

THE REPUBLIC OF TURKEY
MINISTRY OF AGRICULTURE AND RURAL AFFAIRS
General Directorate of Protection and Control

INSTRUCTION ON PROTECTION FROM AND
FIGHT AGAINST
AVIAN INFLUENZA

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INSTRUCTION ON PROTECTION FROM AND FIGHT AGAINST AVIAN INFLUENZA

1- INTRODUCTION

Avian influenza is disease which causes significant economic losses all over the world and is considered to be one of the important viral diseases endangering the fowl breeding sector. It bears importance to provide information to the Turkish Veterinarians, to protect poultry health in Turkey, to fight with this disease and to ensure that all precautions are taken against avian influenza, which has not been in Turkey until now, which is an acute, zoonosis poultry disease that endangers poultry health and develops with high mortality.

In Paragraph (i) of the Regulation on Amendment of Article 116 of the Regulation on Animal Health Security No 3285, the provision “In fight against avian influenza concerning issues not addressed in this Regulation action is taken in compliance with the Regulation on Protection from and Fight against Avian Influenza” is stipulated. This Regulation was prepared within the framework of the said Regulation.

2 –GENERAL INFORMATION ON THE DISEASE

2.1 DEFINITION OF THE DISEASE

Avian influenza is an acute disease caused in poultry animals by A type Avian influenza virus of the Orthomyxoviridae family. It develops with high mortality together with indications of the respiratory and nervous system.

2.2 SIGNIFICANCE OF THE DISEASE

Influenza viruses are observed in human, pig and horse populations in mild climates and in regions near the poles in especially winter, and all the year around in tropical and subtropical regions. However, in poultry and sea mammals influenza epidemics may outbreak anytime. The causative agent of avian influenza, which is a fatal disease, has been isolated in many countries. In the last decade, this disease has occurred in Mexico, Australia, Hong Kong and Italy. There is no similarity in subtypes between these types. This proves that genetic mutations as well as horizontal infections play a significant role on the effectiveness and virulence of the disease. Following the problems in Italy, it has been shown that genetic mutations can cause big problems. In past years, outbreaks have also been seen in the United States of America, Canada, France, Russia, Israel, Germany, Hungary, the Republic of Czechoslovakia, Slovenia, New Zealand and India.

The virus has haemagglutination and haemadsorption characteristics. All strains of the virus grow in the hen egg containing an embryo and kill the embryo. In the infected embryo, prevalent haemorrhages occur. The virus can also grow in tissue culture. This virus is typed according to its ribonucleoprotein antigens. In poultry, more than 80 different influenza viruses, having different properties have been isolated. Among these, the most pathogenic ones are Avian Influenza virus (H7N7), Turkey Influenza virus (H6N2, H8N4), Hen Scot/59 (H5N1), Tern/South Africa (H5N3).

The virus has A, B and C antigenic types. Types B and C cause diseases only in humans. As the findings reached up to now imply, type A cause respiratory track infections in humans, pigs, horses, whales, American bison and poultry. Type A viruses are divided into subtypes depending on Haemagglutinin (HA) and Neurominidase (NA) antigenic structures. 15 different Haemagglutinin (HA) and 9 different Neurominidase (NA) types are known.

Causative agent has mostly been isolated from ducks; however, it has also been isolated from turkey, hens, pheasant, domestic goose, quail, peacock, parakeet, seagull, marsh fowls, partridge, sea fowls, guinea fowl and parrot.

In disease incidences caused by highly pathogenic avian influenza viruses, generally, respiratory difficulties, excessive tear, oedema of the head and face, cyanosis and haemorrhage of the skin, cyanosis of the comb and wattles and diarrhoea are observed. However, in turkeys and hens sudden deaths without any indication may occur.

In disease forms caused by strains of medium pathogenicity, anorexia, depression and drop in egg production are observed. In egg laying animals, 75-80% loss in production and in all poultry animals, 10-100% death is observed.

The recent outbreaks in Iran and Italy, the global structure of the poultry sector, the gene sources, vaccines and biological substances being of foreign origin increase the risk of disease incidents in Turkey.

2.3 SPREAD OF THE DISEASE

In contamination, migratory birds play an important role. Feed, water, tools and equipment and personnel contaminated with faeces and secretions of infected birds and the eggs broken in the hatchery are main reasons of spread.

Horizontal transmission of the disease (from animal to animal) is the most prevalent contamination form. Although there is no certain evidence related to vertical transmission (from hen to the chick via the egg), the agent has been identified in shells of the eggs obtained from infected animals.

Transmission of the virus by the air is restricted to a few kilometres. In addition, the disease is transmitted mechanically from infected animals to sensitive ones by blood sucking flies and rodents. The incubation period may change from a few hours to 2-3 days.

3. DIAGNOSIS OF THE DISEASE

Clinical symptoms, anamnesis, autopsy findings are not sufficient for exact diagnosis. Definite diagnosis of the disease is possible by isolation and identification of the virus in the laboratory. Avian influenza may be confused clinically with infectious bronchitis, infectious laryngotracheitis, fowl cholera, Newcastle disease, microplasma infections and some forms of Marek disease.

3.1 EPIDEMIOLOGICAL INFORMATION

The emergence day of the disease, age of the animals, filiation of the disease, to which breeder it belongs, the number of animals infected and dead, the vaccines applied, application date and method, egg production rate, feed consumption and hygienic conditions of the coop are determined.

3.2 CLINICAL EXAMINATION

The incubation period is short. The disease shows itself generally within 24-36 hours. The infected animals die within 1-7 days. In infected animals the case temperature rises, the feathers are ruffled and severe diarrhoea is observed. Egg production decreases, even stops. The infected animals lose their appetite, their eye lids may close; conjunctiva is swollen and red. Characteristic oedema and cyanosis is formed in the comb and wattles and around the eyes. Oedema may diffuse to the neck and abdomen. Oedema in the glottis may cause respiratory difficulty that may lead to suffocation. Together with respiratory difficulty, a greyish and bloody exudate is secreted from the nasal holes.

