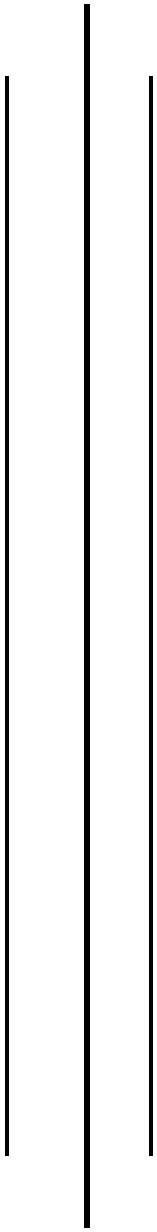
National Surveillance Plan for Highly Pathogenic Avian Influenza (HPAI)



Ministry of Agriculture and Cooperatives
Department of Livestock Services
Hariharbhawan
July 2008

National Surveillance Plan for HPAI

Department of Livestock Services,
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Disclaimer: text used within this plan has been freely adapted from a multitude of sources including, but not limited to: DLS, DLS-AICP, FAO reference documents, and FAO, CMC mission reports so as to best serve DLS for a comprehensive HPAI surveillance plan.

The Ministry of Agriculture & Cooperatives, Department of Livestock Services (DLS), coordinates HPAI surveillance among poultry and works collaboratively with wildlife agencies regarding wild birds. Development of this plan will help identify strengths and weakness of the surveillance approach, communicate the approach to stakeholders and assist in managing the implementation and financial management of activities. This plan shall be reviewed as and when needed by a surveillance plan review committee appointed by DAH and updated to coordinate DLS and donor work plans.

Acronyms and case definitions

AGPT	Agar gel precipitation test
AI	Avian influenza
Backyard birds	Birds reared as scavengers to feed on left over grains, grasses, vegetables and insects, primarily kept for cash and household consumption
Buffer zone	Is an area around the lake or wetland used by migratory birds and is specified by the wild life authority. OR An area about 1-2 Km around the lake where migratory birds visit
CBO	Community based organization
Clinical examination	Examination of flocks/birds based on the history and clinical signs and symptoms
Confiscated birds	Birds which have been informally transported into Nepal and intercepted by various government officials
Commercial chickens	Reared within a confined house, shed, or fence and feed on commercial rations, primarily for commercial purposes
CVL	Central Veterinary Laboratory
DAH	Directorate of Animal Health
Dangerous contact	Premises which are linked through physical proximity or movement of birds with the infected premises. Such premises may be located in the infected (up to 3 Km) or high alert zone (3-10Km) or outside the high alert zone
DLS	Department of Livestock Services
DLSO	District Livestock Services Office
DIT	Disease Investigation Team
FAO, UN	Food and Agriculture Organization of the United Nations
FMD and TADL	Foot and Mouth Disease and Transboundary Animal Diseases Laboratory
High Alert Zone	Surveillance zone of 3-10 Km out side of the infected zone
HPAI	Highly Pathogenic Avian Influenza (H5, H7)
HA/HI	Hemagglutination / Hemagglutination Inhibition
Infected Premises	Infected premises can be an individual commercial farm, a household; a live market or where birds are not confined within a specific area, such community area as may be determined by the competent authority where HPAI has been confirmed.
Infection Zone	An area within a radius of 3 KM from premises or epicenter where HPAI has been confirmed. Such area shall be determined by the competent authority.
NARC	National Agriculture Research Council
OIE	World Organization for Animal Health
PCR	Polymerase chain reaction
RDLS	Regional Directorate of Livestock Services
RVL	Regional Veterinary Laboratory
Premises	Premises can be a household or households, live markets and/or community areas.

SC	Service Center
SSC	Sub-Service Center
VDC	Village Development Committee (includes municipalities)
VEC	Veterinary Epidemiology Center
Wet market	Is a place or community area or local *haat-bazar* where live poultry of mixed species and/or the fresh meat of poultry being sold
Wild water bird zones	Major wild water bird zones (national park, lakes, wetlands) along the plain districts close or bordering India used by migratory water birds for the purpose of HPAI surveillance

Rationale

To date, highly pathogenic avian influenza has not been detected in Nepal. However, there is concern that the country is at risk of infection because of its proximity to Bangladesh, where the disease has persisted. Nepal also shares a long, porous border with India, which has experienced sporadic outbreaks over the past two years. During 2008, India has declared several outbreaks within 50 kilometers of the Nepal border. A few HPAI outbreaks have been reported in Tibet, but with a sparse human and poultry population and minimum trade with Nepal, it does not pose a significant threat for HPAI transmission. Migratory birds also appear to play a role is some cases of HPAI transmission, and Nepal is within the continental migratory pathways for wild waterfowl. Therefore the objectives for the national surveillance plan are as mentioned below. The sampling framework and numbers of samples selected under this plan is assumed to give an adequate level of confidence in the results obtained.

Objectives of sampling

- Early detection of clinical disease and infection
- Assess temporal and spatial patterns of the disease to improve effectiveness of control efforts
- Help define and control risks to public health
- Maintain the viability of subsistence level poultry production and help assure food security

Key stakeholders

- Department of Livestock Services
- National Agriculture Research Council
- Wildlife Department
- Zoological and aviary collections
- Animal health related NGOs (National and International)
- Poultry producers and related organizations
- Farmers and farmer organizations, CBOs
- Others such as civil society, consumer's forum etc

OIE HPAI Definition

Avian influenza (AI) is a highly contagious viral disease affecting several species of food producing birds (chickens, ducks, turkeys, quails, guinea fowl, etc.), as well as pet birds and wild birds.
The AI viruses are divided in two groups based on their ability to cause disease (pathogenicity). Highly pathogenic avian influenza (HPAI) virus spreads rapidly, may cause serious disease and may result in high mortality rates (up to 100% within 48 hours). The low pathogenic avian influenza (LPAI) can causes mild disease that may be undetected or no symptoms at all in some species of birds.

