Study framework to identify the cause of morbidity and mortality in chicken

Introductory notes

If disease outbreak is happed in any given poultry flock, investigation should be as early as possible since many poultry diseases are highly contagious, easily transmitted and may kill significant numbers within a short time.

Visible clinical signs would be identified & registered on farm either by the framer owners (attendants) or by the nearby veterinarian as clinical signs help diagnosis. Since many poultry diseases show similar clinical signs, confirmation should be done by laboratory analysis of samples. Similarly, the laboratory analysis should be quickly done and should help identification of the disease and recommendations for the control of the outbreak.

In Ethiopia the standard laboratory to do animal disease outbreak investigation is the Animal Health Institute laboratory which is located in Sebeta, Ethiopia.

There should at least be two purposes in development of this specific study frame: 1) Regular follow up of chicken flock to collect data on general management of chicken flock (feed/feeding, housing), disease prevention mechanisms (vaccination, disinfection, avoiding contact with other flock), occurrence of disease (periodic or persistent) and disease symptoms (early warning), and sampling (from live & dead) for the diagnosis of potential causes of morbidity and mortality in chicken. In this part, total and average number of chicken died during regular follow up, and cause associated with the death (disease/non-disease) and symptom of the diseases will be determined. Future prevention methods will be recommended (For case definition of common poultry diseases that oocur repeatldy in Ethiopia and causes significant mortality please look at annex one. For history, clinical and production parameter data please look at annex two at the end of this document).2) The second purpose is disease outbreak investigation (when it occurs). Accordingly, the number of samples to be taken and the sampling procedure would vary according to the purpose of the proposed action (For Standard method of sample collection, preservation and transpiration please look at annex three. Details of post mortem (necropsy) technique and ,samples collection form necropsy is presented in separate document and that can be referred) ,

1) Regular follow up to monitor persistent disease occurrence, clinical symptoms and sampling for early diagnosis

Sick chicken can be identified from multiple aspects of abnormal manifestation, such as abnormal respiratory signs, feces color & consistency, presence of paralysis (general appearance) and feeding behaviors. Evaluation of clinical data should be done either by local veterinarians or by trained and well experienced chicken owners.

Study population

Chicken populations to be followed up are all chickens (both sex, all age) distributed by TPGS in different districts of Ethiopia and managed under extensive production system

Selection of villages and house holds

Villages covered by TPGS will be either preferably purposively selected if there is previous occurrence of disease in chicken they received or villages will be randomly selected for expected occurrence of chicken disease if there were no previous history of any d isease.

Households from selected village will be proportionally selected and be included. The proportionality sampling technique designed by Arsham (2007) which uses the formula $N= \frac{0.25}{SE^2}$, where N is the sample size and SE is the proportional standard error can be used to include households. Using 0.01 or 10% proportional standard error, 25 households from total of 36 households of given village will be regularly followed up for occurrence of disease and sampling for early diagnosis

Sample size and sampling units

Since the numbers of chicken population distributed by TPGS are small (in a range of 25 to 75) there is no need of calculation (probability sampling) for representative unit and all the chicken

will be regularly observed. However, when a disease symptom is observed and samples have to be collected from live chicken, sample size can be calculated using online (ex. WinEpi, FreeCalc – EpiTools, available to perform sample size calculation for different sampling purposes (http://winepi.net/winepi2) by using the number of animals with disease symptom, 95% confidence interval and total population in the flock. Example, if in a household who has received 25 chicken TPGS and if 5 chickens had developed similar disease symptoms, using 95% confine interval and 25 chicken as starting population, 11 chicken should be sampled. If there are freshly dead chickens one can sample as much as possible based on the facility at hand (for clinical data collection use annex two; for sample collection use annex three; for necropsy sample use postmortem and necropsy technique).

Clinical data collection in the follow up

The visit frequency to collect clinical or general data should better be daily done to have data bank and to plot the information on graph, however, depending on the labor power available data can be collected weekly or may be monthly. But in any condition one should have to have data by different season as many diseases may have season pattern in distribution. Data should be collected by veterinarian but experienced and trained owners can also collect data. However any necropsy data must be collected only by veterinarian as that need gross morphologic descriptions and result interpretations.

One should carefully note the sick chick/chicken actions (without disturbing) which include way of eating, and drinking (decreased feed/water consumption) and carefully listen to any abnormal **vocalization** (sneezing, coughing, gurgling, rales) which is the commonest symptom of respiratory disease in poultry. Since respiratory rale is seen in more than 90% chickens infected with Newcastle disease, infectious bronchitis disease and avian influenza (Banakar et al. 2016),

Careful observation should be made on feces color, shape, water content and consistency as it is a good indicator of digestive system disease which is among the most widespread diseases in chicken farming (Ducatelle *et al*., 2018). Fecal characteristics together with the age of chicken (young affected most) are good basis for the early warning of chicken gastrointestinal disease and should be included in data collection (flock & individual data).

