RATIONALE FOR AVIAN INFLUENZA SURVEILLANCE IN WILD BIRDS IN CANADA IN 2006:

Surveillance for Avian Influenza in wild birds serves several important purposes.

- Protection of Canada’s export trade in poultry and poultry products
  Mitigation efforts to minimize the socioeconomic impact of the most recent occurrence of Avian Influenza in poultry in BC (domestic ducks, fall 2005) benefited greatly from background information on virus strains in wild ducks generated in the 2005 survey. It is predicted that current, credible data on Avian Influenza strains in wild birds in Canada will similarly reduce the socioeconomic impacts of future outbreaks of Avian Influenza in commercial poultry in Canada. Conversely, absence of such data is an important risk factor for larger socioeconomic impacts.

- Early detection of highly pathogenic Avian Influenza strains
  Surveillance based on wild birds found dead is the most sensitive surveillance method available for detection in wild birds of HP strains of Avian Influenza, such as the Eur-Asian H5N1 strain, irrespective of how these strains may arrive in Canada. Detection of such strains as early as possible upon their arrival or evolution in Canada is one of the most critical mitigation factors in reducing their human health and socioeconomic impacts.

- Maintenance and improvement of national laboratory and surveillance capacity for Avian Influenza viruses
  Rapid analysis of wild duck samples in 2005 identified many weaknesses in Canada’s national capacity to manage Avian Influenza in any species, but the survey also produced many solutions and improvements to national capacity. Continuation of the wild bird survey will play a critical role in maintaining and improving overall national Avian Influenza surveillance capacity, including field, laboratory, and communication components, and contributions to government policy.

- Risk analysis and risk communication
  The risk factors posed by Avian Influenza viruses in wild birds in Canada to
human health, human economies and natural resources can only be assessed on the basis of correct and detailed information about the range of Avian Influenza virus strains, their distribution among species and regions, their variation among years, regions and species, and the extent of interchange with pools of virus strains in Asia and Europe. Wild bird Avian Influenza surveillance is the only means of obtaining this information.

- International contributions and obligations
  - In the current climate of concern regarding potential pandemic Avian Influenza, Canada has an obligation to contribute to the global understanding of Avian Influenza virus strains in wild birds since these birds represent the ultimate global reservoir of the Avian Influenza gene pool. Canada is geographically situated such that early detection of foreign virus strains arriving in the Americas via transatlantic bird migration can only be achieved by surveillance conducted in Canada.

OBJECTIVES OF THE 2006 SURVEY

1. To achieve a high level of vigilance for the early detection of highly pathogenic (HP) strains of avian influenza virus, particularly the Asian/European/African strains of highly pathogenic H5N1 virus, through enhanced detection, collection and medical examination of wild birds found dead in all parts of Canada, including the Arctic.

2. To determine if foreign strains of avian influenza viruses are carried to the Americas by transatlantic migrant wild birds by means of collection of cloacal swab samples from Europe-wintering Eastern High Arctic Brant (Branta bernicla hrota) and Red Knots (Calidris canutus) passing through Iceland on spring migration to the Canadian High Arctic, from Greater Snow Geese (Chen caerulescens) in Nunavut on their Arctic breeding ground in summer and hunter-killed birds in autumn in Quebec, from Common and King Eiders (Somateria sp.), some resident in the St Lawrence Estuary and some migrating to and from Greenland and nesting in Canada, from Arctic-nesting migrant geese (Chen sp, Branta sp) passing through southern Manitoba in autumn, from migrant Canada geese which moult-migrate in Greenland, from Africa-wintering Arctic Terns (Sterna paradisaea) nesting in New Brunswick and from eastern Arctic-nesting Semipalmated Sandpipers (Calidris pusilla) on post-nesting southward migration in summer in New Brunswick and/or Nova Scotia. Some additional sampling and testing may occur if opportunities and resources permit.

3. To repeat the 2005 live duck survey in four of the six regions sampled in 2005 in order more fully to document this immense reservoir of avian influenza viruses in Canada, and to measure year-to-year variation by comparison of results from 2005
and 2006.