It is especially noteworthy that the respiratory track is clogged in turkey poult. In case a sudden fright occurs among the animals, mass deaths may occur. The infected animals usually die within 2 days. In animals that come through the acute phase, nervous disorders including excitation, convulsions, turning and ataxia and clinical indications such as incoordination, not being able to walk or stand up are observed. In addition to the decrease in egg production, decolourization, quality deformation and thinning of the egg shell and production of eggs without shells are noteworthy factors.

In addition to suddenly emerging respiratory and digestive system problems, high temperature might remind the Newcastle disease. Avian influenza can not be diagnosed by clinical indications.

3.3 AUTOPSY FINDINGS

Pathological findings change according to the virulence of the virus and the poultry species. Stiffening immediately occurs following death. In sudden deaths, lesions can not be identified; in advanced stages dehydration, haemorrhage in muscles, including gizzard fat, and internal organs, subcutaneous oedema of the head and neck, fluid exit from the nares and oral cavity, petechia on the inner lining of the sternum, haemorrhage in kidneys, ovaries, proventriculus, gizzard and especially in the intestinal mucosa. Removing skin from the carcass will show a clear straw-coloured fluid in the subcutaneous tissues. Blood vessels are usually engorged. In the liver, spleen and kidneys, foci from yellow to grey, haemorrhage and necrotic degenerations are observed. Especially in events with long term infection, sinusitis, fibrinous pericarditis and peritonitis are seen. In laying hen, haemorrhage or degeneration together with necrotic foci are seen.

3.4 LABORATORY ANALYSES

For a definite diagnosis of the disease, laboratory analyses are required.

3.4.1 Virus isolation and identification

The specimens taken from dead poultry should include tracheal, cloacal and oro-nasal swabs. Samples taken from lungs, air sacs, intestines, spleen, brain, ovary and the heart can be treated together or separately. In small and delicate poultry, swab sampling may damage the animal; thus, faeces might be an adequate alternative.

The specimens should be taken into isotonic phosphate buffered saline (PBS), containing antibiotics and with pH 7-7.4. The antibiotic may change according to local conditions. For tissues and tracheal swabs, penicillin (2000 units/ml), streptomycin (2 mg/ml), gentamycin (50 mg/ml) and mycostatin (1000 units/ml) should be used. For faeces and cloacal swabs, these concentrations should be increased five times. After the addition of antibiotic, the pH of the solution should again be set to 7-7.4. The concentration of faeces and particulate tissues should be 10-20% w/v in the antibiotic solution. The suspensions should be kept at room temperature for 1-2 hours and then must immediately treated. If this is not possible, they may be stored at +4°C for a few days. If they should be stored as frozen, this should be done at -80°C.

The method preferred to grow avian influenza virus is inoculation into eggs containing SPF (specific pathogen free) embryos. The supernatant obtained by centrifuging faeces and tissue suspensions at 1000 rpm is inoculated into the allantoic gap of the eggs containing SPF embryos. These eggs are incubated at 35-37°C for 4-7 days. The allantoic fluid of eggs containing dead or dying embryos and at the end of the incubation period, of all eggs should be tested with respect to haemagglutination activity after they

are cooled to +4°C. The existence of Haemagglutinin (HA) activity shows probable infection by influenza A or an avian paramyxovirus. Fluids giving negative results should be repeated at least once in the egg.

The existence of influenza A virus is confirmed by determining nucleocapside or matrix antigen by Agar Gel Immuno-Diffusion (AGID) test. These two antigens are common for all influenza A viruses. The antigen used in this test is obtained by concentrating the infected allantoic fluid or extraction of corio-allantoic membrane. The antigen prepared is tested with known positive and negative sera. Avian influenza virus grows in a limited number of cell cultures; in isolation studies, of primary cell cultures, chick embryo fibroblast (CEF) cell culture or MadinDarby Canine Kidney (MDCK) cell lineage, which is a continuous cell lineage are also used. If avian influenza virus is isolated, it should be eradicated from the flock as soon as possible. The virulence of the isolates strain should be determined to identify the most suitable strategy. Because of this, the isolated viruses should be subjected to pathogenicity tests:

3.4.1.1 Pathogenicity tests

Highly pathogenic avian influenza viruses cause over 75% mortality within 8 days in 4-8 week chicken following intravenous/intramuscular (IV/IM) injection. However, viruses having low pathogenicity in the laboratory may high pathogenicity capacity in the field through mutation. Thus, such viruses are called potential pathogen viruses.

The International Epidemics Office (OIE) has added the following criteria in classifying highly pathogenic avian influenza viruses:

- a) Of 8 sensitive chicks of 4-8 weeks, death of 6-7 within 10 days after intravenous inoculation of 0.2 ml amnioallatoic fluid containing sterile virus.

- b) If the isolates kill 1-5 chicks and are not H5 or H7 subtype, the following additional tests are required:
- Cytopathic effect in tissue culture, or
 - Plaque formation in the absence of trypsin. If growth is not observed, it is decided that the isolate is not highly pathogenic avian influenza (HPAI) virus.

If it is observed that the viruses other than the H5 and H7 subtypes grow in cell cultures not containing trypsin, amino acid sequences of Haemagglutinin peptide bonds should be determined. If the result is the same as that of the HPAI viruses, the tested strain is highly pathogenic.

Additionally, intravenous pathogenicity index test (IVPI) should also be conducted. If the intravenous pathogenicity index test (IVPI) is > 1.2 in 6 week-old chicks indicates that the strain is highly pathogenic.

3.4.1.2 Intravenous Pathogenicity Index Test (IVPI)

The infected amnioallantoic fluid is diluted with sterile phosphate buffered saline (PBS) to 1/10 and 0.1 ml of this is injected intravenously to 10 chicks of 6 weeks old. The chicks are monitored for 10 days. In these observations, each chick is scored as given below:

Normal	: 0
Ill	: 1
Severely ill	: 2
Dead	: 3

Normally, an “ill” chick should show one of the below symptoms, and a “severely ill” chick should show more than one of the below symptoms. These symptoms are respiratory labouring, depression, diarrhoea, cyanosis, oedema of the head and face and nervous symptoms.