Yellow-brown colored and watery feces strongly indicated Avian Influenza; the Yellow-green colored and watery indicate ND; Lime watery indicates IBD; White and watery indicates IB; Grass-green colored and watery indicates Avian cholera, White mushy indicate Pullorum disease, and Brown-red thin or blood (coccidiosis) (Ducatelle *et al*., 2018).

Careful observation on any nervous sign which include torticollis (neck twisting), Opisthotonus (arching of neck /back), wing paralysis, leg paralysis (one/jumpers or two legs), complete paralysis help the diagnosis of nutritional deficiencies and many infectious diseases.

Particular attention should be given if the paralysis was in one leg or both. One leg stretched forward and one leg backward is a typical presentation of leg paresis in a chicken with Marek's disease. Presence of the paralysis of neck muscles with twisting (torticollis), paralysis of extensors (opisthotonus), and wing paralyzes all should be included in data collection. Other posture abnormality like abnormal sitting (sittingon haunches or lying down), wing paralysis, and ruffled/loss of feathers should be included in data (Knowles *et al*.,2008).

Careful observation on mortality in relation with time of disease onset (number died, percent died) (expect a mortality of about 2 percent in baby chicks, & mortality after three weeks of age should not exceed 1 percent per month) should be made as that help the diagnosis. Also, egg production, eggs shell strength, color, and shape should carefully be observed.

The counting and species identification of the parasite in feces are also an important task of the detection of intestinal diseases in poultry (Zhang *et al* 2014: Li *et al*., 2019). Fecal examination can help to diagnose intestinal round worms like Ascaris, Capillaria and oocytes of Eimerian coccidian (for detail of these parasites and their predilection please look at postmortem materials presented in separated chapter).

Other disease symptoms like sudden death, general weakness (lethargy) expressed by not eating and moving, dull and/ or closed eyes, abnormal discharges (nasal, ocular), discolorations (head, wattle, comb) and/or staining (vent), weight loss, sudden production lose/egg laying, abnormal

gait/lameness,and/or any local swelling and related symptoms are providing early warning of the diseases and should be included in gathered data to evaluate the presence of chicken disease (for detail of this look table 1 below and annex two & annex three).

Table 1. Details of samples to be collected (by disease symptoms) during follow up visit

Disease symptom	**Sample to be collected ***	**Sample transport, fixation**	**Disease targeted**	**Diagnostic tests**
Respiratory disease signs	Orophryngeal swab/cleft palate swabs, tracheal swabs, choanal swabs in VTM/ mycoplasma medium/broth without antibiotics (postmortem) Oculonasl swabs in VTM/ broth without antibiotics (live chicken/antemortem) Oculonasal exudate swab in VTM/ mycoplasma medium/broth without antibiotics (postmortem) Pulmonary/tracheal parasites Fresh (molecular)/ fixed (for histopathology)	Transport in cold chain Do not free at -20^0c freezer with automatic defrost If Freeze at -70^0c & transport on ice pack better	NewCastle Disease/Infectious bronchitis/Infectious laryngotracheitis/ infectious coryza/ Mycoplasma gallisepticum/synovia/aspergylosis/ parasites	1st Clinical signs//necropsy lesions/ 2nd infectious agent isolation & identification/molecular tecnniques/histopayhology
Diarrhea	Cloacal swabs/ Fresh feces in VTM/ Broth without antibiotics (live chicken) Intestinal swabs in VTM/ Broth without antibiotics Gall bladder swabs Egg shell/egg contents Tissue of Cecal tonsils/ bursa/ liver spleen/ovary/lung/heart Intestinal impression smears	Same as mentioned above	Fowl typhoid/Pullorum, IBD/Newcastle/cocidiosis	1st Clinical signs//necropsy lesions/ 2nd infectious agent isolation & identification/molecular tecnniques/histopayhology
Nervous signs, leg/wing	Feed sample; intestinal/ventricular content (in plain containers	Same as mentioned above	Nutritional deficiency, NCD, Marek's, musculoskeletal	Same as mentioned above

/neck paralysis; general weakness	Fixed sciatic & brachial plexus; spinal cord; thoracolumbar vertebrae; fixed tibia/tendon/muscle			
Facial swelling without/with mild respiratory sign	Fresh & fixed nasal sinus Nasal sinus swab in VTM/broth without antibiotics/mycoplasma mediaum Subcutaneous swab in VTM/broth without antibiotics	Same as mentioned above	Infectious coryza/ CRD/aspergylosis	Same as mentioned above
Sudden death	Feed sample in plain bag Ventricular/intestinal contents in plain bag Different tissues (fresh) & fixed Exudates(swab) in VTM/broth without antibiotics	Same as mentioned above	Many hyper acute infectious diseases/poisons	Same as mentioned above Same as mentioned above
Anemia	Feed sample in plain bag Blood smear/fixed tibia or halved bone marrow Ectoparasites	Same as mentioned above	Nutritional deficiency/infectious anemia virus/parasites	Same as mentioned above
Poor feather/ Itching	Skin scraping/ fresh &fixed tissues	Same as mentioned above	Skin disease/ectoparasite	Same as mentioned above

***** Always collect whole blood and serum/plasma ; VTM virus transport medium; NCD Newcastle disease; IB Infectious Bronchtitis; LT Laryngo Tracheitis; CRD Chronic Respiratory Disease

2) Investigation of common chicken disease outbreaks & sampling techniques

In investigation of chicken diseases, selection of individuals to be tested and/or sampled, sample size, the type/s of sample/s to be collected, time of sample collection and selection of correct diagnostic tests are largely matters the diagnostic results.

