Global Livestock CRSP

AVIAN FLU SCHOOL

International Course Guide

Version: May 18, 2007

MODULE 2: SURVEILLANCE

Wildlife Health Center and Cooperative Extension
School of Veterinary Medicine
University of California, Davis

Acknowledgments

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CONTENTS
Module 2: Surveillance

Instructor Notes	Course Material

In addition to this Course Guide, you will need the following for this module:

PowerPoint Presentation (Module 2)

Room set up with tables for 4-5 people each (see room setup diagram)

This module includes the following training methods:

- Lecture
- Visuals
- Small and Large Group Discussion
- Exercises
  - Exercise 2-1: Check Your Understanding
  - Exercise 2-2: Review Key Concepts
  - Exercise 2-3: Surveillance Planning
- Appendix:
  - Guidelines for sample size calculation
  - Tables 1–6

Introduce this module by welcoming the participants to Module 2 of the Avian Flu School.

Introduce any new instructors or participants for this module and ask each to briefly describe his or her relevant experience.

TARGET AUDIENCES

This module is intended for individuals involved in the development or implementation of surveillance activities, including government veterinarians, animal health educators, epidemiologists, wild life surveyors and personnel involved in the eradication or control of animal diseases.

Instructor Notes	Course Material

MODULE OBJECTIVES

At the conclusion of this module, you will be able to:

- Design a national H5N1 HPAI surveillance plan (for domestic and wild birds), based on guidelines
- Modify the surveillance plan as the disease situation changes
- Identify types of surveillance for inclusion in a surveillance plan
- Identify appropriate sampling procedures
- Understand the importance of collecting appropriate sample sizes

TIMELINE

A suggested time plan for this module is as follows:

Introduction and Module Overview: 10 minutes

Lesson 1: 30 minutes

Lesson 2: 20 minutes

Lesson 3: 45 minutes

Lesson 4: 30 minutes

Lesson 5: 10 minutes

Conclusion and Final Exercise: 45 minutes

Total Time: 3 Hrs.

Instructor may add or omit material to customize length of module as necessary.

MODULE PREVIEW

- H5N1 HPAI surveillance plans may be different than surveillance strategies for other AI.
- Surveillance approaches for captive and free-living populations are different.
- Surveillance efforts require resources and, as such, should be prioritized and targeted. Costs can be reduced by combining surveillance with other activities.
- There are two main types of surveillance used for data collection: passive and active surveillance.
- Surveillance can be classified according to the objective, which changes with time through the course of an outbreak.
- The right number of samples should be collected to detect H5N1 HPAI in a population.

Answer questions, then continue.

Instructor Notes	Course Material
TRANSITION *In the Overview Module, we introduced surveillance and told you that the ability to efficiently control the spread of a highly infectious disease such as H5N1 HPAI is dependent upon the capacity to rapidly detect the virus if introduced.*	DEFINITION: **Surveillance** is a systematic form of data recording with three distinct elements: 1. Sampling, recording and analysis of data 2. Dissemination of information to interested parties, so that 3. Action can be taken to *control disease*

ADDITIONAL NOTES

LESSON 1
H5N1 HPAI SURVEILLANCE

Instructor Notes	Course Material

TIME: 30 MINUTES

START TIME:________

END: ________

TRANSITION

As discussed in the overview, it is optimal to survey both poultry and wild birds, since H5N1 HPAI may spread into uninfected areas through their movements.

Successful surveillance planning includes specific strategies for each the following:

- **Locating** susceptible populations.
- Efficient **detection**, reporting and assessment of morbidity and mortality events.

IMPORTANT POINT

To have effective H5N1 HPAI emergency management, it is critical to be ready to respond to an outbreak. Surveillance is vital for early detection.

A surveillance plan should be ready to be applied **before**, **during** and after an **outbreak**.

Successful surveillance planning includes specific strategies for each the following:

1. Locate:

____

IMPORTANT POINT

Keeping an accurate and up to date database of all commercial premises, backyard poultry, wild bird congregations, zoos, and pet shops is vital to allow the quick identification and surveillance of at risk populations within an area after infection is detected.

2. Detect:

____

Instructor Notes	Course Material
• A system for the rapid **collection** and **transport** of samples from suspected cases to a laboratory. • Rapid **diagnosis** and **reporting**. For this to happen, there must be the technical capability to diagnose H5N1 HPAI. • **Traceability** of the samples, so that result and animal of origin can be matched without mistake. • Recording, managing and **analyzing** diagnostic and surveillance data. • **Dissemination** of information.	3. Collect and transport: ____ 4. Diagnose and report: ____ 5. Trace back: ____ 6. Analyze: ____ 7. Disseminate: ____

EXERCISE 2-1: CHECK YOUR UNDERSTANDING

Refer to Exercise on next page.