4. To sample wild ducks within the Lower Fraser Valley of British Columbia in order to obtain information on virus strains in wild ducks in this area of intense poultry farming where epidemic HP avian influenza occurred in poultry in 2004.

5. To assess other potentially important avian reservoirs in influenza A viruses in Canada through collection of samples from live or hunter killed wild geese, both arctic-nesting populations (via objective no. 2 above) and temperate nesting populations in close contact with large human populations, and through collection of samples from crows (American Crow - *Corvus brachyrhyncos*, and Northwestern Crow - *Corvus caurinus*) available through Canada’s West Nile Virus surveillance program.

6. To complement and extend, but not to duplicate unnecessarily, surveillance for avian influenza in wild birds that is to be carried out by the United States. Reciprocal sharing of survey results among Canada, the United States and Mexico is included in this objective.

ANTICIPATED PARTICIPANTS:

It is intended that this surveillance program will continue to build and enhance health management capacity in Canada through collaboration among public health, agriculture and wildlife agencies within federal and provincial/territorial governments, and with universities.

Primary Federal Participants:
- Public Health Agency of Canada
- Canadian Food Inspection Agency
- Environment Canada - Canadian Wildlife Service

Primary Provincial Participants:
- Provincial Departments responsible for Public Health
- Provincial Departments responsible for Agriculture (animal health)
- Provincial/Territorial Departments responsible for Wildlife

Primary Non-government Participants:
- Canadian Cooperative Wildlife Health Centre (Canada’s veterinary colleges)
- Centre for Coastal Health (Malaspina University, Nanaimo)
- Ducks Unlimited Canada
- Wildlife Rehabilitation Stations

International Participants:
SURVEY GOVERNANCE

Steering Committee: All participating agencies will be members of the Survey Steering Committee. This Committee met by teleconference on 23 January 2006 to assess the need for a Survey in 2006. The Steering Committee will receive and comment on all documents and will meet by teleconference as necessary.

Survey Executive Committee:
This Committee will consist of members representing primary federal and provincial/territorial participant groups. There will be one member each from the Canadian Food Inspection Agency, the Public Health Agency of Canada, Environment Canada, from each the provincial/territorial committees of Chief Veterinary Officers, Chief Medical Officers of Health, and Wildlife Directors, and the CCWHC as survey coordinator

Survey Coordination:
The Canadian Cooperative Wildlife Health Centre will coordinate program design, and will implement and coordinate the surveillance program itself, in accordance with the Steering Committee’s decisions and direction from the Survey Executive Committee.

SURVEY COMPONENTS AND PARTICIPANT RESPONSIBILITIES

I. Enhanced Surveillance in Birds Found Dead

1. Sample Collection:

In general, provincial and territorial wildlife agencies will take the lead in each province and territory in establishing protocols and assuring collection of dead birds and shipment of these to participating laboratories. The Canadian Wildlife Service also will be a major participant.
● Each Province and Territory will establish a protocol of communications with communities, the public and participating agencies, and for the detection, collection and shipment of specimens, assisted by the CCWHC as survey coordinator.

The protocols for communications and for detection, collection and shipment of dead birds in each province and territory will aim at a balance between the need for enhancement of surveillance (a 2-3 fold increase in the number of dead wild birds examined per year), and the limits on resources, and both agency staff and laboratory capacities. Judgements will have to be made concerning which specimens to include in the survey and which to exclude.

● In general, priority will be given to:

  - Bird species which use aquatic or wetland habitats
  - Mortality events that appear unusual in some way for the region and location
  - Mortality events involving more than one species
  - Mortality events involving notable numbers of birds

● Wildlife rehabilitation stations often are the first to receive reports of dead wild animals from the public. These stations also often function after hours and on weekends, when most calls from the public will be received. As a consequence, these stations will certainly receive notifications and inquiries from the public regarding dead birds and the Wild Bird Influenza Survey, and each will require specific information on the Survey protocols to be followed in their province or territory.