Samples collected from long-standing dead chicken in the flock are not suitable for laboratory test and instead, specimens should be collected from recently died, from sick (moribund) chickens and few apparently healthy and live birds.

It is also worth to note that there is no universally accepted sampling method to all chicken diseases and hence methods of sampling and samples to be collected are varied by diseases.

Sample size and sampling units to investigate disease outbreaks

To collect sample from freshly dead birds one can collect as much as possible samples. However, to do postmortem and collect sample from moribund chicken, sample size can be calculated using WinEpi tool or software (de Blas and Muniesa 2010). Assuming 95% certainty, and given that the diagnostic test is 100% sensitive and specific and the prevalence of a given disease is 50% in a population of 75, 50 and 25 chicken (TPGS distributed) the sample size needed are respectively 5 chicken, 5 chicken and 4 chickens (de Blas and Muniesa 2010). In a population of 75 chickens and 50 chickens we should collect samples from at least 5 chickens in order to detect the disease.

Sampling technique, sample types and transportation for major chicken diseases that are known for repeatedly occurring as an outbreak and causing devastating mortality in Ethiopia chicken farming are briefly indicated below.

Sample collection to investigate Newcastle disease investigation

To collect sample for detection of APMV1 brain heart infusion (BHI) broth is the recommended VTM as it contains a protein component which protects the virus from degradation during storage and shipping. Howevr, any salt balanced media with protein component such as Tris-buffred tryptose broth (TBTB), nutrient broth (NB) and peptone broth (PB) can be used (OIE manual, 2018).

Sample collection Method and Samples to be collected

For detail of sample collection please look at annex three and for case definition of the diseases please look at annex one.

Swab collection

Cotton free swab that are synthetic or semi-synthetic (e.g., polyester, rayon, nylon) should be used as cotton swabs are inactivate the virus

During sample collection target samples from recent mortalities, sick birds and when these are not available sample apparently health birds next to building inlets or in cages adjacent to sick/dead birds

Oropharyngeal swabs (this swab is preferred) : Swab the oral cavity and opening of the trachea avaoinding the esophagus, and bring the swab up through the choanal cleft where the sinuses drain to capture material from the upper respiratory tract (for practal detail of oropharyngeal swab collection please look at fig 1 in annex four).

Tracheal swab (only from fresh carcasses): after carefully and aseptically opening the the trachea collect the sample

After collecting the sample swirl the swab vigorously in the VTM (3-5ml BHI with antibiotics/without antibiotics), squeeze the excess liquid from the swab inside the specimen tube and collect the swab in an appropriate container for proper disposal at the laboratory. Avoid leaving the swab or other collection devices in the tube as swab left in the media may reduce the

volume available for testing. Clearly label the container with appropriate ID using waterproof marker.

Pooling procedures: swab samples may pooled by the same species and the same premises and by the same sapling route. Note: do not pool tracheal, oropharyngeal and coanal swabs together.

Typically pool 5 swabs /pool in at least 3ml of VTM or upto 11 swabs/pool in at least 5ml of VTM (valid only for tracheal or oropharyngeal swabs for gallinaceous species). Collect cloacal swabs of up to 5swabs/pool in at least 3ml of VTM pooled by sample route and species.

Tissue specimen: Pool by system (respiratory, enteric, reproductive) typically from a single bird; It is not recommended to pool tissue from more than one bird (especially for free living birds)

Specimen preservation, transport and storage

Specimen for isolation and identification of the virus and for detection of viral genetic materials should always be maintained and transported in cold chain. Immediately after collection specimens should be held on ice pack and should be transported within a short time to laboratories for processing or for freezing. All tube should be transported in an upright position. When kept at 4^0C (refrigerator) APMV1 has been shown to be stable for up to 96 hours. If samples have been frozen (-70ºC), they should remain frozen until delivered to the testing laboratory. Specimen should never be stored in the freezer portion (-20ºC) of a standard refrigerator unit with automatic defrost cycle (specimen will undergo through repeated thawing that is detrimental to the virus & viral nucleic acid)

Infectious bursal diseases outbreak detection

Description: Infectious bursal disease (IBD) is acute, highly contagious and immunosuppressive diseases of young chicken usually at 3 to 6 weeks old.

