the Director General
Department of Animal Production and Health

15th March 2006

All Provincial Directors of Health Services,
Provincial Directors of Animal Production & Health
Deputy Provincial Directors of Health Services,
Veterinary Investigation Officers,
Animal Quarantine officers,
District veterinary Surgeons,
Heads of Institutions,
Regional Epidemiologists,
Port Health Medical Officers,

Joint Circular on Guidelines for Collection and Transport of Specimens

Avian influenza is an infectious disease of birds caused by type A strains of the influenza virus. The disease, which was first identified in Italy more than 100 years ago, occurs worldwide.

All birds are thought to be susceptible to infection with avian influenza, though some species are more resistant to infection than others. Infection causes a wide spectrum of symptoms in birds, ranging from mild illness to a highly contagious and rapidly fatal disease resulting in severe epidemics. The latter is known as “highly pathogenic avian influenza” (HPAI). This form is characterized by a sudden onset, severe illness, and rapid death of affected birds/flocks, with a mortality rate that can approach 100%.

All known subtypes of influenza A virus cause infection in aquatic avian species, thus providing an extensive reservoir of influenza viruses. From time to time these viruses spill over from this natural causing outbreaks of disease in other species.

Migratory waterfowl – most notably wild ducks – are the natural reservoir of avian influenza viruses, and these birds are also the most resistant to infection. Domestic poultry, including chickens and turkeys, are particularly susceptible to epidemics of rapidly fatal influenza.

Direct or indirect contact between domestic flocks and wild migratory waterfowl has been implicated as a frequent cause of epidemics in poultry populations. It is generally

accepted that wild birds act as reservoirs for many of the avian influenza subtypes which can be transmitted to domestic populations of birds and to commercial poultry. Live bird markets can also play an important role in the spreading avian influenza viruses.

The quarantining of infected farms and destruction of infected or potentially exposed flocks are standard control measures aimed at preventing spread to other farms and eventual establishment of the virus in a country's poultry population. Apart from being highly contagious, avian influenza viruses are readily transmitted from farm to farm by mechanical means, such as by contaminated equipment, vehicles, feed, cages, or clothing. Highly pathogenic viruses can survive for long periods in the environment, especially when temperatures are low. Stringent sanitary measures on farms can, however, confer some degree of protection.

At present, there is no concrete evidence to confirm sustained human-to-human transmission of avian influenza. The influenza virus type A and its various subtypes can, in the presence of another influenza virus, merge with it through mixing and re-assortment. This can result in a new virus with different characteristics than the parent viruses, to which the population has no immunity. These new viruses can lead to human-to-human transmission of a severe disease resulting in a pandemic situation which will be a cause for extreme concern.

All reported human cases so far have been linked to direct exposure to dead or infected birds or contaminated surfaces. A few exceptional cases have been associated with food preparation. No cases have been reported following consumption of properly cooked meat or eggs.

Suspicions that human-to-human transmission may have taken place usually arise when cases occur close together in time and place among persons, such as family members or health care workers, known to have had close contact with a case. Such clusters of cases have been detected on several occasions during the 2004 outbreaks. All such instances involved family members.

Collection of specimens of sick/dead birds for investigation

When a MOOH or any other health worker is informed of a dead/sick birds, it should be brought to the notice of the area veterinary officer. Collection of specimen should always be carried out with the guidance of a veterinary officer. When a veterinary officer cannot be contacted the MOH/area PHI should attend to it with the guidance of the Regional epidemiologist/District veterinary surgeon, Epidemiologist/Director General of Animal production and health adhering strictly to the guidelines.

Provincial/District/MOH level committees for IEC activities on AI and Coordination of action

The Regional Epidemiologist/MOOH should take action to form the above committees at different levels in collaboration with relevant area veterinary officer/other relevant officials of other ministries/departments/organizations to function as a joint team, with regard to AI preparedness.

Avian Influenza in Birds / Animals

Collection of Specimens from Birds / Animals
The success of virus detection largely depends on the quality of the specimen and the condition for transport and storage of the specimen before it is processed in the laboratory.

Suspected Cases in a Poultry Farm
Carcasses to be handled with care and usage of protective items including gloves and masks recommended. Carcasses have to be put inside a thick polythene bag, air-tightened and subsequently placed inside another thick polythene bag. This has to be kept inside a rigiform box having ice and transported to the relevant Veterinary Office or Veterinary Investigation Centre.

Dead Birds in other Places
Dead birds found in other places to be reported to the Veterinary Surgeon in that area. Alternatively, this can be collected with proper sanitary measures in double layered thick polythene bags and transported to the relevant Veterinary Office.