Part I
Surveillance - Absence of HPAI

Avian populations at risk

Commercial Birds–chickens and ducks
Nepal has a domestic poultry population of approximately 24 million birds, 45% of which are raised commercially and 55% as backyard birds (MOAC, 2008). The majority of the layer flocks and broiler flocks are in Chitawan and Kathmandu Valley. There are an estimated 58 hatcheries, 800 poultry producers, and 400,000 domestic ducks in Nepal. The commercial hatchery industry normally obtains parent birds from Europe, Asia and Australia. Some of these countries have suffered occasional HPAI incidents, however grandparent flocks in most of these countries are under strict biosecurity measures and national HPAI surveillance and response capacity is of a high standard. Such grandparent flocks are highly unlikely to become infected with HPAI virus. The possibility of domestic birds coming from Tibet is negligible due to long distances and minimum trade. There are minimal biosecurity practices across the industry, chicks most likely are being transported informally from India due to lower costs, and migratory wild birds often use the same water areas as domestically raised ducks.

The risk for HPAI transmission to commercial chickens and ducks is moderate to high

Backyard Birds – chickens, ducks, pigeons, and other species
There is no specific population estimate or density distribution estimate for backyard birds in Nepal. They are mostly raised locally, but can be transported great distances and illegally may enter from India, in addition to being exposed to migratory wild birds. The possibility of domestic birds coming from Tibet is negligible due to low production, difficult terrain, long distances and minimum trade.

The risk of HPAI transmission to backyard birds is considered moderate to high.

Wild Birds –
In some countries, HPAI virus has been detected in dead wild water birds (shore birds and waterfowl). Investigation of unusual mortalities in wild birds can provide an early indication of HPAI infection. Nepal lies on the migratory flyway of large numbers of wild water birds. A map prepared by DLS presents 6 major wild water bird zones (lakes, wetlands) along the lower elevation districts close or bordering India used by migratory water birds and will serve as sites for HPAI sampling (see Figure XX). These zones will be reviewed during the 2008/2009 sampling season and updated as necessary. Domestic chickens and ducks in these zones are expected to act as sentinels for potential HPAI transmission. Recognized zones are:

1. Suklaphata Wild Reserve, Kanchanpur
2. Ghodaghodi Tal/Lake, Kailali
3. Bardia National Park, Bardia (Bishajari Tal/lake)
4. Chitwan National Park, Chitwan
5. Jagdishpur Tal/Lake, Kapilbastu
6. Koshi Tappu, Sunsari

It would be beneficial to sample wild bird species most likely to be infected with HPAI. Due to limited ability to identify wild bird species and the mixing of species at the sampling zones, no species stratification of sampling is planned.

The risk of HPAI transmission to backyard and commercial birds from wild birds is considered low.

For this plan, however, wild birds migrating into Nepal are considered as an unknown risk for HPAI transmission until testing confirms otherwise.

Live Markets

The majority of the birds in the live markets are backyard poultry with a small number of commercial chickens. There is no location mapping of markets currently or their most likely sources of birds. This data will be available in the future through DLS. It is understood that these birds do travel considerable distances within Nepal and the potential exists that some birds may be illegally transported from India The possibility of domestic birds coming from Tibet is negligible due to long distances and minimum trade.

The risk of HPAI transmission to commercial and backyard poultry from live market birds is moderate.

While no specific testing program is in place for live markets, birds in live markets will be sampled under the backyard bird surveillance strategy when sick and dead birds are identified. It may be difficult to accurately trace back the origin of positive samples obtained from swabbing feces in cages. Nevertheless the procedure has value in indicating the presence of virus in the market's catchments area and can provide clues for intensive investigation to identify the source.

Confiscated Birds

These birds will be sampled by DLS or animal quarantine staff under the Backyard Birds surveillance.

Birds informally transported into and within Nepal are considered high risk for HPAI transmission until testing confirms otherwise.

Surveillance Strategy

Risk Category for each District

There are 75 administrative Districts in Nepal. Each District is classified as high, medium, and low risk based on the following factors and will be updated as and when needed (See Figure 1 and Table 2):

- Shared border with India and proximity to Bangladesh
- Documented informally transportation of birds
- Commercial chicken populations
- Domestic duck populations
- Backyard bird populations

- Number and activity of live markets
- National parks/wetlands/lakes or Tal used by migratory wild birds and their proximity
- Road/highway access
- Disease situation in neighboring area across the border.

HPAI case definition

Many infectious diseases of bird resembles in symptoms. Early detection and prompt reporting may help stop the spread of disease. Signs to be observed and reported include: sudden death, high flock mortality, reduced egg production, diarrhea, depression and decreased appetite, breathing difficulties such as coughing, sneezing and gasping, nervous twitching or dropped wings or paralysis, and swelling of the head with purple colored combs and wattles, and subcutaneous haemorrhage on legs.

Poultry health triggers points for the purpose of surveillance

Commercial farm:

- Either food and water intake reduced by 20% or greater within two days, OR
- Mortality of 10 % or greater within two days OR
- Egg production drop of 20 % within two days

Backyard poultry:

- Either mortality of 5% or greater within two days, OR
- Death of more than 10 birds within two days in a ward or in a cluster of houses.

Type of surveillance samples

- Clinical examination consists of visual inspection looking for sick and dead birds in flocks or villages. If the poultry health "trigger points" are exceeded, DLSO shall undertake a field investigation or request the assistance of the disease investigation team if assed essential. Appropriate samples as mentioned below must be collected and sent to the laboratory, if the history and clinical signs are suggestive of HPAI.

- Swab samples:
    - It can be tracheal, Cloacal or fresh, wet feces.
    - Tracheal samples are best for species with the virus accumulating in the respiratory tract (chickens).
    - Cloacal swabs are best for species with the virus accumulating in the intestinal tract (ducks).
    - Fresh, wet feces swabs are useful for birds that are not handled (wild birds) or where it it uncommon to see sick or dead birds (live markets and wild)

- Blood (serological) samples:

For this surveillance plan serum samples should only be taken from avian species that do not always show clinical signs such as ducks (wild and domestic) and other wild water birds.