Allow 2-3 minutes for individuals to complete the True/False assessment of their current understanding of surveillance concepts.

After 2-3 minutes, inform the participants that we will repeat the exercise at the end of the module and compare their responses now with their responses later.

EXERCISE 2-1: CHECK YOUR UNDERSTANDING

Purpose: This exercise allows you to assess your current knowledge of the topics to be included in this module and identify topics of particular interest.

Instructions: Review the list of surveillance concepts below and determine whether you believe them to be True or False. Check one True or False box after each statement. We will repeat the exercise at the end of the module and compare your responses now with your responses later.

	Surveillance Component	**True**	**False**
1	The use of targeted surveillance for H5N1 HPAI optimizes the budget spent on surveillance	o	o
2	Active surveillance of chickens is crucial for the early detection of H5N1 HPAI	o	o
3	Surveillance plans for H5N1 HPAI are no different from the plans for any other type of AI	o	o
4	Clinical surveillance may fail because people don't know where to report cases	o	o
5	Serosurveillance is key to the detection of H5N1 HPAI in unvaccinated commercial chickens	o	o

Instructor Notes	Course Material

TRANSITION

To give you the foundation for surveillance, we will define some of the main characteristics of surveillance plans, how to classify them, and how they specifically apply to H5N1 HPAI.

GRAPHICS

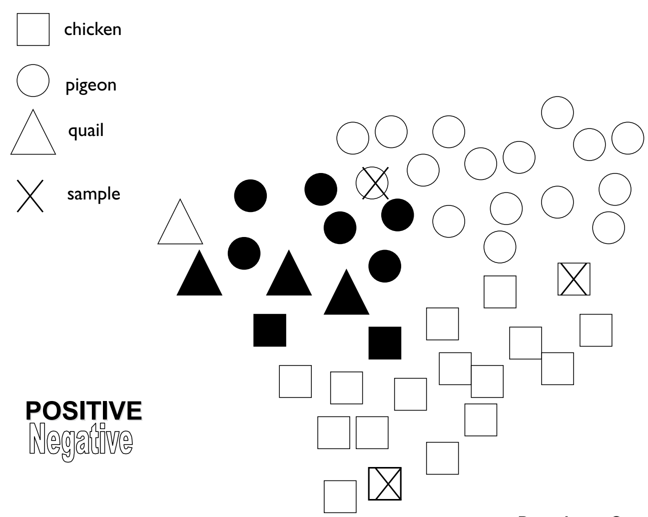
The graphics explain how targeted surveillance works:

- Each shape represents a bird species.
- Quails have the highest prevalence of H5N1 HPAI, while pigeons and chickens have much lower prevalence.
- When taking three random samples, the chances are that no infected bird will be sampled.
- When targeting the birds that are most likely to be infected (quails), the chances of sampling an infected individual will increase.
- When sampling pigeons or chickens by their proximity to quails (which have the highest prevalence) the chances of sampling an infected bird will increase.
- This is an approximation of a live bird market situation.

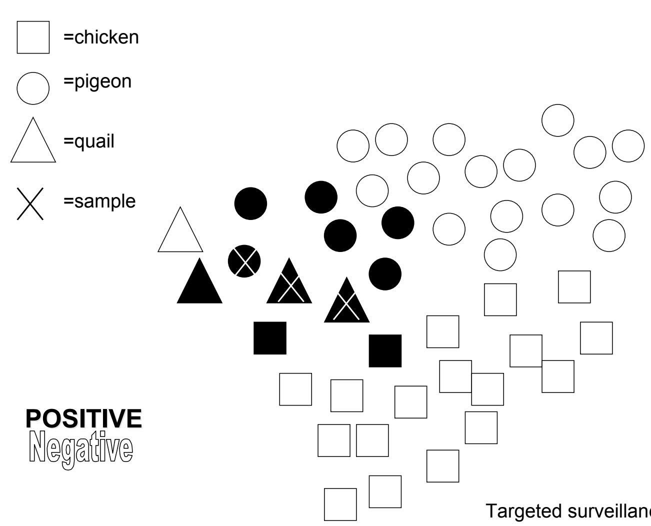
IMPORTANT POINT

Targeted surveillance is the tailoring of a surveillance program based on increased likelihood of infection in a particular species or area rather than doing a completely random sampling (OIE 2005a).