2. Specimen Examination

Veterinary diagnostic laboratories which participate in Canada’s general national wildlife disease surveillance program will receive and examine the dead birds collected as part of the 2006 Survey. These laboratories are the CCWHC Regional Laboratories at Charlottetown PEI, St-Hyacinthe QC, Guelph ON and Saskatoon SK, Animal Health Centre in Abbotsford BC (Ministry of Agriculture, Food and Fisheries) and the Veterinary Services Laboratory in Winnipeg (MB Ministry of Agriculture, Food and Rural Initiatives).

● Each bird, or a sub-sample of birds from large-scale mortality events from which many specimens are sent to the laboratory, will be examined to determine cause of death and each also will be tested specifically for Influenza A viruses regardless of cause of death. When the number of birds received exceeds the capacity of the diagnostic laboratory to examine them on a priority basis, specimens for influenza assessment will be extracted and processed, and the carcass then will be frozen and examined for cause of death at a later date.

● For Influenza assessment, two tissues – the entire cloaca with its content, and a sample
of lung no smaller than 1 cm$^3$ – will be collected in a single container, and either passed immediately to a participating virology laboratory or frozen and shipped immediately to a participating virology laboratory.

- A complete record of each examination, and all findings with respect to cause of death, will be entered into the national wildlife disease database by the participating diagnostic laboratory as soon as these data are known.

- Participating virology laboratories will treat the combined cloaca and lung as a single, pooled sample, and will not analyse the two tissues separately. The participating virology laboratories will be the same laboratories of the National Avian Influenza Laboratory Network which will analyse swab samples collected in the 2006 Survey (see below) and will follow the same protocols for immediate PCR analysis for Matrix Protein, followed by PCR for H5 and H7 proteins if positive for Matrix Protein. (See virology section for live bird survey, below). Isolation of viruses from Matrix positive but H5/H7-negative samples and submission to NCFAD/NML will be done as for samples from live wild birds

- Because the dead bird survey is the component of the 2006 Survey through which pathogenic strains of influenza are most likely to be detected, virology laboratories will give first priority for Matrix, H5 and H7 PCR analysis to samples received from the dead bird survey

II. Samples from Live, Healthy Wild Birds, from Hunter-killed Birds and from Dead Crows

1. Sample Collection:

- Samples will consist of two (2) swab samples taken from each bird, one a swab of the pharynx (back of the mouth/throat) and a second of the inside of the cloaca (feces). These two swabs from each individual bird will be placed together in a single sample vial and thereafter analysed as a single sample. These two-swab samples will taken from live and from hunter-killed, healthy wild birds, or from West Nile virus-negative dead crows submitted to the West Nile surveillance program. Immediately upon collection, the swab tips will be placed in vials containing virus transport medium. Swabs and transport medium will be supplied by the CCWHC. Once collected, the sample vial will be kept ice-cold for up to 1-2 days and frozen as soon as possible. Samples will not be permitted to thaw and will be delivered to participating laboratories in a fully frozen state.
Complete field records will be kept for each sample, including all data required by the Survey database. These records will be entered directly into the Survey database or will be sent immediately to the CCWHC for entry.

The Canadian Wildlife Service (Environment Canada) will take the lead in obtaining samples from wild ducks trapped for banding and other purposes as part of its on-going waterfowl management programs.

- The sampling protocol used in the 2005 Survey will be repeated as exactly as possible (same sites, same target numbers of species: 500 Mallards, 300 other duck species, emphasis on hatch year birds) in 2006 in Interior BC, Alberta, Quebec and the Atlantic Provinces. Because of the excessive collection effort required to secure 500 samples from mallards in Atlantic Canada, 500 samples will be obtained from Black Ducks instead, in the Atlantic region only.

- An additional collection of 800 samples, following the same protocols, will be made from wild ducks trapped on the coastal marshes at Delta BC, at a location at least 10 km distant from commercial poultry farms.