Blood sampling does have other applications, but for the purposes of this sampling plan, its use will be confined to ducks, whether healthy or showing any signs of disease.

- Collection of dead birds:

A fresh whole carcass is extremely valuable with any species of bird.

Method of surveillance

- **Passive**
  - Poultry producers and associations, community organizations, wildlife officials, NGO participatory groups, and village animal health workers are advised to report "trigger" points in poultry and other species of bird. The suspicion ("trigger points") of HPAI (notifiable disease) is mandatory by law for all poultry producers to report in the production cycles. However, this is not being practiced currently. DLS will attempt to create awareness campaign to improve these practices.

- **Active**
  - Collecting swab samples from some sick and dead birds from specific bird populations at risk
  - Visiting commercial farms, backyard poultry and live markets for clinical examinations
  - Collecting blood samples from healthy wild birds (waterfowl) at risk that typically don't show HPAI clinical signs.

National Sampling Strategy - **(see Annex for background calculations)**

Commercial chickens

- **Clinical examination shall be carried out in high and medium risk districts**
- **Scheduled swab sampling shall be carried out in High and medium risk districts**
- **On request swab sampling in response to disease report shall be carried out in all districts**

Step 1 – DLSO shall determine the population (number) and distribution of commercial chicken farms in their district

Step 2 – DLSO shall assign number (such as 1,2,3,….N) to each commercial chicken farm in the district . Based on the knowledge of local risk factors of the district, high risk farm shall be given top priority and these farms shall be over sampled. DLSOs shall submit a list of commercial chicken farms to DAH for approval.

Step 3 – Clinical examination shall be carried out by DLSO on a random subset of 10% of the total number of commercial farms each week.

Step 4 – Tracheal swabs shall be collected from up to 10 sick or dead birds per farm, at 1% of the selected farms (from Step 3)

Step 5 – Respond to all reports by poultry producers, participatory groups, etc of sick or dead birds and collect sample as appropriate. (Estimated up to 500 tracheal samples per year)

Estimated total number of laboratory samples per year for this population = up to 5700

Backyard birds

- **Clinical examination shall be carried out in High and medium risk districts**
- **Scheduled swab sampling shall be carried out in the high and medium risk VDCs of high risk districts**
- **On request, swab sampling shall be carried out in all districts**
- **Live bird markets and commercial duck farms including confiscated birds are also included here for sampling purpose.**

Step 1 – DLSO shall assign a number (such as 1,2,3…. N) to each VDC in the District
Step 2 – DLSO shall assign a defined risk classification to each VDC
Step 3 – Each DLSO shall submit a list of VDCs and their risk classifications to DAH for its approval
Step 4 – Randomly select 10% of the high risk VDCs in the District each month.
Step 5 – For each high and medium risk VDC, select 3 out of 9 wards where backyard poultry is present and visit at least once in a month.
Step 6 - For each selected ward, talk to the people of the ward and ask about trigger points to warrant an investigation, and then ask to clinically examine their backyard birds. The major live birds market of each ward selected must be visited for sick or dead bird and collect appropriate sample.
Step 7 – Out of 10% of the selected wards samples shall be taken from 3 premises or cluster of houses or live market from each ward. Premises can be homes, live markets and community areas. Up to 5 Sample shall be collected from birds per house,or cluster of houses, or live market.
Step 8 - Tracheal swabs shall be taken from sick or dead chickens.
Step 9 – Blood samples shall be collected from healthy ducks.
Step 10 – Respond to all reports by poultry raisers, participatory groups, etc of sick or dead birds and sample as appropriate. (Estimated up to 500 tracheal samples per year)
Step 11 – Samples from All confiscated birds shall be collected. If more than 25 birds, then sub-sample as per VEC direction.

Rationale: Live market and confiscated birds are included in the Backyard bird surveillance strategy due to programming ease. It is judged that neither of these sample types warrants a separate strategy. It is recognized that live markets can play an important role in the early detection of circulating virus amongst the poultry population through the use of serological sampling. As it has been determined that only sick and dead birds will be sampled for this plan and such birds are anticipated to be uncommon in the markets, low number of samples are expected. Likewise, it is expected that few birds per year shall be confiscated due to the secretive nature of illegal poultry trade across borders.

Estimated total number of laboratory samples per year for this population = up to 5900

Wild birds and Domestic ducks in Buffer Zones

- **Sampling on wild dead birds shall be carried out in all Districts**
- **Fecal samples from wild birds nesting places shall be conducted in Wild water bird zones**
- **Blood samples from domestic ducks will be collected from buffer zones (national park, lake and watershed areas)**

Freshly dead birds - carcasses
Wildlife officials, conservation organization members, participatory groups and the public residing in the vicinity of water body are requested to report dead birds to DLS for sampling. After proper wrapping Whole carcasses shall be submitted for testing.
Estimated total number of laboratory samples per year for this population < 100

Live birds – feces samples
Migratory waterfowl will be sampled by collecting fresh wet feces from areas used overnight by the birds in conjunction with wildlife officials. 10 pooled samples each containing feces samples are to be taken once monthly at each designated wild bird zone (see Figure XX) during the wild bird migration season of September to March of each year. Five separate samples are to be placed in one media tube (pooled) and repeated 10 times for each sampling zone each month.

Total number of laboratory samples per year for this population = 420

Live birds – blood samples or cloacal swabs
The possible live capture of wild birds would require evaluation by the appropriate wildlife officials. Currently, there are no plans for this type of surveillance in Nepal.