In clinical IBD characteristic clinical sign (sudden onset, morbidity, droopy appearance), and especially postmortem lesions (muscular hemorrhage, enlarged edematous or hemorrhagic bursa) will help the diagnosis.

Sample: Collect bursa (high virus than other organ), and spleen in sterile ziplock bag (ideally within 1st three days of the appearance of clinical signs), transport in cold chain and freeze at -800C as soon as possible. Fix the bursa in 10% buffered formalin for histopathology.

Confirmation of clinical cases of IBD is through isolation and characterization of IBV from bursal tissue, virus detection by TR-PCR, and histopathological detection of bursal lesions.

Infectious Bronchitis diseases outbreak detection

Description: IB is an acute, contagious disease characterized primarily by respiratory signs in growing chickens and decreased egg production and egg quality in egg laying hens. Several strains of the virus are nephropathogenic and may produce interstitial nephritis and mortality.

Sampling: Sample should be collected as soon as signs of clinical diseases are observed.

Tracheal swab: Swab the oral cavity and opening of the trachea avoiding the esophagus, and bring the swab up through the choanal cleft where the sinuses drain to capture material from the upper respiratory tract (same as sampling for ND)

Tracheal & lung swab (only from fresh carcasses): after carefully and aseptically opening the trachea collect the sample.

Kidneys or oviduct sample: For birds with nephritis or egg production problems, samples from the kidneys or oviduct, respectively, should be collected in addition to respiratory specimens. In situations where IB-induced nephritis is suspected, kidney samples should also be selected from fresh carcases.

Blood samples from acutely affected birds as well as convalescent chickens can also be submitted for serological testing???.

Fowl typhoid & Pullorum disease outbreak detection

Description: Pullorum disease is a bacterial disease (S. Pullorum) of young chicken (2-3 weeks of age) with very high mortality (may be 100%). Fowl typhoid is an acute septicaemic bacterial (S. Gallinarum) disease of mature chickens characterized by depression, labored breathing, anemia and diarrhea causing adherence of feces to the vent.

Sampling: For optimal recovery of the organisms, the birds being sampled should not have been treated with antimicrobial drugs for approximately 2–3 weeks previously.

Samples: Cloacal swabs from sick live chicken in transport media (without antibiotics)

Post-mortem tissues taken from the spleen, liver, gall-bladder, kidneys, lungs, heart, ova, testes, alimentary tract or joint lesions should be collected in sterile bottle or after tissue surface is seared with a hot spatula and a sample is obtained by inserting a sterile cotton swab or sterile loop through the heat sterilised surface

The preferred tissues for routine investigation are liver, ileo–aecal junction and ovaries/oviduct..

Fresh egg materials, fresh feces, or any contaminated materials from housing, incubators or transport boxes may also be taken

Pooled Tissue collection

Pool from tissues from a number of birds, and, for routine testing, five ileo-caecal junction samples may be pooled. Larger numbers of aseptically collected non-intestinal samples can be pooled, but for practical purposes, composite samples of liver, spleen and ovary from five birds are often tested

Cloacal swabs & fresh feces from live birds: Swabs dipped in nutrient broth are suitable; small swabs being used for young chickens.

Hatcher fluff, debris and macerated egg/chick waste samples and chick box liners or floor faecal or litter samples should be collected.

Fowl pox disease outbreak investigation

Description of the disease: Fowl pox is slow-spreading disease of chickens characterized by the formation of proliferative lesions and scabs on the skin, and diphtheritic lesions in the upper parts of the digestive and respiratory tracts In the diphtheritic form, proliferative lesions involving the nasal passages, tongue, larynx or trachea can result in respiratory distress and death from suffocation.

Sampling: Samples to be collected are tracheal swabs, skin lesions, tissue impressions on commercially available cellulose paper cards and formalin fixed tissues.

Comparing to other diseases fowl pox can be easily diagnosed by detecting FPV (cytoplasmic inclusions) from stained tissue smear from lesions. The inclusions can also be demonstrated in sections of cutaneous and diphtheritic lesions by the use of haematoxylin and eosin (H&E), acridine orange or Giemsa stains.

A smear technique for fowl pox

Stock solutions

i) Stock solution for primary stain: a solution of basic fuchsin (5 g) in 95% ethanol (100 ml) is slowly added to a second solution of crystalline phenol (10 g) in distilled water (900 ml). This stock solution, kept in a tightly screw-capped glass bottle, is incubated for 48 hours at 37°C, and then stored at room temperature.

ii) Phosphate buffer, pH 7.5: NaH 2 PO4 H2 O (2.47 g) and Na2 HPO4 (11.65 g) are added to distilled water (1000 ml) and stored at 4°C.