The Canadian Wildlife Service also will take the lead in securing samples from Atlantic Brant and Red Knots on spring migration to Canada during staging in Iceland, collecting 600 samples from eastern arctic nesting Semipalmated Sandpipers during fall staging on the Bay of Fundy, collecting 300 samples from hatch year Greater Snow Geese on their arctic nesting ground and 300 from hunter-killed birds in Quebec, collecting 600 samples from hunter-killed arctic nesting geese in southern Manitoba, and collecting 600 samples from hatch year temperate nesting, urban Canada Geese in southern Ontario and Quebec. Further, the Canadian Wildlife Service may obtain samples from Lesser Snow Geese, Cackling Geese, Ross Geese, and Atlantic Brant, Tundra Swans of unknown wintering affiliation, Greenland-wintering King and Common Eiders, St. Lawrence Estuary nesting Common Eiders, and migrant Canada Geese in northern Quebec which may moult-migrate to Greenland.

The CCWHC and its collaborating laboratories in BC and AB will collect samples from American and Northwestern Crows in British Columbia, Alberta, Ontario and the Maritime Provinces.

2. Primary Virology:

Initial testing of samples for the presence of Influenza A viruses will be done in a laboratory (agriculture, health, university) that routinely serves the province or region in which the samples are collected. These are:
- British Columbia’s Animal Health Centre (Ministry of Agriculture, Food and Fisheries)
- Alberta’s Agri-Food Laboratories Branch, AAFRD
- Manitoba’s Veterinary Services Branch (Ministry of Agriculture, Food and Rural Initiatives)
- Saskatchewan’s Prairie Diagnostic Services
- The Animal Health Laboratory at the University of Guelph under contract from the Province of Ontario
- Quebec’s INSA - Réseau des laboratoires (Ministère de l'Agriculture, des Pêcheries et de l'Alimentation)
- The Atlantic Veterinary College under contract from the Provinces of New Brunswick, Newfoundland and Labrador, Nova Scotia and Prince Edward Island.
- National Centre for Foreign Animal Diseases (NCFAD): Samples received from Iceland will be analysed directly by NCFAD because these samples represent a formal international collaboration. For these samples, NCFAD will follow the protocols established for primary virology carried out in regional laboratories such that selected influenza viruses present in these samples are identified and partially characterized, whether they are of H5/H7 or of other H types.

● Primary screening will be via PCR, followed by isolation in embryonated SPF hen’s eggs of all influenza viruses detected by PCR. PCR analyses will be performed as quickly as possible once samples have been received by the laboratory. Although samples from wild birds found dead will be given first priority for analysis, these swab samples also will be given very high priority for analysis.

● PCR analysis for Matrix Protein gene sequences will be done on all samples. All samples shown to contain influenza A virus(es) by this analysis will immediately be analysed by PCR for H5 an H7 protein gene sequences. All samples thus identified as containing H5 or H7 virus(es) will be shipped immediately to NCFAD for complete identification. Regional laboratories will do no further work on these H5/H7-positive samples.

● Results of analyses will immediately be entered into the Survey database.

● Virus isolation will be performed by regional laboratories on samples that are Matrix Protein- positive but H5/H7 negative, and five aliquots of each of the viruses isolated will be sent to the Canadian Science Centre for Human and Animal Health, where three aliquots will be placed in a national archive of influenza viruses maintained by the Public Health Agency of Canada (PHAC), and one each will be distributed to the NCFAD and to the National Microbiology Laboratory (PHAC).

● All laboratories will follow identical PCR and virus isolation protocols. NCFAD will be responsible for establishing primary virology protocols and quality assurance procedures.
through the *Avian Influenza Virus Laboratory Network*, collaboratively with the participating regional laboratories.

3. Further Characterization of Viruses:

The NCFAD will undertake the following.

- Isolation and characterization of selected H5 and H7 viruses, including pathogenicity testing if required (requirement determined by CFIA).
- H and N typing of selected viruses isolated in the 2006 Survey
- Immediate entry of data in the Survey database
- Establish and maintain the primary virology protocols and quality assurance procedures for regional laboratories through the *Avian Influenza Virus Laboratory Network*, collaboratively with the participating regional laboratories.
- Maintain an archive of all viruses derived by NCFAD from the wild bird survey.