Samples for Laboratory diagnosis
Veterinary Investigation Officers to collect appropriate samples as shown below and to transport the same to the Veterinary Research institute.

Dead Birds:
Intestinal contents (faeces) / cloacal swabs
Oropharyngeal swabs
Samples from trachea, lungs, air sacs, intestines, spleen, kidney, brain, liver and heart.

Live Birds:
Tracheal and clocal swabs
In case of small birds collection of faeces is suggested as swabbing may harm the birds. At least 1gm of faeces to be collected and placed in Phosphate Buffer Saline(PBS) – pH 7-7.4

Transport and Storage of Specimen
Specimens should be collected and transported in a suitable transport medium with antibiotics in ice. Cell Culture Medium, Phosphate Buffered Saline, Tryptose-Phosphate Broth, 50% Glycerol Saline are commonly used transport media. They should be

supplemented with protein, such as Bovine Serum Albumin (BSA) or Gelatin, to a concentration of 0.5% to 1%, to stabilize the viruses.

Materials Required for Transport of Specimen
1-3ml Plastic Screw Capped Tubes
Sterile Cotton Swabs
Viral Transport Medium
Instrument for Post Mortem Examination

Preparation of Sample Vials
To sterile vials dispense 1-2ml of transport media.
It is preferable to store these media at -20^{o}C until used, but they can be stored at 4^{o}C (or room temperature for short period of 2-3 days)

All the necessary information (type of animal sampled, species, type of sample, date, time, location of sample, number of deaths) should accompany the specimen.

Clinical specimens should be collected as described below and added to transport medium.

Tracheal Swabs
The trachea of live birds is swabbed by inserting a dry cotton swab into the trachea and gently swabbing the wall, and the swab is placed in transport medium as above.

Storage of Carcasses of Birds
The Storage of Carcasses should be done in -20^{o}C in a deep freezer in a refrigerator. However it is advised to send the carcasses to the diagnostic lab without any delay.

Infection control precautions for HPAI

Infection control for HPAI involves a two-level approach:

- Standard precautions which apply to ALL patients at ALL times, including those who have HPAI; and

- Additional precautions which should include:

    - droplet precautions,
    - contact precautions (WHO4), and
    - high-efficiency mask and negative pressure room if possible.

Standard precautions

Treating all patients in the health care facility with the same basic lever of “standard” precautions involves work practices that are essential to provide a high level of protection to patents, health care workers and visitors.

These include the following:

- hand washing and antisepsis (hand hygiene);
- use of personal protective equipment when handling blood, body substances, excretions and secretions;
- appropriate handling of patient care equipment and soiled linen;
- prevention of needlestick/sharp injuries;
- environmental cleaning and spills-management; and
- appropriate handling of waste.

Additional (transmission-based) precautions

Additional (transmission-based) precautions are taken while still ensuring standard precautions are maintained. Additional precautions include:

- droplet precautions; and
- contact precautions. (including the use of high efficiency masks – negative pressure rooms if possible)

A combination of these precautions will give the appropriate level of precaution for HPAI. The precautions should be implemented while the patient is infectious.

- Adults > 12 years of age – precautions to be implemented at time of admission and continued until 7 days have lapsed since onset of symptoms.
- *Children ≤12 years of age – precautions to be implemented at time of admission and continued until 21 days have lapsed since onset of symptoms.* *Shedding of virus can be at high titres for up to 21 days in young children.

Transportation of patients

Limit the movement and transport of patients from the isolation room/area for essential purposes only. If transportation is required out of the isolation room/area within the hospital, the patient should wear a mask and a gown where possible. All staff transportation outside the health care facility is required, the patient should wear a surgical mask and gown and where there is contact with surfaces, these surfaces should be cleaned afterwards. For example, if a patient has been transported in an ambulance, the ambulance may be cleaned inside with a disinfectant such as 70% alcohol or with bleaching solution(0.5%).(WHO 2006 infection control guide lines)

Specimen collection for human samples

Following **standard precautions**, all specimens should be regarded as potentially infectious and staff should adhere rigorously to protective measures in order to minimize exposure.

Collection of human specimens for avian influenza infection

Respiratory virus diagnosis depend on the collection of high-quality specimens, their rapid transport to the laboratory and appropriate storage before laboratory testing. Virus is best detected in specimens containing infected cells and secretions. Specimens for the direct detection of viral antigens or nucleic acids and virus isolation in cell cultures should be taken preferably during the first 3 days after onset of clinical symptoms**. Soon after the collection of specimens they should be packed in ice or stored at 2-8°C until transported to the laboratory. If specimens cannot be processed within 48-72 hours,they should be kept at or below -70°C.All the samples should be labeled and sent with a request form.**

Type of specimens

A variety of specimens are suitable for the diagnosis of virus infections of the upper respiratory tract,

Nasal swab
Nasopharyngeal swab
Nasopharyngeal aspirate
Nasal wash
Throat swab

In addition to swabs from the upper respiratory tract, invasive procedures can be performed for the diagnosis of virus infections of the lower respiratory tract where clinically indicated.