Individual deaths should be evaluated according to score 3. The average of the observations of a chick gives the IVPI. If the index is 3, all the chicks died within 24 hours. If the index is 0.00, none of the chicks show symptoms of the disease within the 10 day-observation period.

3.4.2 Serological tests

Agar gel Precipitation (AGP), Haemagglutination Inhibition (HI), Virus Neutralization (VN) and Enzyme Linked Immuno Sorbent Assay (ELISA) tests are made use of. Especially in the first isolation, the HI test bears practical significance to eliminate the probability of the Newcastle disease. Serological tests are especially used in epidemiological studies and determination of epidemics under field conditions.

3.4.2.1 Agar Gel Precipitation Test (AGPT)

This is a widely used test inn diagnosis of avian influenza. All avian influenza viruses have common nucleocapside and matrix antigens. This test allows for the determination of presence or absence antibodies formed against any influenza virus. In the test, concentrated antigen containing nucleocapside and matrix is used. The matrix antigen diffuses more rapidly than the nucleocapside antigen. AGP test is used widely in routine in determination of infection in turkey or chicken flocks by identification of specific

antibodies. Usually, preparations obtained from corio-allantoic membrane of the hen eggs containing embryo and enriched in nucleocapside. For this, infected hen eggs, 10 day-old, containing embryo, are homogenized; frozen and melted three times; and centrifuged at 1000 rpm. The supernatant liquids are inactivated by adding 0.1% formalin or 1% betapropiolactone, re-centrifuged and used as antigen. Some poultry species may not produce precipitating antibodies following infection with influenza viruses.

The tests are conducted generally by using a solution of 1% agarose (w/v) (mega purified agar) and 8% sodium chloride (w/v) in 0.1 M phosphate buffer (pH 7.2). the nutrient medium is poured into Petri dishes or on microscopic slides to have a thickness of 2-3 mm. Using a template and a piercer, holes of 5 mm diameter with distances of 2-5 mm between them are opened. Each suspected serum must be adjacent to a known positive serum and n antigen. This will establish a continuous precipitation line between the known positive serum, the suspected serum and the ribonucleoprotein antigen. Into each hole, approximately 0.050 ml reagent should be put.

The precipitation lines become identifiable after 24-48 hours, but this also depends on the concentration of the anticase and the antigen. The precipitation lines formed can best be observed on black background lit from the behind.

If the precipitation lines formed between known positive sera connect to the lines between the test hole and the antigen, the result of the test is positive. Crosswise lines are interpreted as the absence of specific antibodies in the test serum in the positive control hole.

3.4.2.2 Haemagglutination (HA) and Haemagglutination Inhibition (HI) Tests

Variations of HI and HA test procedures may be used in different laboratories. In the examples below represent tests, in which V-based plates are used and for which the final volume is 0.075 ml. the necessary reagents for these tests are the following:

Isotonic PBS (0.1 M pH 7.0-7.4)

Washed hen erythrocytes 1% (v/v)

Positive and negative sera

Haemagglutination antigen

a. Haemagglutination (HA) Test

1. Into each cell of the 96 celled U or V based microplates 0.025 ml PBS is put.
2. Into the first cell, 0.025 ml virus suspension (infective allantoic fluid) is put.
3. The virus is diluted **to twice**.
4. Again 0.025 PBS is put into the cells.
5. 0.025 ml of 1% hen erythrocyte is placed into all cells.

6. The plate is shaken slightly and left for 40 min for the erythrocytes to precipitate (at room temperature of 20°C, or in locations where the air temperature is higher at +4°C), until the control erythrocytes form an obvious button.
7. The HA value is determined. The highest serum dilution that gives a complete HA is considered as the titre. This represents 1 HA unit.

b. Haemagglutination Inhibition (HI) test

1. Into each cell of the 96 celled U or V based microplates 0.025 ml PBS is put.
2. Into the first cell, 0.025 ml serum is put.
3. The virus is diluted **to twice** until the 12th cell.
4. Into each cell, 0.025 ml of the virus diluted 4 HA units and left for 30 minutes at room temperature (20 °C) or for 60 minutes at 4°C.
5. Into each cell, 0.025 ml of 1% hen erythrocyte is placed. After mixing slightly, the plate is left for approximately 40 min for the erythrocytes to precipitate and until an obvious button formation is observed in the control cell (at 20°C or when the air temperature is higher at 4°C).
6. The highest serum dilution that inhibits the antigen diluted 4 HA units is the HI titre. Exact determination of agglutination is assessed by tilting the plate. It is assumed that the cells which are in the same condition as the control cell containing 0.025 ml erythrocyte and 0.05 ml PBS indicate inhibition.

7. The validity of the results should be assessed according to the positive and negative sera. Negative control serum should be in a known dilution titre.

Some sera obtained from poultry species other than chicken may have a non-specific haemagglutination effect for hen erythrocytes. These agglutinins may be taken out by absorption hen erythrocytes of the test serum or the erythrocytes of the related poultry species may be used.

3.4.2.3 Neurominidase Inhibitor (NI) Test

NI test is used to identify the other important surface antigens of the influenza virus. This is a complex test, compared by the HI test.

Neurominidase subtypes are made using antisera prepared against 9 known neurominidases.

For definite identification of the avian influenza isolate, purified neurominidase and haemagglutinin antigens are necessary.

3.4.2.4 Virus Neutralization (VN) Test

Although this is a sensitive method in determining anticase titres against avian influenza virus, it is not practical for routine applications because it is expensive and depends on intense labour. The test is conducted by alpha and beta methods. Since the antibodies used to neutralize the virus are prepared against surface antigens, results of the virus neutralization test have similar specificity with the haemagglutination inhibition test. In the test, SPF hen eggs with 9-10 day embryos. Following inoculation, the eggs are incubated for 3-4 days at 37 °C. Embryo deaths, haemagglutination activity or both are considered to be the indication of embryo infection.