Total number of laboratory samples per year for this population = 0

Domestic backyard ducks - 6 buffer zones (national park, lake and wetland areas)

It is planned to blood sample 10 ducks per month chosen at the sampler's discretion in the buffer zone of each of the 6 wild water birds zones during the wild bird migration season (September1st to March 31th) in Nepal. Four of the 6 wild water bird zones have established buffer zones. However sample collection from ducks shall be restricted within 2 kilometers area of the wetland, lake, or national park.

Total number of laboratory samples per year for this population = 420

Surveillance data collected, analyzed, monitored, and reported

- All DLSO Chiefs are responsible to fax or email their data forms to DAH/VEC on every Thursday. DLSO shall also submit the report to the concerned laboratory when samples are collected and submitted.
- All Central, Regional, and Avian Laboratory Chiefs are responsible to fax or email their data forms to DAH/VEC on the last day of the month.
- Data will be compiled, analyzed, monitored, and a report created by DAH/VEC. This report will be in electronic form and be sent by the 4th of each month to: DLS, RDLS, RVL, and all DLSO.
- Please refer Annex for all reporting forms (district, laboratory and VEC) used in this plan.

Part II
Surveillance – HPAI outbreak or suspected outbreak

Once the HPAI infection is detected, or is strongly suspected, there is a need to intensify the active surveillance activities. Specific surveillance strategies will be applied to the Infected and High Alert zones. This plan does not describe other activities like culling, cleaning, and disinfection during an outbreak.
While demarcating the Zones, it may not need not be circular, may be distorted based on risk contouring. Most importantly tracing from the identified point of outbreak shall be conducted. Backward tracing will help to establish the source of infection. Forward tracing will help to identify dangerous contact premises.

1. **Infection Zone Activities:** (0-3 kilometers)

- HPAI testing during culling activities as per VEC direction
- Emergency Disease Investigation Team mobilized
- After culling, cleaning, and disinfection, sampling will occur as per VEC direction
- When re-stocking is allowed, sampling design shall be carried out as per direction from VEC
- Visit any infected commercial premises weekly for 90 days to inspect the sealed gate, burial site, and to confirm no restocking of poultry

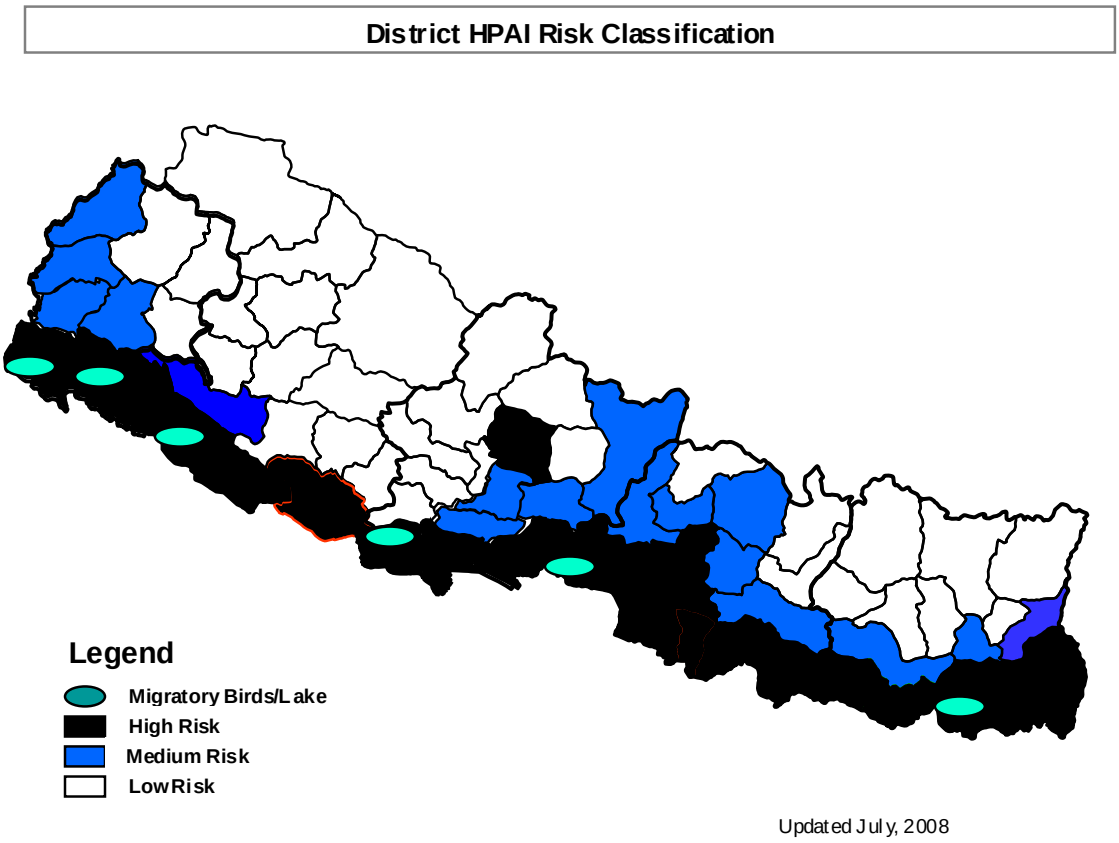
2. **High Alert Zone Activities**: (3 -10 kilometers)

- Visit all commercial poultry premises- clinical surveillance followed by sampling of sick dead birds (twice weekly) for 90 days.
- Visit live bird markets, poultry distributors, slaughter facilities, and other key stakeholders (twice weekly) if this activity is allowed to occur.
- Visit all villages within High alert zone -conduct community dialogue and sample as indicated (twice weekly) for 90 days.
- Emergency Disease Investigation Team shall be mobilized

3. **Dangerous Contacts Activities: (can be within or beyond each of the above zones)**

- HPAI sampling design during culling activities shall be as per the VEC direction
- Emergency Disease Investigation Team mobilized
- After completion of culling, cleaning, and disinfection activities sampling design shall be carried out as per direction from VEC .
- Visit any infected commercial premises weekly over a 90 day period following completion of decontamination procedures to inspect the sealed gate, burial site, and to confirm no restocking of poultry
- When re-stocking is allowed, sampling design shall be carried as per VEC direction