Test procedure

i) Place a drop of distilled water and the lesion (cutaneous or diphtheritic) on a clean slide. Prepare a thin smear by pressing the lesion with another clean slide and rotating the upper slide several times.ii) Air dry and gently fix the smear over a flame. iii) Stain the smear for 5–10 minutes with freshly prepared primary stain (8 ml stock solution of basic fuchsin mixed with 10 ml of phosphate buffer, pH 7.5, and filtered through Whatman filter paper No. 1). iv) Wash thoroughly with tap water. v) Counterstain with malachite green (0.8% [w/v] in distilled water) for 30–60 seconds. vi) Wash the smear with tap water and then dry. vii) Examine the smear under oil immersion. The elementary bodies appear red and are approximately 0.2–0.3 μm in size

Sampling for investigation of a given disease outbreak is also largely facilitated by having a complete history of the flock and this should give a clear picture of the flock management and should help to select the information that relates to this particular disease outbreak (see anexx two).

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Annex one

Case definition for chicken diseases that are known to occurred repeatedly, and cause morbidity and mortality in Ethiopian chicken production.

Disease	Case definition	Suggestive clinical signs
Newcastle disease	Severe, systemic, and fatal viral disease of poultry due to virulent strains of avian paramyxovirus type1	**Respiratory signs**: Gasping and coughing, copious mucoid discharge, edema of tissue aroundeyes, throat and face, cyanosis of comb and wattles. **Nervous signs**: Convulsions, torticollis, opisthotonus, drooping of wings, paralysis of legs, and wings, **Enteric signs**: greenish diarrhea are frequently seen.
Infectious bursal disease	Highly contagious immunosuppressive illness of young chickens (2-3WKs) with high mortality, reduced growth and higher susceptibility to other diseases	Sudden onset and short duration, the tendency for some birds to pick at their own vents, whitish, watery mucoid diarrhea followed by dehydration, soiled vent feathers, anorexia, severe depression and ruffled feathers,trembling,, inc oordination, prostration and finally death with characteristic bursal lesion
Marek's disease	Lymphoproliferative disease of chicken ch aracterized by lymphomas (skin &visceral organ), peripheral nerve enlargement and paralysis caused by MDV	Asymmetric progressive paresis, later complete paralysis of one or more of the extremities. Bird stretches one leg forward and other backward as a result of paralysis of the leg. Drooping of the limb in case of wing involvement. Dilation of crop and gasping if vagal paralysis. If nerves controlling neck muscles are affected, the head may be held low
Infectious bronchitis	IB is an acute, highly contagious chicken disease characterized primarily by respiratory signs in growing chickens and decreased egg production and decreased egg quality in hens	Respiratory form: clinical signs include depression, huddling under heat lamps, conjunctivitis, facial swelling, dyspnea, and. decreased feed consumption and reduced weight gain. Infection with nephropathogenic strains can cause initial respiratory signs, then later depression, ruffled feathers, wet droppings, greater water intake, and death. In layers,

		egg production may drop by as much as 70%, and eggs are often misshapen, with thin, soft, wrinkled, rough, and/or pale shells, and can be smaller and have watery albumen.
Infectious Laryngotracheitis	Infectious laryngotracheitis is an acute, highly contagious, viral disease chicken, primarily affecting the upper respiratory tract and is characterized by high morbidity(100%), moderate mortality(50%), weight loss, and decreased egg production	In mild manifestation, the clinical signs are with nasal discharge, respiratory rales, conjunctivitis, and little to no mortality. The clinical signs of the severe form include dyspnea, coughing of bloody mucus, along with periocular sinusitis and severe conjunctivitis. Layer hens demonstrate a decrease in egg production, while the growth of young poultry is affected
Fowl pox	Fowlpox is a slow-spreading viral infection of chickens characterized by proliferative lesions in the skin that progress to thick scabs (cutaneous form) and by lesions in the upper GI and respiratory tracts (diphtheritic form).	Cutaneous form (Dry Pox): generalized lesions (thick scabs) on wattle, comb and unfeathered parts of the skin. Diphtheritic form (Wet Pox): White or opaque eruptions in the mouth, nares, pharynx, esophagus, larynx and trachea, caseous white patches coalesce and expand rapidly and become ulcerated. Mucous membranes undergo an extensive fibrin-necrotic process and develop diphtheritic membrane. Dyspnea, gasping and suffocation due to caseous material in the larynx. Death occurs due to suffocation
Coccidiosis	Coccidiosis is *a protozoal disease causing diarrhea (sometime bloody), weight loss and decreased production in poultry*	**Cecal coccidiosis**: Mainly caused by E. tenella in chickens up to 12 weeks old. Mortality may run as high as 50%. Infected birds are listless, have bloody droppings and a pale comb and show a lack of appetite. Laboratory examination will show hemorrhages in the cecal wall. After severe bleeding, a core will be formed in the lumen. **Small intestinal coccidiosis**: Caused by E. acervulina, E. brunetti, E. maxima, E. necatrix, E. tenella and E. mitis. May affect birds of any age. E. acervulina is not normally very pathogenic, but in some cases, considerable mortality may be seen. Birds infected show loss of weight, combs may be shriveled and a drop or even cessation of egg production in layers may be seen.