3.4.2.5 Enzyme Linked Immuno Sorbent Assay (ELISA) Test

Indirect ELISA test, which allows testing of a high number of serum samples and which has high specificity and sensitivity, is used in serological diagnosis of the disease in hens and turkeys.

4. PRINCIPLES OF SENDING PATHOLOGICAL MATERIAL TO THE LABORATORY

The necessary epidemiological information on the coops in which the disease emerged; name, address, raising type and capacity of the establishment; date of emergence and symptoms of the disease; autopsy findings; the number of animals that have died, that are ill or infected; the duration between death and sampling of the infected material; the infected materials sent, etc. should be completely written in the infected material delivery protocol.

Since the diseased or dead animals contain a high concentration of virus, to prevent contamination, the autopsy should be conducted under special conditions, taking the necessary measures. It is preferred that the animals are sent to the laboratory as a whole, without conducting an autopsy. When the animals are sent to the laboratory as a whole, the diseased animals that show clinical symptoms should be delivered in cages, the sides and bottom of which are closed and the dead animals should be delivered in plastic bags, in ice boxes or dry ice, within at the latest 12 hours. When the animals are sent to the laboratory not as a whole, the trachea, lungs, spleen, kidneys, heart, liver, ovaries, brain and intestines of the animals showing the symptoms of the disease should be taken out under aseptic conditions, and put into jars containing 10% formol for histopathological analyses and physiological saline with 50% glycerine for other analyses.

When swabs are taken, caecal and tracheal swabs taken at least from 5 animals should be placed into their special tubes containing transport medium.

For serological analyses, blood should be taken from 1% of the flock. The number of serum samples sent from low capacity establishments should not be less than 20. After the blood taken coagulates, they should be scratched to get the serum and if possible the sera should be sent.

5. PROTECTION

5.1. TAKING PROTECTIVE MEASURES

There is no treatment for avian influenza. It is among the notifiable diseases in many countries and is based on state control. In order to increase the resistance of the flock vitamins are added to their feed and water, and antibiotics are added to their feed to prevent secondary infections.

For protection against the disease, animals should not be imported into and exported out of the flock. Entrance of free living fowls and rodents into the flock should be prevented; no person other than the caretakers should be allowed into the coops; containers with disinfectants should be placed at the entrance of the coops. The areas around the coops should be made of materials that are easy to clean and disinfect.

5.2 GENERAL HYGENIE RULES

The disease agent can survive a long time, especially in cold and humid environments. However, since the virus is surrounded by a membrane containing lipids, it is inactivated easily and stabilized by ultraviolet rays. The infectivity of the virus can be destroyed by suitable disinfectants. The virus may become inactive in hot and dry environments and at high and low pH.

The virus spreads around through nasal secretions and faeces of infected animals and remains viable for 5 weeks under coop conditions. It survives for a long time in tissues, faeces and water. Therefore, the most important problem in control of the disease is manure. The virus survives longer in the high moisture content and low temperature of faeces. In the infected manure, it remains viable for 30-35 days at 4°C; 7 days at 20°C; and 105 days in winter in wet faeces. Therefore, periodic disinfection, cleaning and ventilation of the coops, food and water hygiene; suitable temperature and moisture in the coop; clean work clothes and disinfected boots and gloves for the caretakers should be ensured.

5.3 VACCINATION

Vaccines prevent development of the clinical symptoms but can not prevent emergence of the infection. In general, when animals carrying strains of low pathogenicity are vaccinated, the probability that these strains transform into highly pathogenic strains through mutation exists. The vaccinated animals continue spreading the virulent virus and at the same time hinder serological control. Therefore, vaccination is not advised.

6. FIGHT

6.1 PROCEDURES TO BE FOLLOWED IN THE ESTABLISHMENT UNTIL THE PUBLIC VETERINARIAN ARRIVES

In cases the coops are suspected to be infected by the avian influenza virus or in case disease is observed in the coops, in compliance with the Articles 9 and 10 of the Law on animal Health Security, No 3285, the authorities informed shall immediately communicate this to the Provincial Directorate of the Ministry in provinces and to the District Directorate in the districts.

The freelance veterinarian or the veterinarian employed by an establishment is also liable to inform the outbreak. Such veterinarians should inform the public veterinarian of the suspect. As they wait for the public veterinarian to initiate, they should take all the necessary measures to prevent spread of the disease.

Until the public veterinarian arrives, the authorities and the responsible personnel in the villages, towns, cities, farms and establishments in the outbreak location take the following precautions.

- a) The diseased and the healthy poultry are kept in separate places. Entrance into and exit from the places where the diseased poultry are kept is prevented.

- b) The dead animals are retained for examination and diagnosis. If they are putrefying, they are transported to a place where animals do not dwell, without dragging. They are limed and buried into pits dug 2 metres deep.

- c) Taking out all kinds of animal feed, materials and things that belong to the diseased and that the diseased have contacted is banned.

- d) Personnel adequate in number are given duty for the care of the diseased animals. No one is allowed to enter the isolation areas other than those appointed. Contact of the personnel appointed for care of the diseased animals with healthy animals and with materials belonging to healthy animals.

- e) In villages, the village headman; in cities and towns, the municipality, the ward headman and animal owners; in farms and establishments, animal owners, establishment manager and caretakers are responsible of implementation of the measures taken.