Figure 1 Classifications of Risk Districts and wild water bird zones



26 High Risk Districts
18 Medium Risk Districts
31 Low Risk Districts
6 Wild Water Bird Zones

Distribution of risk districts based on the 5 development region

Risk districts	EDR	CDR	WDR	MWDR	FWDR
High risk districts (26)	Ilam	Sarlahi	Kaski	Dang	Kailali
	Jhapa	Mahottari	Kapilvastu	Bardiya	Kanchanpur
	Sunsari	Dhanusha	Rupandehi	Banke	
	Morang	Kathmandu	Nawalparasi		
	Siraha	Lalitpur			
	Saptari	Bhaktapur			
		Makwanpur			
		Chitwan			
		Parsa			
		Bara			
		Rautahat			
Medium risk districts (18)	Dhankuta	Sindhuli	Syanja	Rukum	Darchula
	Udayapur	Dhading	Tanahu	Surkhet	Baitadai
	Pachthar	Nuwakot	Palpa		Dadeldhura
	Shankhuwashava	Kavre	Gorkha		
		Sindhupalchok			
Low risk districts (31)	Bhojpur	Dolakha	Manang	Humla	Bajhang
	Terathum	Ramechap	Lamjung	Mugu	Bajura
	Solukhumbu	Rasuwa	Mustang	Kalikot	Achham
	Okhaldhunga		Myagdi	Jumla	Doti
	Khotang		Parbat	Dolpa	
	Taplejung		Baglung	Rolpa	
			Gulmi	Salyan	
			Arghakhanchi	Pyuthan	
				Dailekh	
				Jajarkot	

EDR= Eastern Development region
CDR= Central Development region
WDR= Western Development region
MWDR= Mid Western Development region
FWDR= Far Western Development region

HPAI Sampling SOP

- **Commercial/backyard/live market chickens (sick or dead) – tracheal swabs, fresh whole carcasses**
- **Confiscated birds – fresh whole carcasses if dead and tracheal swabs if sick and suspected**
- **Commercial/backyard ducks – blood samples if sick and health and cloacal swab & whole carcass if recently dead**
- **Ducks in wild water bird buffer zones – blood samples (sick and healthy) cloacal swab & whole carcass (recently dead)**
- **Wild birds – if dead, collect the fresh whole carcasses, if alive, sample fresh wet feces**

Equipment needed:

- Sample collection kit, ***(items included in the kit shall be as approved by CVL)***
- cool box along with ice packs
- PPE
  - 1 pair Tyvek Coveralls
  - 1 pair Boot Covers
  - 2 pair Nitrile Gloves
  - 1 Plastic Apron (in plastic pouch)
  - 1 N-95 Particulate Respirator
  - 1 pair Goggles
  - 4 Alcohol Wipes
  - 1 PDI Sanitary Viricidal Wipe
  - 1 Infectious Waste Bag (red)

Tracheal/cloacal/fresh wet feces swabs

1. Wear PPE
   a. If HPAI unknown status – a minimum of N95 mask, gloves and boots
   b. If HPAI is confirmed – full equipment required
2. Purpose of the collection of sample will be explained with farmer's family, farmer's group and user's group at farm to get help and people participation.
3. Collect history of flock (species, breed, flock size, number of sick, number of dead, egg production and duration of sickness) on the sample form.
4. Inspect and select sick or recently dead bird for sampling.
5. Hold the bird and insert swab through mouth into the trachea. Rotate the swab gently around the tracheal wall. **OR..**
6. Hold the bird and insert swab deeply into the vent and rotate swab gently along the vent wall. The swab should be deeply stained with fecal material.

   OR..
7. Find fresh wet feces and insert swab into the center and rotate swab. The swab should be deeply stained with fecal material.
8. Open transport media tube and hold screw cap in the hand.
9. Insert the swab into transport media and cut excess portion of stick of swab by scissor.

10. Close the sample tube containing swab and media by applying screw cap
11. Label the sample tube (species code, type of swab, date, and place of collection) and pack properly.
12. Keep the sample into the cool box at 4°C.
13. Wipe out outer surface of cool box with sprit or spray with decontaminant.
14. Remove PPE and wash hand properly by using detergents.
15. Dispose of PPE by burning and burying on sampling premises
16. Transport the sample to the designated lab within 48 hours from collection.
17. Inform the lab about the arrival time of sample at as far in advance as possible.