Fowl typhoid	Fowl typhoid is bacterial disease of later growing and mature chicken which is characterized by acute septicemia, depression, diarrhea and death	Signs may include: decreased appetite, depression, dehydration, weight loss, ruffled feathers, and watery to mucoid diarrhea. A progressive loss of condition can lead to anemia with pale, shrunken combs. Post-mortem lesions include swollen, friable liver, with or without necrotic foci, enlarged spleen and kidneys, anemia, and enteriti
Pullorum disease	Pullorum disease is *a bacterial disease of* young chicken characterized by very high mortality, huddling near the heat source, anorectia, weakness, depression, diarrhea and white fecal material pasted to the vent	Large numbers of dead in-shell chicks or chicks die shortly after hatching. Loss of appetite and huddling together. Sagging of wings and distorted body appearance, pot-bellied, chalky white excreta (white diarrhea), vent pasting, labored breathing or gasping.

Annex two: History, clinical and production parameter data collection sheet

In the field, diagnostic procedures should be initiated as soon as flock health is compromised, using both individual chicken and flock history and as well as clinical signs, morbidity and mortality related to diseases.

Flock history and disease history collection Form

GENERAL INFORMATION

Date of data collection _____number of birds in the flock_____Breed_____age_____ _____housing type_____, ventilation_____,Feed type & feeding program_____Vaccine used so for & vaccination schedule_____, hyhiene and biosecurity process _____routine medication (yes/no)_____, Vaccine used so for & vaccination schedule_____, Newcastle _____ At what age (1st vaccine)_____ Vaccine type(1st vaccine) _____, Booster vaccines (vaccine types, frequency & ages at each vaccination)_____; _____; _____, Infectious bursal

disease_____ At what age (1st vaccine)_____ Vaccine type(1st vaccine) _____, Booster vaccines (vaccine types, frequency & ages at each vaccination)_____; _____; __,Marek's disease (do they be vaccinated at hatchery?) _____ Infectious bronchitis_____ Infectious laryngotracheitis _____, Fowl typhoid _____ (1st vaccine type and age) _____booster vaccines (frequency & age per each)_____; _____, Fowl Pox (age 1st vaccine) _____ _____, other vaccine if any_____ _____

DISEASE HISTORY

Date disease started _____ number ill)_____number died _____ most recent used treatment _____

Mortality in one area of the house (yes/no) _____ Mortality only at night? (yes/no) _____Mortality affecting only females? (yes/no) _____ reduction in egg production? (yes/no_____ Abnormal egg shape, poor shell quality? (yes/no)_____ outcome of treatment(if any)_____

CLINICAL DATA (should be collected by veterinarian or experienced poultry owner)

Head / face swelling? (yes/no)_____ Respiratory disease signs? _____Abnormal comb / wattle_____ feather quality?_____ Birds appear dehydrated?(yes/no)_____ Abnormal fecal consistency? (yes/no)_____ wet or liquid feces(yes/no)_____ feces with undigested feed (yes/no)_____ dry feces (yes/no)_____ Mobility concerns? lameness(yes/no)_____ deformed legs(yes/no)_____ foot lesions (yes/no)_____ Evidence of insect/rodent/(yes/no)_____

PRODUCTION PARAMETER DATA

Feed intake issues? reduced feed intake (yes/no)_____Weight loss(yes/no)_____, reduced growth(yes/no)_____, reduced egg production(yes/no) _____ under fleshed? (yes/no)_____ over fleshed? (yes/no)_____ Water intake issues? higher water consumption (yes/no)

_____ reduced water intake (yes/no)_____, litter quality concerns?_____ damp litter(yes/no)_____ wet litter(yes/no)_____ fungal contamination (yes/no)_____ biosecurity concerns?_____

Annex three

Standard method of sample collection, preservation and transpiration

The veterinarians and/or experienced chicken owners must select, aseptical procures, appropriate sample, adequate number of specimens, proper preservation and transport of specimens for the isolation or demonstration of a causative agent.

Samples for the diagnosis of chicken diseases include whole blood, serum, formalin-fixed tissue and swabs (tracheal, choanal, oropharyngeal, cloacal, organs and joints). For molecular detection of diseases causing agents Fast Technology for Analysis of nucleic acids (FTA) cards can be used to collect feather pulp, whole blood or isolates from any type of swab.

BLOOD COLLECTION

For routine serological monitoring, serum samples should be collected from normal, healthy birds. During a potential disease investigation, however, blood samples should be collected from birds that are exhibiting the clinical signs or lesions of the suspected pathogen or syndrome.

Volume of the Blood Sample

Normally, 2 to 3ml of whole blood (1 to 1.5 mL of serum) is enough for serological test of many chicken diseases. Sufficient serum should be kept frozen for future use.

To collect blood from Wing vein use 0.5–1.0 inch 20–22 gauge and for collection from Cardiac puncture use 1.5 inch 18–20 gauge needle.