6.2 PROCEDURES TO BE FOLLOWED BY THE PUBLIC VETERINARIAN BEFORE GOING TO THE OUTBREAK LOCATION

- a) When the public veterinarian is informed of a suspect concerning avian influenza, he determines the identification of the informing person. If this person is the animal owner, the public veterinarian inquires the following particulars:
- The place of the establishment, raising type and intensity,
 - The persons and motor vehicles in the establishment,
 - Whether factors that may cause contamination such as vehicles, animals or people have entered into the establishment recently,
 - Whether there are disinfectants and disinfection equipment in the establishment.
- b) The public veterinarian gives instructions to the informing person to stop movement of the persons, animals and things in the suspected establishment. In addition, the public veterinarian conducts the followings:

- He informs the Regional Institute Directorate.
 - He takes the equipment necessary for sampling infected material with him.
 - He contacts the disinfection team and gives information on appropriate disinfectants.
- c) The public veterinarian, after being informed of the outbreak, goes to the outbreak location using the possible quickest means, within 24 hours. The Provincial or the District Director is liable to supply such means. When the Provincial or the District Directorate does not have the necessary possibilities, the civilian and local administrations and the Animal Health Security are liable to provide all the necessary help and facilities.
- d) The public veterinarian, after being informed of the outbreak, shall make the necessary preparations and then go to the location of the incident. He shall show the necessary meticulousness to park the vehicle he takes to an appropriate place or distance outside the establishment.
- e) The establishment should be entered after wearing the disposable garments kept available at the establishment. At the point where the clothing is changed, a box made of cardboard or another impermeable, appropriate material, 2 plastic bags of adequate size, appropriate disinfectant solution and a pair of gloves made of latex should be kept ready.

6.3 PROCEDURES TO BE FOLLOWED WHEN THE PUBLIC VETERINARIAN ARRIVES AT THE ESTABLISHMENT

The public veterinarian should conduct the followings when he arrives at the establishment.

- a) In order to take over the disease until the laboratory results arrive, the Arrest Minutes in Annex 1 is filled and signed. Until definite diagnosis is given and the necessary measures are taken, the contact of the animals with the surrounding areas is prevented. The public veterinarian inspects the animals within temporary band and the measures taken. He supervises the burial of the animals that have died or putrefied until then. Such animals should be moved appropriately to location where animals do not dwell, which are not near streams, and which shall not contaminate ground waters. The dead poultry are limed and buried in pits at least 2 metres deep. The public veterinarian ensures that the diseased and suspected poultry are kept in separate places and takes measures to prevent the spread of the suspected infection. He ensures that the coops, feed containers and drinkers are disinfected. He ensures that antiviral disinfectants are added to the drinking water of animals.

- b) The public veterinarian takes samples of the infected materials to be sent to the laboratory. When sampling the infected material, he should wear a hat, goggles and gloves, considering that the disease has a zoonotic character.

- c) The public veterinarian restricts the number of personnel in the establishment. He prevents contact of the caretakers with the other coops. He makes the personnel in the establishment submit a written commitment, an example of which is given in Annex 2 on not contacting other receptive (sensitive) animals for 3 days.

- d) If possible, vehicles should not be allowed into the establishment. If it is necessary for the vehicles to enter, the public veterinarian shall specify appropriate points in the establishment for washing and disinfecting the vehicle at the exit. For disinfection of such vehicles, a point where the washing water used in the process does not mix into the water ways. The vehicles of the establishment shall be disinfected from the outside and if possible on the inside.

- e) The public veterinarian shall specify appropriate points for washing and disinfecting of the personnel at the exit. He gives the necessary instructions for the personnel and his shoes to be washed at the exit and if possible, he ensures that the personnel wear overalls. Such personnel must wash the clothes he wears as soon as he reaches his home.

- f) The public veterinarian shall not visit the other poultry farms until the suspect ends or if the suspect is verified, until 3 days after his last contact day with the infected establishment.

If an expert veterinarian is appointed to the suspected establishment by the Institute, this expert veterinarian shall take with him the necessary equipment for taking samples of infected material and assistant personnel. The assistant personnel do not enter the establishment. The vehicle used is parked away from the establishment. The establishment should be entered after wearing the disposable garments kept available.

The expert veterinarian appointed by the Institute to investigate the subject may visit only the establishments suspected of avian influenza. If he has been appointed for more than one incident within the province, he should visit the establishments starting with the weakest suspect and continuing with the stronger suspect respectively. This expert veterinarian shall not visit the other poultry farms until the suspect ends or if the suspect is verified, until 3 days after his last contact day with the infected establishment, even if there are other reasons.

When the visit to the establishment is over, the health personnel firstly disinfect their personal overalls at the point they had changed clothes. If as a result of the investigations the suspect is verified, the followings shall be conducted:

- a) Disinfection of the resistant equipment that had been previously separated to be sterilized later, used and put together in a bag;
- b) Collection of all the materials that can not be disinfected (for example materials that are made of paper and shall be used again) in transparent plastic bags;
- c) Collection of overalls and all kinds of material to be destroyed in an appropriate plastic bag that will be kept at the establishment at that moment.

The expert veterinarian from the Institute shall take with him the necessary materials and equipment. He takes the necessary measures to reduce the infection in the establishment to the lowest level, conducts the necessary investigations and ensures that the samples taken are transported in the shortest time possible.

When there is suspicion related to virulent avian influenza or when the disease is observed, the materials and blood samples taken from diseases or dead animals for diagnosis and the infected material protocol, completed with accurate particulars, are sent to the laboratory in the quickest way, if possible within 12 hours, in the appropriate form.

6.4 PROCEDURES TO BE FOLLOWED WHEN THE OUTBREAK OCCURS

As soon as the existence of the disease is confirmed by the laboratory report, the local Animal Health Security Committee holds a meeting in accordance with Article 91 of the Regulation. In the decisions of the Animal Health Security Committee issues such as the disease outbreak report prepared by the public veterinarian public veterinarian, announcement of the outbreak, the general and border coordinates of the protective band, the items that are free, conditional or banned within the zone of the protective band, procedures to be implemented for animals sent to the slaughterhouse and for transit passages, the points where the “THERE IS AVIAN INFLUENZA HERE” shall be put, the means to inform the neighbouring villages, towns, provinces, cities and farms, the status of the animal markets and fairs if they exist, how to conduct killing and destroying and disinfection methods shall be individually and separately mentioned.

The local Animal Health Security Committee continues its studies until avian influenza is completely eradicated.

6.4.1 Issues to be considered when taking Animal Health Security Committee decisions

6.4.1.1 Announcement of the outbreak location and general measures to be taken

When the location of outbreak is being announced, around the infected establishment, the borders for a control zone of at least 3 km radius and a surveillance zone of 10 km, including the said 3 km, shall be specified. During this zoning, geographical, administrative, ecological and epizootological factors related to the disease and the control structures shall be taken into account.