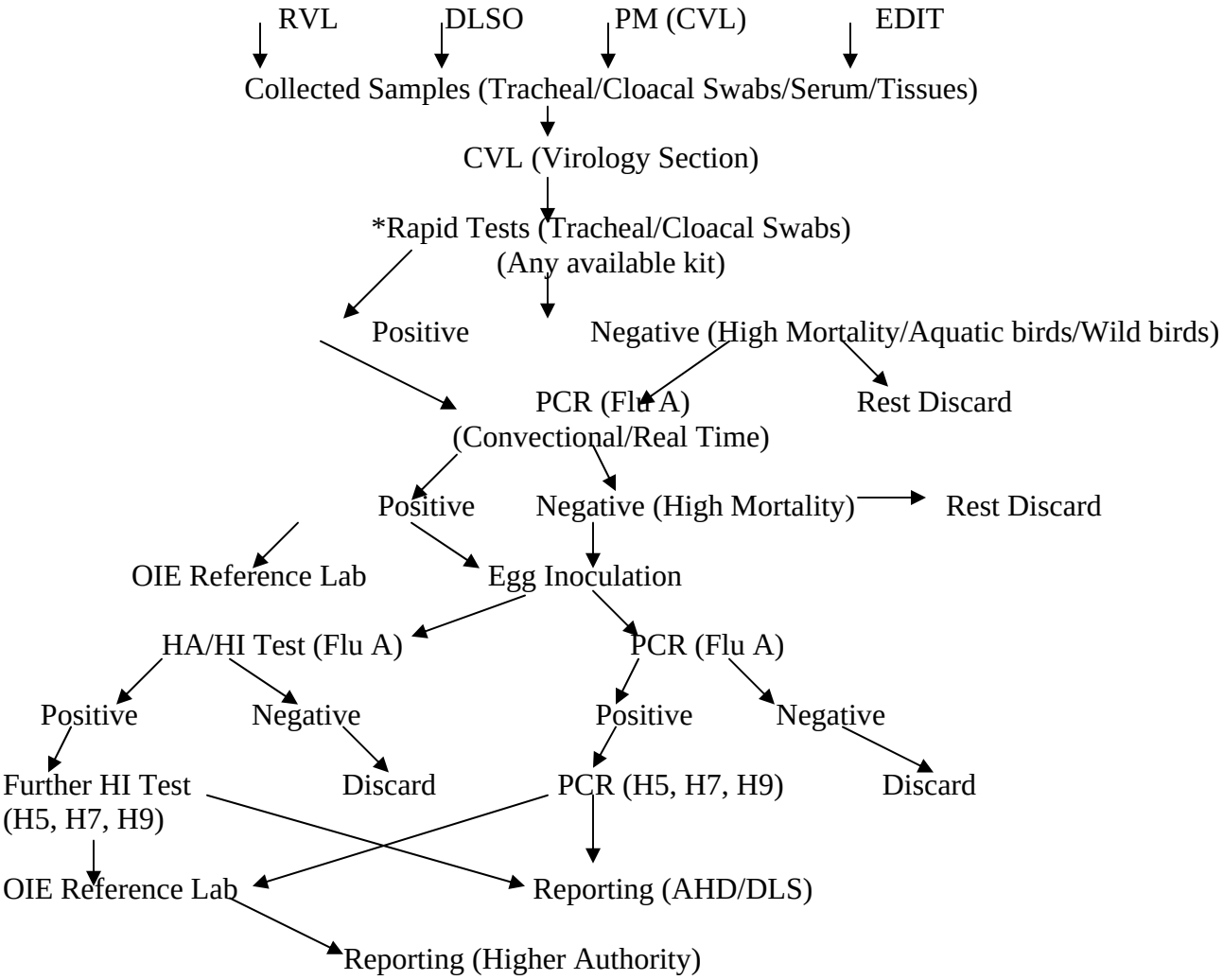
Whole fresh carcasses

1. Wear PPE
    - If HPAI unknown status – a minimum of N95 mask, gloves and boots
    - If HPAI is confirmed – full equipment required
2. Recently died birds are placed in a double plastic bag, with each one sealed
3. The other bag is labeled with a tag
4. Label the sample container (species code, type of swab, date, and place of collection) and pack properly.
5. Store carcass at 4 degree Celsius in cool box
6. Dispose of PPE by burning and burying on sampling premises
7. Transport the sample to the designated lab within 48 hours from collection.
8. Inform the lab about the arrival time of sample at as far in advance as possible.

Blood samples

1. Wear PPE
    - If HPAI unknown status – a minimum of N95 mask, gloves and boots
    - If HPAI is confirmed – full equipment required
2. Purpose of the collection of sample will be explained with farmer's family, farmer's group and user's group at farm to get help and people participation.
3. Collect history of flock (species, breed, flock size, number of sick, number of dead, egg production and duration of sickness) on the sample form.
4. Blood will be collected either from brachial (wing) vein or jugular (neck) vein.
1. Serum will be separated from blood by centrifugation at 3000 rpm for 10 minutes, if a centrifuge is not available, create a small air space in the blood collection syringe, leave the syringe containing blood in a slant position overnight at room temperature, or until the blood has become clotted and the serum is separated.
2. Pour or pipette serum from the syringe/tube and place it in a storage tube.
3. Label the sample tube (species code, type of swab, date, and place of collection) and pack properly.
4. Serum can be stored at 4° Celsius for one week, otherwise it shall be stored frozen at -20° C
5. Transport the sample to the designated lab within 48 hours from collection.
6. Inform the lab about the arrival time of sample at as far in advance as possible.

Future Strategic Plan for testing Samples Suspected of AI



* Regional Laboratories will also be performed Rapid Test. All samples positive for Avian Influenza A tested in RVL shall be sent to CVL for cross-checking and virus isolation. Whereas 10% of samples found negative shall also be sent to CVL for further confirmation.

Note: Disease Diagnosis for the following disease shall be conducted as differential diagnosis for AI in case of negative samples

- New castle disease
- IBD
- IB
- DHV
- DEV
- Salmonella
- Mycoplasma

Annex 1: Calculations for the National Surveillance Strategy

Background note on statistics for sample size:

Sample sizes at commercial poultry facilities and backyard premises are dependent on what assumptions are made regarding effective population sizes (if below 1000 individuals), expected prevalence, and desired confidence of detection. As the table below presents, a wide range of sample sizes could be assigned as targets based on the desired level of statistical significance, practicality of field sampling, and financial resources.

This national surveillance plan has established up to 10 sick or dead birds on commercial farms, 10 healthy ducks in wild water bird buffer zones, and up to 5 birds at backyard bird premises as sample targets. The assumption being if HPAI is present within the flock, the prevalence will most likely be above 20%. It is understood that there will be exceptions to this assumption. It is also understood that if HPAI is first detected in Nepal, it will most likely not be present in a large number of premises. It is much more likely to be found at a small number of clustered premises (within same District or VDC). The dilemma faced with defined resources is in deciding the merits of sampling more birds at a smaller number of premises across the country or to be more modest with samples at each premises, relying on a more likely higher flock prevalence, and allowing a greater distribution of samples. This effort is helped by stratification of the Districts and VDCs by risk classification.