Chicken blood collection methods

1. Wing (brachial) vein (for birds 4 weeks and older)

- Hold bird by both legs.
- Place legs under elbow of nondominant hand.

- Free both hands to gain access to underside of wing.
- Remove feathers to better view the brachial vein.
- Visualize the brachial vein.
- Orient needle in alignment with vein, bevel pointed up, with tip of needle pointed toward wing tip.
- Needle should be inserted first under the skin and then into the vein mid-way between elbow and shoulder joints.
- If needle is within the brachial vein, blood will fill syringe with minimal pull on syringe plunger. Pulling back on plunger with too much force will create high negative pressure, causing the vein to collapse and stopping the flow of blood into the needle.
- Once the needle is removed from the vein, the application of slight pressure with a finger over the injection site will promote more rapid clotting
- If blood is not flowing into the syringe:———
    - Needle is not in the vein.
    - Needle is plugged with a clot.
    - Vein has been punctured and a hematoma is forming.

Wing vein puncture using a scalpel blade——————

- Although this method can provide more rapid blood collection, it does have the potential to induce more trauma than using a needle and syringe.
- A #11 scalpel blade inserted into a #3 or #4 scalpel blade holder is used to puncture the brachial vein just above the elbow joint.
- A blood tube is used to collect the blood as it hemorrhages from the cut (likely to result in sample contamination/Wiping the skin with rubbing alcohol prior to the cut).
- Depending on the size of the cut, this method can cause significant trauma (blood loss, stress, etc.) to the bird and involves risk of severing the brachial artery and nerve.

Cardiac method of collection (not recommended for routine procedure)

Once a blood sample has been collected into a syringe, the sample should be carefully transferred to a tube to promote clot formation. The needle should be removed from the syringe before the

blood is pushed into the clotting tube (hemolysis), Slowly inject the blood into the clot tube, allowing it to run down the side of the tube, keep the right time and remove serum.

SWAB COLLECTION

Cotton free swab that are synthetic or semi-synthetic (e.g., polyester, rayon, nylon) should be used as cotton swabs are inactivate the virus

During sample collection target samples from recent mortalities, sick birds and when these are not available sample apparently health birds next to building inlets or in cages adjacent to sick/dead birds

Oropharyngeal swabs (this swab is preferred) : Swab the oral cavity and opening of the trachea avaoinding the esophagus, and bring the swab up through the choanal cleft where the sinuses drain to capture material from the upper respiratory tract (see fig 1 in the annex).

Tracheal swab (only from fresh carcasses): after carefully and aseptically opening the the trachea collect the sample

After collecting the sample swirl the swab vigorously in the VTM (3-5ml BHI with antibiotics/without antibiotics), squeeze the excess liquid from the swab inside the specimen tube and collect the swab in an appropriate container for proper disposal at the laboratory. Avoid leaving the swab or other collection devices in the tube as swab left in the media may reduce the volume available for testing. Clearly label the container with appropriate ID using waterproof marker.

Pooling procedures: swab samples may pooled by the same species and the same premises and by the same sapling route. Note: do not pool tracheal, oropharyngeal and coanal swabs together.

Typically pool 5 swabs /pool in at least 3ml of VTM or upto 11 swabs/pool in at least 5ml of VTM (valid only for tracheal or oropharyngeal swabs for gallinaceous species). Collect cloacal swabs of up to 5swabs/pool in at least 3ml of VTM pooled by sample route and species.

Tissue specimen: Pool by system (respiratory, enteric, reproductive) typically from a single bird; It is not recommended to pool tissue from more than one bird (especially for free living birds)

HISTOPATHOLOGY SAMPLE COLLECTION

Histology can be a valuable tool in assessing flock health. Some poultry diseases can only be diagnosed by histopathology. For example, the clinical presentation of infectious laryngotracheitis virus or wet pox within a flock can be virtually identical, but the diseases cause distinctly different and characteristic histopathologic changes that allow a definitive diagnosis. Successful use of histopathology as a diagnostic practice requires the availability of appropriately selected and preserved samples.

Avoid collection from deteriorated or decomposed tissues and also do not collect samples from chickens that have been previously frozen as freezing and thaw processes can disrupt cellular features, leading to poor quality slides.

Samples should be collected using a scalpel or razor blade that is sharp and sterile as scissors and blunt blades causes traction which destroy cellular details.

An individual sample should be no larger than 1 cm3 (1x1x1 cm) to allow for adequate penetration of the tissue with fixative.

When a particular disease is suspected based on flock history, tissues associated with that disease may be collected, even if they appear normal. Tumors and other masses, focal discolorations, and organs that are enlarged, atrophied, or otherwise abnormal should be sampled.

For commonly occurring chicken diseases in Ethiopia one should collect all lesions with visible lesion but must not miss the following tissue samples.