6.4.1.1.1 Control zone

The control zone is the establishment, in which the disease is observed (the disease centre) and the area which includes the establishments which are assumed to have contact with this centre and suspected of contamination and/or disease. The mentioned terms are explained as follows:

Centre: This term means the establishment in which the infection is determined and verified by laboratory findings.

In the centre the quarantine and disinfection rules are applied more strictly. In the centre, taking out the poultry animals is banned and these animals are destroyed at the incident locality as far as possible. Movement of all the materials that might be contaminated shall be prevented and are decontaminated. At the same time, free living birds, which might transmit and spread the disease, shall be destroyed and controlled.

Establishment suspected of disease: These are the establishments which surround the centre and in which there are animal that show non-obvious and incomplete symptoms.

Establishment suspected of contamination: These are the establishments which have contact with the centre and which have exchanged materials effective in contamination, such as tools, equipment, feed, etc.

A circle of 3 km radius shall be drawn on the map and the control zone shall be related to details within the frame work of the related instructions and the ideal geometrical line to be reported, drawn considering the special points such as highways and roads, railways, natural barriers, bridges, fixed constructions and other reference factors. The epidemiological observations concerning the potential spread risk of the infection shall also be taken into account.

In identification of the control zone, the following factors shall be considered:

- Intensity of poultry population,
- Surveillance of direct and indirect contacts (other establishments, traders, markets, etc)
- Self-sufficiency with respect to services (slaughterhouses, transformation premises, etc).

Measures to be taken within the control zone:

- a) All establishments in which there are poultry are identified.
- b) Periodical visits are made to all establishments in which there are poultry. Samples for laboratory analyses are taken from these establishments and if necessary, these poultry are clinically examined. The results of the visits and inspections conducted are recorded.
- c) All poultry animals are sequestered in the establishments they are in or in any other place that they can be kept in isolation.
- d) Appropriate disinfection means are applied at the entrance or exit of the establishments.
- e) The movement of the vehicles used for movement of poultry animals, poultry carcasses and eggs within the zone and the individuals appointed for transportation are controlled.
- f) Movement of poultry on public and private roads, except transit through the zone by air, railway or highways.
- g) Opening of fairs and markets related to poultry or other fowls and organization of exhibitions and competitions are banned.
- h) Except the assumptions below, the movement of poultry and hatching eggs outside the establishment is banned.

The public veterinarian allows for movement only for the below situations:

- a) The poultry in other establishments and coops which are in the control zone and which are not infected are allowed to be slaughtered for immediate slaughter in a slaughterhouse resident within the control zone. In case this is not possible, such poultry are allowed to be slaughtered in another slaughterhouse suggested by the competent authorities outside the control zone.
- b) Transportation of one-day old chicks and ready chicken to be sent to establishments which are within the surveillance zone but which do not contain any poultry. However, this establishment is subject to official control like the control zone.
- c) The eggs in other establishments which are in the control zone and which are not infected and the packages containing them are allowed to be transported to hatcheries specified by the competent authorities, after their disinfection (formol fumigation for 30 min or washing with 2.5-3% sodium hypochlorite solution)

In cases where it is necessary to move poultry or animal products out the control zone, the public veterinarian appointed to monitor the disease is applied. Before allowing for such movements, it is necessary that the public veterinarian examines these in the establishment. The public veterinarian records this in a report. The report is reviewed by the concerned Animal Health Security Committee. If deemed as appropriate, movement is allowed. The transportation of the poultry or eggs allowed to be transported is conducted under official control and in transportation vehicles which do not have contact with the surroundings, disinfecting them before and after usage.

6.4.1.1.2 Surveillance zone

The surveillance is the area which includes and borders the control zone and which has a radius of 3-10 km depending on the geographical structure of the region, establishment density, slaughterhouse, feed factory and other ecological and epidemiological factors. On the border of this zone, signs that bears the warning “THERE IS AVIAN INFLUENZA HERE” are placed. In general, inspection is less. This zone is a buffer zone between the control region and the other industrial regions.

Measures to be taken in the surveillance zone:

- a) All establishments in which there are poultry animals are identified.
- b) The movement of the poultry animals and hatching eggs within this zone is allowed upon permission.
- c) Movement of poultry animals outside this zone is banned except for direct transportation to a slaughterhouse resident outside the surveillance zone, specified by the competent authorities.
- d) The eggs are allowed to be transported only to hatchery centres specified by the competent authorities. Before delivery, the eggs and their packages shall be disinfected.
- e) Manure and poultry basements that have been used are not allowed to be moved out of the zone.

- f) Movement of feed and equipment outside the zone is allowed upon permission.
- g) Installing fairs, exhibitions, competitions and market places related to poultry and other fowls in this region is banned.
- h) Transit passages by air, highways and roads or railway are allowed upon permission, provided that the origin and destination are outside the control and surveillance zones and that the animals are not unloaded within the announced area. If the animals are to be arrested obligatorily within the surveillance zone, such animals are considered to be suspected and their subsequent movements are re-considered carefully.

6.4.2 Total annihilation of poultry animals

Total annihilation of poultry animals is necessary, considering possible contact of free living fowl (especially nightingale species) with infected structures, related to the warranty of less spread of the virus and effect rate. Thus, total annihilation procedures, discarding animals and manure and disinfection procedures should be conducted while the doors and windows of the buildings and coops closed as far as possible. This way, entrance of free living birds to and then exit from these contaminated places is prevented. In addition, such processes shall include anaesthetic procedures to minimize suffering of animals and to establish a sudden death.

Widespread methods used for total annihilation of poultry animals

- a) For annihilation of small poultry groups (family type establishments), intrapulmonary injection of drugs combining narcotic, curaric and anaesthetic products is advised. This way, a death within a few seconds without suffering is ensured (0.6 ml/kg).

- b) For larger groups, using gas or hypnotics for anaesthesia and then mass methods providing death by asphyxia is advised. The animals may be killed in the coop or in an impermeable container of the type used in transportation of high risk wastes.