Table1: Adapted from Cannon and Roe, 1986 Livestock Disease Survey

Population Size	Expected Prevalence %	Confidence of Detection	Sample Sizes
100	5	90/95/99	37/45/59
1000	5	90/95/99	44/57/86
100	10	90/95/99	20/25/36
1000	10	90/95/99	22/29/43
100	20	90/95/99	10/13/19
1000	20	90/95/99	11/14/21
100	40	90/95/99	5/6/9
1000	40	90/95/99	5/6/9

Annex 2: Calculation of Commercial Chickens:

Total number of farms = estimated 1000 nationally
10% of these farms for clinical examination each week

1000 farms X 10% = 100 farms per week
100 X 52 weeks = 5200 visits
Each farm will have clinical examination approximately 5 times each year.

1% of these farms for swab sampling of sick and dead birds each week

1000 farms X 1% = 10 farms
10 farms X up to 10 birds per farm = 100 birds per week
100 birds per week X 52 weeks = up to 5200 samples per year

In addition, participatory surveillance sampling – will depend on the cooperation of producers and associations, but may yield approximately 500 samples per year

- **Estimated total number of laboratory samples per year for this population = up to 5700**

Annex 3: calculation of backyard birds:

Estimate the effective target population
Estimated 24 million domestic birds in Nepal
Estimated approximately 55% are backyard raised
Estimated 8 of the 13 million backyard birds are in the High and Medium Risk Districts
Estimated 1% of these are sick or dead each year
Target population = 80,000 birds
Estimated 1000 high and medium risk VDC in the High Risk Districts

10% of these farms for clinical examination each month for High and Medium Risk Districts

1000 VDC X 10% = 100 VDC
100 VDC X 12 months = 1200 visits
Each VDC will have clinical examination approximately 1 time each year.

1% will have swab sampling each month – only in High Risk Districts

1000 VDC X 1% = 10 VDC
10 VDC X 3 wards/village = 30 wards
30 wards X 3 premises/ward = 90 premises sampled per month
90 premises X up to 5 birds/premises or cluster of premises = up to 450 samples per month
450/month X 12 = 5400

In addition, participatory surveillance sampling in High and Medium Risk Districts – will depend on the cooperation of producers and associations, but may yield approximately 500 samples per year

That means we are sampling 10-15% of the effective population per year (80,000/5900 = 15%)

Backyard birds in Medium Risk Districts will be sampled at the owner's request (participatory), but not scheduled swab sampling as the High Risk District backyard birds. This is based on the combination of epidemiological assessment of disease transmission risk and using judgment to allocate finite financial resources.

- **Estimated total number of laboratory samples per year for this population = up to 5900**

Annex 4: calculation of wild birds:

Dead birds – very few will be found and turned in, estimate less than 100/year

Wild water birds – 6 identified sampling zones
50 fresh, wet feces swabs samples per sampling zone collected in 10 pooled samples (5 samples per pool)
Sampling for 7 months of the year
6 X 10 (5 samples per pool) fresh, wet feces X 7 = 420 samples per year

Domestic ducks within the sampling zones
10 (blood samples or fresh, wet feces swabs) samples per sampling zone
Sampling for 7 months of the year
6 X 10 X 7 = 420 samples per year

- **Estimated total number of laboratory samples per year for this population = up to 940**

Summary of total number of samples for national surveillance plan (absence of HPAI)

1. **Commercial poultry**
   - Estimated total number of laboratory samples per year for this population = up to 5700
2. **Backyard poultry**
   - Estimated total number of laboratory samples per year for this population = up to 5900
3. **Wild freshly dead birds from wild life areas/national par**
   - Estimated total number of laboratory samples per year for this population < 100
4. **Wild water birds** (fresh wet feces of live birds to be collected in the resting sites of migratory birds from 6 wild water bird zones
   - Total number of samples per year =420

5. Domestic backyard ducks - 6 Wild Water Bird buffer zones

- Total number of laboratory samples per year for this population = 420

Total number of samples from all the population = 12540

Table 2: District Risk Classification

High Risk	Districts	Border	Highway	Poultry population	Duck population	Other 1	Other 2
1	Jhapa	X	X	362669	12149	illgeal birds	
2	Ilam	X	X	215800	566	illgeal birds	
3	Morang	X	X	663632	62200	Koshi Tappu/Lake	
4	Sunsari	X	X	472614	52200	close to Koshi Tappu	
5	Saptari	X	X	255250	10270	close to Koshi Tappu	big live market
6	Siraha	X	X	402900	7200	close to Koshi Tappu	
7	Dhanusa	X	X	182988	7899		lake
8	Mahatori	X	X	293250	5900		
9	Sarlahi	X	X	149031	7512		
10	Bara	X	X	235240	17619		
11	Rautahat	X	X	275455	8579		
12	Parsa	X	X	181769	9550		busy border
13	Makwanpur	NO	X	464330	3847	close to wildlife	
14	Kathmandu	NO	X	2302650	3024	previous LPAI	illegal birds
15	Lalitpujr	NO	X	879885	4523	movement	illegal birds
16	Bhaktapur	NO	X	585990	5655	movement	illegal birds
17	Chitwan	NO	X	2784500	19500	national park	lake/river
18	Nawalparasi	X	X	457436	24200	close to wildlife	
19	Rupendahi	X	X	507545	25060		busy border
20	Kapilbastu	X	X	235559	5120	lake/watershed	previous LPAI
21	Kaski	NO	X	649686	9599	lake/ wild birds	illegal birds
22	Dang	NO	X	545460	7850	watershed	
23	Banke	X	X	468304	2100		busy border
24	Bardia	X	X	350030	2302	wildlife	rivers
25	Kailali	X	X	373190	6850	lake/park/illegal	close to 2 national park
26	Kanchanpur	X	X	181800	1380	wild life	river
Medium							
1	Darchula	X	X	96692	242	river as a barrier	
2	Baitadi	X	X	41200	134	river as a barrier	
3	Dadeldhura	X	X	39500	507	river as a barrier	
4	Palpa	NO	X	275675	460	link road to Pokara	
5	Syanja	NO	X	225672	520	link road to Pokara	
6	Gorkha	NO	X	299876	1330	link road to Pokara	
7	Tanahu	NO	X	431542	2555	link road to Pokara	
8	Dhading	NO	X	475693	2446	link road to Pok/KTM	