Disease suspected	Primary tissue to be collected
IBD	1^0, Bursa, Thymus, 2^0 thigh/pectoria muscle, proventrucluc & other organs with lesions
NCD	Velogenic viscerotropic: 1^0, Proventriculus, gizzard, gutassociated lymphoid tissue & intestine with lesion. 2^0 any tissue with lesions Velogenic neurotopic : 1^0 trachea, larynx, conjunctiva, peripheral nerves & brain. 2^0 any

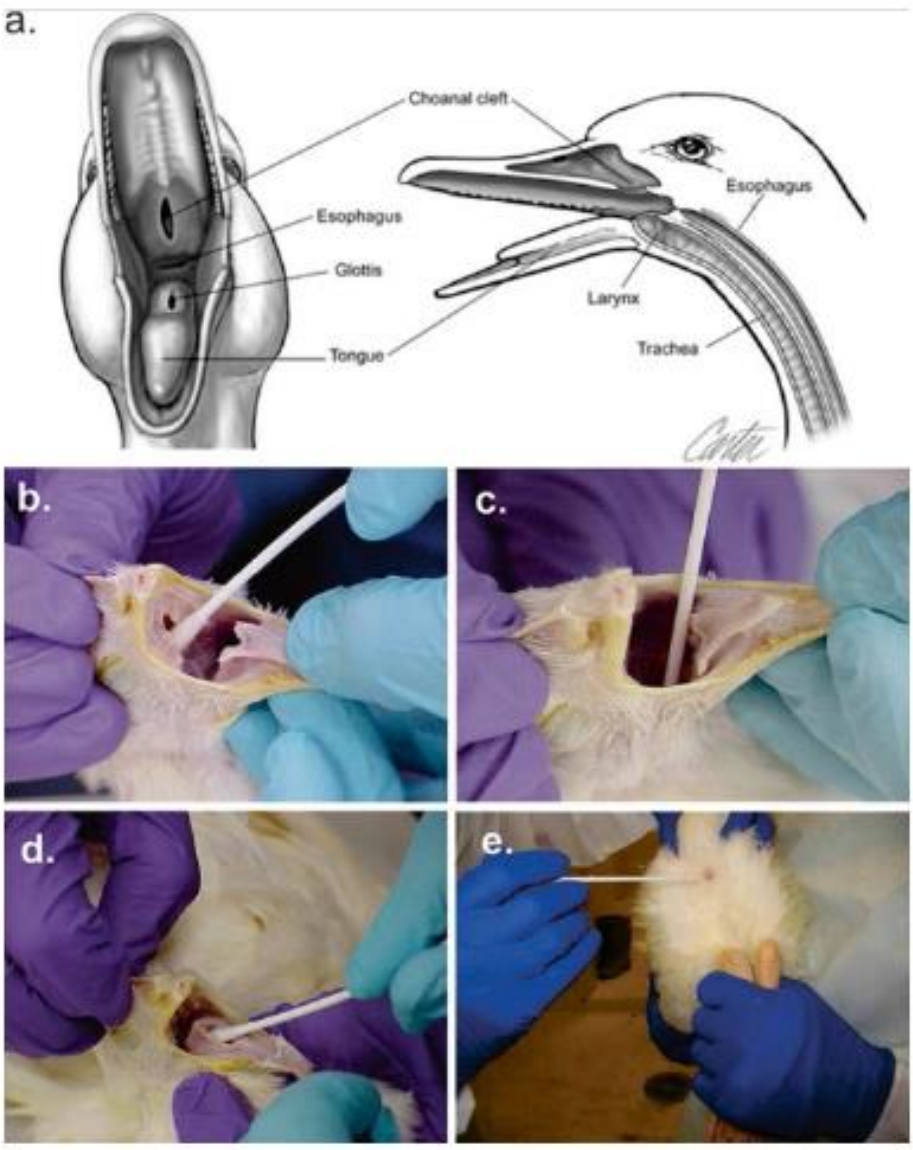
	tissue with lesions
Marek's disease	1^0 Sciatic nerve, brain, eye, tumors (skin & internal organ nodules)
Fowl pox	Dry for: skin scab (wattle/comb/face). Wet form: Trachea larynx
Infectious laryngotracheitis & infectious bronchitis	1^0 trachea, larynx, conjunctive, 2^0 any tissue with lesions In infectious bronchitis kidney
Coccidiosis & any enteritis	Portions of the intestine with visible lesion
Pullorum disease/Fowl typhoid	1^0Intestinal lesions, Liver (nodule), gall bladder, gonads, in young chicks yolk sac 2^0 any tissue with lesions

Samples should be promptly submerged in a solution of 10% neutral buffered formalin (volume of formalin is at least 10 times the volume of all tissues). Lung tissue and other air-containing tissues may be wrapped gently in absorbent cotton to aide immersion.

When submitting sample please write the name and address of the owner, name, address and phone number of submitter and complete description of history, clinical signs, breed, sex, and other information.

Annex four

Diagram that indicate oropharyngeal swab collection from live chicken



Schematic diagram for swab collection (Courtesy of Spackman *et al*., 2008, Methods in molecular biology)