Transtracheal aspirate
Bronchoalveolar lavage
Lung biopsy
Post-mortum lung or tracheal tissue

Specimens for the laboratory diagnosis of avian influenza A should be collected in the following order of priority.

Nasopharyngeal aspirate
Acute serum
Convalescent serum

Specimens for direct detection of viral antigens by immunofluorescence staining of infected cells should be refrigerated and processed within 1-2 hours. Specimens for use with commercial near-patient tests should be stored in accordance with the manufacturer's instructions. Specimens for virus isolation should be refrigerated immediately after collection and inoculated in to susceptible cell cultures as soon as

possible. If specimens cannot be processed within 48-72 hours, they should be kept frozen at or below –70C.

Respiratory specimens should be collected and transported in virus transport media. A number of media that are satisfactory for the recovery of a wide variety of viruses are commercially available.

Procedures for specimen collection

Materials required

Sputum/mucus extractor
Suction machine
Polyester fibre-tipped applicator
Plastic vials with VTM
Tongue depressor
Sterile cotton swabs

Preparing to collect specimens

Clinical specimens should be collected as described below and added to transport medium. Nasal or nasopharyngeal swabs can be combined in the same vial of virus transport medium. When possible, the following information should be recorded on the request form: general patient information, type of specimens, date of collection, and contact information of person completing the form, etc.

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. Specimens from both nostrils are obtained with the same swab. The tip of the swab is put into a vial containing 2-3ml of virus transport medium and the applicator stick is broken off.

Nasopharyngeal Swab

A flexible, fine-shafted polyester swab is inserted into the nostril and back to the nasopharynx and left in place for a few seconds. It is then slowly withdrawn with a rotating motion. A second swab should be used for the second nostril. The tip of the swab is put into a vial containing 2-3ml of virus transport medium and the shaft cut.

Nasopharyngeal Aspirate

This is the best method of collection of specimens for diagnosis of avian influenza.

Nasopharyngeal secretions are aspirated through a catheter connected to a mucus trap and fitted to a vacuum source. The catheter is inserted into the nostril parallel to the palate. The vacuum is applied and the catheter is slowly withdrawn with a rotating motion. Mucus from the other nostril is collected with the same catheter in a similar manner. After mucus has been collected from both nostrils, the catheter is flushed with 3ml of transport medium.

Nasal Wash

Materials required

Sterile screw capped specimen cups

Petri dish

The patient sits in a comfortable position with the head slightly tilted backward and is advised to keep the pharynx closed by saying "k" while the washing fluid (usually physiological saline) is applied to the nostril. With a transfer pipette, 1-1.5ml of washing fluid is instilled into one nostril at a time. The patient then tilts the head forward and lets the washing fluid flow into a specimen cup or a Petri dish. The process is repeated with alternate nostrils until a total of 10-15ml of washing fluid has been used. Dilute approximately 3ml of washing fluid 1:2 in transport medium.

Throat Swab

Both nostrils and the posterior pharynx are swabbed vigorously, and the swab is placed in transport medium as described above. (WHO 2006)

Transportation of specimens

Specimens for transport must be placed in leak-proof specimen bags, which have a separate sealable pocket for the specimen (i.e. a **plastic biohazard specimen bag**.) or Ziplock bags could be used as an alternative. Personnel who transport specimens should be trained in safe handling practices and decontamination procedures in case of a spill.

The accompanying request form should be clearly marked as "suspected or probable HPAI" and the laboratory notified by telephone that the specimen is "on its way." Specimens should be hand delivered where possible.

All MOOH are advised to contact the Divisional Veterinary Surgeons in case of need for any animal laboratory investigation. It is the responsibility of the MOH to guide the public health staff and the public on animal laboratory surveillance when such a need arises.

For details, please contact Director General, Department of Animal Production and Health / Veterinary Research Institute (tel: 081-22388195, fax: 081-2388619), Animal quarantine office, tel 2448683-Epidemiologist, Epidemiological Unit (tel: 011-2695112, 2681548,4740490,4740492,fax: 011-2696583), Molecular Medicine Unit, University of Kelaniya (tel: 011-2960483).

Director General of Health Services
Ministry of Health and Nutrition

Director General
Department of Animal Production and Health

District Emergency Team - DAPH

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