- c) Feeding gas into the container may be accomplished by connecting tubes to the water collection discharge pipe of the cooling group, through the valve on the cooling group. The number of animals for one metre cube of gas used should not exceed 150 (average weight 1.8 kg).

The most commonly used gases for this purpose and their methods of usage are given below:

- a) **Carbon dioxide (CO₂):** 17.5 kg/1000 m³: saturates the medium within 30 min and death is realized within 15 min. This is one of the most commonly used gases.

- b) **Carbon monoxide (CO):** 8 kg/1000 m³: saturates the medium within 30 min and death is realized within 15 min. This is one of the most commonly used gases.

- c) **Hydrocyanic acid (HCN):** 3 kg/1000 m³: saturates the medium within 30 min and death is realized within 14 min. since this gas is highly toxic, it should be used with utmost care.

- d) **Alpha chloralose:** Mixed into feed in 2-6% and causes lethargy in 60 min.

- e) **Sodium Phenobarbital:** Mixed into drinking water (80 mg in 55 ml) causes lethargy and death in 4 hours.

6.4.3 Destruction of poultry animals

In destruction of poultry, a carcass destruction method which provides the highest security is preferred. Thus, it is preferred that burying into pits is not used if the hydrogeological conditions allow for, if the safety of other poultry are not endangered and if it does not require transportation to relatively long distances under that does not have sufficient biological safety.

Incineration on-site is not advised. Incineration may only be conducted in structures built for this. For incineration, incinerators, which have been equipped with post-combustion equipment to incinerate stack gases and which have adequate capacity for carcass destruction are necessary.

Additionally, it is possible to use thermo-destruction facilities for high risk materials.

All personnel, functioning at different stages, shall not visit places containing receptive (sensitive) animals during work and for 3 days following the last contact with the infected poultry.

6.4.3.1 Destruction by burying into a pit

As soon as the diagnosis is verified, preparation of pits for burying the carcasses should start. The place previously chosen should be near or as near as possible the infected centre, preferably away from residences, in places where animals do not dwell, which are away from streams and which shall not contaminate ground waters. The pit should have a width and a depth of at least 2 m.

At such a depth, an area of 1.3 m² shall be necessary for 300 animals of each 1.8 kg. If digging deeper is possible (3.6-6 m), the number of animals per m² may be doubled with every 1 m depth. After the carcasses and/or materials are placed into the pit, they are covered with unslaked lime. Then, the pit shall be covered with soil, caring not to press much since the decomposition reactions and the gases evolved may lead to formation of cracks. All materials that can not be disinfected (e.g. wood, paper, etc.) are buried together with the animals.

6.4.3.2 Destruction in rendering facilities

In case the carcasses are transported to transformation or incineration facilities, trucks having large bodies which are suitable for transportation and which are completely impermeable (including the door) should be used. The animals shall not be transported alive.

The destruction process conducted in transformation facilities for production of meat flour may be accepted after grounding at a minimum temperature of 120°C for half an hour. The controls related to the transformation shall be conducted with controls on the infectivity of the finished product.

Such facilities should have the minimum qualifications specified below:

- a) The roads leading to the facilities should have cement flooring. Maximum care should be given to waste water discharge lines and treatment unit.
- b) The place where the carcasses are unloaded from hermetic bodies, the access plateaus to grounding units and entrances to incinerators should have cement flooring and the access should be closable.
- c) At the entrances to the plateaus, there should be a washing and disinfection point placed at the internal part.

The norms to be followed by the operators are the same with the norms of the operators of the infected fattening animals.

The normal operation of the facility is stopped for the duration necessary for destruction of the carcasses. Before starting commercial activity, samples shall be taken from the meat, bone flour, gelatine, etc. and analyzed with respect to avian influenza.

Transportation of the carcass in destruction

Transportation of the carcasses of animals annihilated totally should be made in impermeable containers having hermetic covers.

The driver of the vehicle should not get out of his vehicle; if he should, he should wear disposable overalls and shoes before he steps out. When getting back into his vehicle, he should hand out the disposable clothes he has worn.

Disinfection in transportation

- a) There should be a mobile disinfection unit at the entrance to the establishment for disinfecting all the vehicles exiting the establishment.
- b) The personnel should change their clothes completely at the entrance and wear the clothes prepared for this purpose. The personnel should change their clothes completely when work is finished and should take a meticulous shower at the mobile showers.
- c) At the loading stage, as the carcasses are loaded into the case, the section should be disinfected with an appropriate disinfectant.
- d) After loading is completed and before the loaded motor vehicle leaves the infected area, the vehicle should be disinfected with utmost care, especially the tyres, case and the bottom parts of the body. It is important that before the vehicle leaves the infected area, the drops on it are let to fall down.
- e) At the point of unloading the carcasses, the vehicle should be washed and disinfected, including the internal parts of the cases. This is conducted by the disinfection station placed in the plateaus. After the truck leaves this area, it is disinfected in this medium. The truck is disinfected again at the disinfection point placed at the entrance to the facility externally.
- f) Concerning the disinfection of the establishment, the instructions specified in the related section shall be complied with after total annihilation is completed.

6.4.4 Destruction of animal products

It is banned to take out the eggs, hen manure, feathers and feed in the infected coop or establishment. Utilization of such animal products is not permitted to be used. If the system of burying the carcasses is used, the materials mentioned below may also be replaced into the same pit and covered with soil or these materials are transported to and destroyed in the incinerators by the same methods provided for transportation of the carcasses.

The materials that should be considered in infected establishments are mainly as follows:

- a) Poultry manure
- b) Eggs
- c) Egg derivatives
- d) Hay
- e) Feed and other nutrients
- f) Feathers
- g) Egg containers
- h) Drugs and vaccines

6.5 Control of the Infected Area

The public veterinarian appointed to monitor the epidemic controls the infected area until the disease is eradicated. Since avian influenza can infect humans, the public veterinarian provides information on the disease to animal owners and the local health organizations.