NML will assist NCFAD when surge capacity is required. In addition, NML will:

- Carry out some limited routine virus genome sequencing to characterize virus isolates at the molecular level.
- Maintain an archive of all viruses isolated by regional laboratories.

III. For All Components of the Survey

1. Avian Influenza Virus Archives

The national archive established in the 2005 Survey will receive samples (3 aliquots) of each virus isolated by the regional laboratories. The purpose of this archive is to provide scientists in Canada or elsewhere access to the full range of virus strains for research purposes.

Survey Executive Committee will establish guidelines for access to these samples by scientists, to assist NML in administration of the archive.

NCFAD also will maintain an archive of viruses isolated within its biosafety level 3 laboratory and which thus can not readily be moved out of these level 3 conditions. NCFAD will
work with other research scientists in various ways, as necessary, to provide access to these viruses, for example by providing RNA extracts of these viruses which can be taken from the level 3 facility.

To enhance scientific access to viruses identified in the Survey, gene sequence data generated by NCFAD and NML will be deposited in GenBank, an open-access gene sequence database, as recommended by the WHO and OIE.

2. Data Management:

Data generated by all participants (sample collection data, PCR data, H and N typing data, gene sequence data, pathogenicity data, etc.) will be entered into the Survey database to which participants will have password-protected access. The database will be further developed and managed by the CCWHC, in consultation with participants and the Survey Executive Committee.

3. Communication of Results

- Policies for communication of results from the 2006 Survey will be established by the Survey Executive Committee (see Survey Governance, above).
- For communications and citation purposes, the name of this surveillance program shall be: “Canada’s Inter-agency Wild Bird Influenza Survey”
- The Survey Database will be the mechanism for immediate reporting of all laboratory results (by regional and federal laboratories).
- Open sharing of results among Survey participants, with other government agencies, with the Canadian public and with the international community, consistent with government policies, is a communications principle for the Survey

4. Public and Media Communications, Human Health and Safety Standards and Messages, and Contingency Plans for Possible Detection of Highly Pathogenic Virus Strains

Each Province and Territory, and the Federal Government, will establish lead agency designations, protocols and procedures that specify:
● By whom and under what circumstances dead wild birds will be secured and shipped to participating laboratories for inclusion in the enhanced dead bird survey.

● The content of avian influenza-related information relevant to public health, hunter safety, Wild Bird Survey personnel safety, and other similar concerns to be disseminated in the province, territory or agency, and the mechanism and responsibilities for its dissemination.

● Contingency plans for actions and communications in response to the possible scenarios of detection of Highly Pathogenic strains of avian influenza viruses, including the Eurasian HP H5N1 strain.

● Communications with politicians, upper government management, the media and the public about the Wild Bird Survey, under routine conditions and under conditions of possible detection of Highly Pathogenic strains of avian influenza viruses in North America.

It is the shared intention among Wild Bird Survey participants that criteria for responding to reports of dead birds, content and mechanisms for communications, and contingency action plans be harmonized among federal, provincial, territorial and non-government agencies and groups through advanced planning and appropriate dialogue.

4. Analysis and Survey Report

The data generated by the 2006 Survey will be analysed and summarized by the staff of the CCWHC and the Centre for Coastal Health (Nanaimo, BC). A report on the survey will be written and made available to all participants.

5. Intellectual Property

Survey participants are encouraged to analyse survey data, perform further work on the isolated viruses, and publish the results. All use of the survey data should cite and acknowledge the source as “Canada’s Inter-agency Wild Bird Influenza Survey.” All use of survey data for analysis and publication must be negotiated on a bi-lateral or multi-lateral basis among those making such use of the data and the individuals and laboratories who have generated the samples and the data, following the norms of ethical scientific practise in Canada.
TARGET SAMPLE NUMBERS:

1. **Enhanced Surveillance of Birds Found Dead**

   - **Target Sample Size:** 2,500 (Canada-wide)

     It is not possible to predict the rate or timing of acquisition of dead birds for examination. It is anticipated that efforts at enhanced detection and submission will result in approximately twice the usual annual submission rate of 1000-1500 dead birds.