9	Sindhuplanchowk	Indo-China	X	291231	799		
10	Nuwakot	NO	X	754697	2138	previous LPAI	close to KTM
11	Kavre	NO	X	713900	5500		close to KTM
12	Sindhuli	NO	X	287651	5500		
13	Shankhuwashava			327030	1179		close to Tibet
14	Udyapur	NO	X	347832	3647		
15	Pancthar	X	X	107860	995		
16	Dhankuta	NO	X	153565	567		
17	Surkhet	NO	X	280986	8060	lake	large duck #'s
18	Rukum	NO	X	164903	4500	lake	large duck #'s
Low							
1	Taplejung			156512	2596		
2	Bhojpur			186375	1483		
3	Terhathum			106400	1363		
4	Solukhumbu			137628	976		
5	Okhaldhunga			168615	1307		
6	Khotang			265709	1896		
7	Dolakha		X	354723	4225		
8	Ramechap			203965	553		
9	Rasuwa		X	88333	224	way to Tibet	
10	Manang			3522	6		
11	Lamjung		X	192425	4393		
12	Mustang			10209	35		
13	Myagdi			198963	920	close to Pokhara	
14	Parbat		X	176086	4652	close to Pokhara	
15	Baglung		X	131300	1791	close to Pokhara	
16	Gulmi		X	175766	937		
17	Arghakhanchi			182185	175		
18	Humla			31050	10		
19	Mugu			43062	212	lake	
20	Kalikot			29300	163		
21	Jumla			26475	270		
22	Dolpa			69637	81	way to Tibet	
23	Rolpa			170020	89		
24	Salyan			361557	2500		
25	Pyuthan			112500	83		
26	Dailekh		X	140986	1123		
27	Jajarkot			496227	345		
28	Bajhang			52664	389		
29	Bajura			47919	865		
30	Doti	NO	X	88218	282	wildlife	
31	Achham		X	100391	73		

Disease Reporting Form for Districts

Sample submitted to CVL/NAL/RVL Date:……. Risk Category……..

Development Region……Regional Code……..District……District Code……

Type of farm/Premises	Name & address of Farm	VDC/ Farm code	Ward No.	Total Birds	No. Affected	No. dead	Major Sign & symptoms	Type and number of Samples Submitted						*Coding with date of sample collection
								TS	CS	S	FF	DB	Other	

* As per coding Guidelines & coding for sample number collected from one farm should be 1 to ……N irrespective of sample type.

Type of Farms (Code):

CD=Commercial Duck
CB=Commercial Broiler
CL=Commercial Layer
BC=Backyard Chicken
BD=Back Duck
LM=Live market Bird
IB= Illegally Imported/Confiscated Birds
WB= Wild Bird

Risk Categories (Code):

HRD= High Risk Districts
MRD= Medium Risk Districts
LRD = Low Risk Districts

Type of Samples (Code):

TS=Tracheal Sample
S= Serum
CS=Cloacal Swab
FF=Fresh Faecal
DB=Dead Bird
Others=ND/Fowl cholera/ILT/IBD/EDS

Farm Code (for commercial Chicken): 1,2,3,4…N

- As per coding Guidelines & coding for sample number collected from one farm should be 1 to ……N irrespective of sample type. .
- **Premises Include:** Premises can be homes, live markets and/or community areas

Signature:
Dtae:

Monthly Disease Reporting Form (CVL/NAL/RVL)

Name of Laboratory: Date:

Risk Category	District Code	Type of farm/ premises	Name & address of Farm	VDC/ Farm code	Ward No	Date		Number of Samples Tested						Result			Referred to:
						Sample Received	Sample Tested	TS	CS	S	FF	DB	Other	+ve	-ve	Others	

Type of Samples:

TS=Tracheal Sample
S= Serum
CS=Cloacal Swab
FF=Fresh Faecal
DB=Dead Bird
Others= Eg. Tissue

Risk Categories: HRD= High Risk Districts
MRD= Medium Risk Districts
LRD = Low Risk Districts

Type of Farms (Code):

CD=Commercial Duck
CP=Commercial Poultry
BB=Backyard Birds
LM=Live market Bird
IB= Illegally Imported/Confiscated Birds
WB= Wild Bird

- As per coding Guidelines & coding for sample, number of sample collected from one farm remain the same with the same type of samples irrespective number of samples.
- **Premises Include:** Premises can be homes, live markets and/or community areas

NO of risk districts
1- High risk districts- 26
2- Medium risk districts-18
3 Low risk districts- 31
4- Wild water buffer zones-6

Monthly Disease Reporting Form for VEC/DAH

Date:

Risk Category	Dist Code	Total Farm/ premises	No Farm/ premises visited	Total Birds	Affected farm/ premises	Affected birds	Dead birds	Number & Type of Samples Submitted					Number of Samples Tested					Result		
								TS	CS	S	FF	DB	TS	CS	S	FF	DB	+ve	-ve	others

Type of Farms (Code):

CD=Commercial Duck
CP=Commercial Poultry
BB=Backyard Birds
LM=Live market Bird
IB= Illegally Imported/Confiscated Birds
WB= Wild Bird

Risk Categories (Code):

HRD= High Risk Districts
MRD= Medium Risk Districts
LRD = Low Risk Districts

Type of Samples (Code):

TS=Tracheal Sample
S= Serum
CS=Cloacal Swab
FF=Fresh Faecal
DB=Dead Bird
Others=ND/Fowl cholera/ILT/IBD/EDS

- As per coding Guidelines & coding for sample number collected from one farm should be 1 to ……N irrespective of sample type.

Premises Include: Premises can be homes, live markets and/or community areas