6.6. Eradication of the Epidemic

The quarantine measures taken for avian influenza are cancelled after 21 days following the last annihilation, after the necessary periodic disinfections are made.

6.6.1 Cleaning and Disinfection

The procedures to be followed concerning cleaning and disinfection of the infected establishments after the total annihilation of poultry are specified below:

- a) The parts in connection with the establishment or the coop (hatching centre, egg and carrier gathering room, place of egg selection and packaging, pasteurization facilities) shall be appropriately disinfected physically or functionally. In addition, the transportation vehicles of the establishment for carrying live animals, hatching or consumption eggs, feed and others should be subjected to the same treatment.
- b) The walls, floors, areas and the ceilings should be meticulously cleaned; all remains of faeces should be scraped off; all structures that can not be disinfected should be demounted to prepare disinfected surfaces and should be destroyed.
- c) The walls and floors of closed places should be washed with an appropriate disinfecting solution, iron structures should be decontaminated using heat and if this procedure is not risky, should be subjected to flames.

- d) In the external parts, all existing organic residues should be scraped; and a thorough cleaning should be conducted. After this, these parts should be washed with an appropriate disinfecting solution.
- e) On open areas not of cement, the same disinfectant solution should be applied more than once. All equipment and machines in the establishment should be gathered together, cleaned and disinfected thoroughly and meticulously. After this stage is completed, appropriate disinfectant should be applied to coops, open areas, etc.
- f) An appropriate disinfectant should be applied to water depots, piping, canals and drinkers and the solution should be retained within the structures being disinfected.
- g) Feed silos should be emptied. Knocking on these silos to make feed residues fall down is necessary. Then, the silos should be washed with a hot water hydro-cleaner from above and then fumigated. The feed containers and nests should be demounted, washed with a hydro-cleaner and then disinfected.
- h) In order to provide disinfection for everyone having access to the establishment, a mobile disinfection station should be available at the entrance to the establishment.
- i) The personnel should behave as if in the total annihilation stage.

- j) The spaces cleaned and disinfected should be fumigated.
- k) After 15 days fumigation and disinfection procedures should be repeated for water and feed distribution systems.

6.6.1.1 Preliminary cleaning and disinfection

- a) As soon as the poultry carcasses are taken to be destroyed, the poultry coops and the related parts, and all parts of the buildings, courts, etc. that have been contaminated during total annihilation and post mortem examination should be disinfected using appropriate disinfectants.
- b) All kinds of poultry tissues and eggs that might have contaminated buildings, courts, tools, etc. should be taken and destroyed together with the carcasses.
- c) The disinfectant used should remain on the application surface at least for 24 hours.

6.6.1.2 Final cleaning and disinfection

- a) Oils and dirt should be cleaned off all the surfaces using an oil remover product and then washed with water.
- b) After washing with water, the above mentioned surfaces should be subjected to a disinfectant again.
- c) After 7 days, an oil remover product should be applied on these spaces, they should be rinsed with cold water, disinfected and rinsed again with water.

6.6.1.3 Major disinfectants used against the disease

- a) **Sodium hypochlorite:** 2% solution in active chlorine. Disinfection of tools, buildings and zootechnical equipment.
- b) **Quaternary ammonium salts:** 4% solution. Application on internal and external walls, spraying on floors, objects, tools and vehicles.
- c) **Potassium peroxymonosulphate + malic acid + sulphamic acid + dodecylbenzylsulphonate + sodium hexametaphosphate complex:** 1% solution. Application on internal structures and disinfection of objects, tools and vehicles.

- d) **Calcium hydrate (Milk of lime):** 3% solution. After preliminary washing and disinfection, spraying on walls and floors.

- e) **Cresylic acid:** 2.2% solution. Spraying on floors.

- f) **Synthetic phenols:** 2% solution. Spraying on floors.

- g) **Formalin + permanganate:** Fumigation in closed spaces.

Other applications in fight with avian influenza are conducted in compliance with the imperative provisions of the Law on Animal Health Security, no 3285 and the Regulation on Animal Health Security.

7. ANNEXES

ANNEX 1 – ARREST MINUTES IN A SUSPECTED DISEASE CENTRE

ANNEX 2 – STATEMENT

ANNEX 1

ARREST MINUTES IN A SUSPECTED DISEASE CENTRE

On the date, informed by Mr/Mrs residing in the address, the owner/responsible person named, born on, of the poultry animals of the coop installed at the address, which is suspected of has been informed of the provisions of Article 47 of the Law on Animal Health Security, no 3285 and has been instructed by the Veterinarian to conduct the followings, while waiting for the related measures of the local health authorities.

1. The owner shall record the existing poultry animals in the establishment and help the veterinarians appointed for epidemiological investigation.
2. The poultry and other animal species existing in the establishment are to be arrested.
3. Separating and isolating the suspected poultry from the healthy ones.
4. Keeping the dead poultry while waiting for other measure.
5. Restricting dogs, cats and court animals.
6. Banning movement of manure outside the establishment.
7. Banning entrance of poultry and other animal species into the establishment.

8. Preventing movement of meat or carcasses of poultry and other animal species, of feed, tools, objects and any other material out of the establishment.
9. Responsibility of protection of existing meat and carcasses in the establishment.
10. Prevention of individuals, materials and vehicles from coop to coop.
11. Prevention of any contact of caretaker personnel with the animals in the coop.
12. Thorough and meticulous disinfection of the dwelling places of poultry and the nearby areas.

Mr/Mrs has been appointed as the caretaker of the suspected poultry and is liable of execution of the above mentioned issues and acceptance of this minutes.

Establishment owner/manager

Headman or Mayor

Public veterinarian

NOTES: This minutes should be notified to the owner of the animals in the establishment suspected of disease or the person responsible of the establishment.

ANNEX 2

STATEMENT EXAMPLAR

Since there is suspicion of avian influenza in the establishment belonging to Mr/Mrs
....., Mr/Mrs
born on, residing at the address
..... commits not to contact other animals
of poultry species for 3 days as of today and to wash and disinfect his/her clothes that
are possible disease carriers. / /

Signature