2. **Live Transatlantic Migrants**

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>Sample</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlantic Brant</td>
<td>Iceland</td>
<td>Swabs</td>
<td>150</td>
</tr>
<tr>
<td>Red Knot</td>
<td>Iceland</td>
<td>Swabs</td>
<td>200</td>
</tr>
<tr>
<td>Swans</td>
<td>Nunavut</td>
<td>Swabs</td>
<td>100</td>
</tr>
<tr>
<td>Gr. Snow Goose</td>
<td>Nunavut</td>
<td>Swabs</td>
<td>300</td>
</tr>
<tr>
<td>Gr. Snow Goose</td>
<td>Quebec</td>
<td>Swabs (Hunted)</td>
<td>300</td>
</tr>
<tr>
<td>Eider</td>
<td>Nunavut</td>
<td>Swabs</td>
<td>200</td>
</tr>
<tr>
<td>Canada/Snow Goose</td>
<td>Manitoba</td>
<td>Swabs (Hunted)</td>
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<tr>
<td>LSN/CDN/Cackling</td>
<td>Nunavut</td>
<td>Swabs</td>
<td>600</td>
</tr>
<tr>
<td>Arctic tern</td>
<td>New Brunswick</td>
<td>Swabs</td>
<td>300</td>
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<tr>
<td>Semiplm. Sandpiper</td>
<td>NB/NS</td>
<td>Swabs</td>
<td>600</td>
</tr>
<tr>
<td>Other</td>
<td>Atlantic</td>
<td>Swabs</td>
<td>200</td>
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</tbody>
</table>

   **Total** 3550

3. **Live Wild Ducks**

   Given below are the target sample sizes; sampling will emphasize young-of-the-year (hatch year) birds. In addition to the 500 Mallard (*Anas platyrhynchos*) samples from each location, it is anticipated that there will be access to other species of ducks during procurement of the mallard samples. Up to 300 samples from other young-of-the-year ducks will be collected and processed in addition to the mallard samples to assess species differences. 500 samples from mallards at each sampling site will permit detection of virus in at least one bird with 99% confidence if the prevalence of infection is at least 0.01 (i.e.1%). In Atlantic Region, 500 samples will be collected from Black Ducks (*Anas rubripes*) due to the predominance of this species compared to Mallard.
### Wild Bird Influenza Survey 2006

#### Region Species

<table>
<thead>
<tr>
<th>Species</th>
<th>BC (L. Fraser)</th>
<th>BC</th>
<th>AB</th>
<th>QC</th>
<th>Atlantic</th>
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<tbody>
<tr>
<td>Mallard</td>
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<td>500</td>
<td>500</td>
<td>500</td>
<td>500*</td>
<td>2000</td>
</tr>
<tr>
<td>Common Eider</td>
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<td></td>
<td></td>
<td></td>
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<tr>
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<td>1000</td>
<td>800</td>
<td>4200</td>
</tr>
</tbody>
</table>

* In the Atlantic Region, 500 Black Ducks will be sought instead of 500 Mallards

#### 4. Peri-Domestic Species

<table>
<thead>
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<th>Species</th>
<th>Location</th>
<th>Sample</th>
<th>Number of Samples</th>
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</thead>
<tbody>
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<tr>
<td></td>
<td>AB</td>
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</tr>
<tr>
<td></td>
<td>ON</td>
<td>Swab</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>Atlantic</td>
<td>Swab</td>
<td>300</td>
</tr>
<tr>
<td>Canada Geese</td>
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<table>
<thead>
<tr>
<th></th>
<th>Total</th>
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</thead>
<tbody>
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<td>Total of Samples:</td>
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</tbody>
</table>

Total of Samples: 12,050