Surveillance for H5N1 HPAI should be mainly targeted, because surveillance can be very expensive. By targeting the highest-risk individuals, costs can be reduced.



Random Surveillance



Targeted surveillance

Instructor Notes	Course Material

Methods of data collection
Passive vs. active surveillance:

Passive surveillance is the testing of sick or dead birds that are reported.

- it is generally less expensive than active surveillance.
- it is a good way to identify new and emerging diseases.

However, when a specific disease starts spreading, active surveillance becomes more important for early detection.

It is key to know how H5N1 HPAI spreads globally to assess the risk of introduction of H5N1 HPAI into your country or region and determine what species or compartments should be targeted. That way you can adapt your surveillance plan.

There are several sources of current information available:

- National government agencies
- Promed
  http://www.promedmail.org/
- OIE
  http://www.oie.int/
- BBC (and other news media)
  http://news.bbc.co.uk/

Methods of data collection: Passive vs. active surveillance

PASSIVE SURVEILLANCE:

IMPORTANT POINT

Passive surveillance can be greatly improved by education. The people who may encounter sick or dead birds should be taught what to look for and who to report to.

In order to be aware of the H5N1 HPAI global status, there are several sources of current information available:

Instructor Notes | Course Material

Active surveillance is the systematic testing of the birds or animals, including those that are healthy.

The frequency of active surveillance will depend on the situation, but should be at least every 6 months (OIE 2005).

Surveillance Types

Surveillance can be classified depending on the diagnostic techniques used. The main surveillance types are:

1. **Clinical (or syndromic) surveillance**
   - It also includes the necropsy of birds.
   - Can be an inexpensive way to detect early outbreaks, particularly in areas where other methods are not feasible.
2. **Virological surveillance** should be used to:
   a. monitor at risk populations
   b. confirm clinically suspect cases
   c. follow up on positive serological results
   d. determine causes of mortality.

The frequency of virological surveillance usually depends on the risk of the presence of AI in the area, but may also be influenced by personnel, equipment and economic considerations.

IMPORTANT POINT

Active targeted surveillance should be conducted when AIV prevalence is highest, usually the coolest and wettest months of the year.

ACTIVE SURVEILLANCE:

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Surveillance Types

1. **Clinical or syndromic surveillanc**e focuses on detecting the clinical signs or lesions of the target disease.

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2. **Virological surveillance** tests for the presence of AI virus. It should be used to:

a. ____________________

b. ____________________

c. ____________________

d. ____________________

Instructor Notes	Course Material
3. **Serological surveillance:** • Positive serological tests must be viewed with caution. • Not all birds and/or species develop measurable antibodies.	3. **Serological surveillance or serosurveillance** tests for the presence antibodies to the AI virus circulating in the bloodstream.

EXERCISE 2-2: REVIEW OF KEY CONCEPTS

See exercise on next page.

Purpose: This exercise will allow you to assess learning gains about methods of data collection and surveillance types.

Instructions: Follow the steps below to conduct this exercise:

1. Have participants complete the review exercise in which they are to identify the method of data collection and surveillance type that apply to each scenario.
2. Allow 2-3 minutes for individuals to complete.
3. Ask participants to compare their answers with the persons seated next to them (2-3 minutes).
4. Review responses in the larger group.

IMPORTANT POINT

Ducks, wild and domestic, are difficult and expensive to survey for H5N1 HPAI for several reasons:

1. They may be asymptomatic carriers, thus clinical surveillance cannot be used
2. They don't reliably seroconvert
3. There may be more than one H5 subtype, thus gene sequencing must be done to determine the specific virus that has been detected. This will increase costs and the level of training required to analyze the results.

EXERCISE 2-2: REVIEW OF KEY CONCEPTS

Purpose: This exercise will allow you to assess learning gains about methods of data collection and surveillance types.

Instructions: Follow the steps below to conduct this exercise:

1. Select from the list below, and write into the table the method of data collection and surveillance type that apply to each scenario.

Data Collection Methods

Passive
Active

Surveillance type

Clinical surveillance
Virological surveillance
Serosurveillance

2. Compare your answers with the person next to you.

3. Be prepared to report key findings back to the larger group.

Data Collection	Scenario	Surveillance type
	A: A hiker reports a dead swan to the local authorities, who then collect it and send it to the lab	
	B: Swabs are collected from all live bird markets in Los Angeles, CA that have both chickens and ducks housed together.	
	C: A farmer observes a decrease in egg production and sends 30 blood samples to the national diagnostic lab.	

Which surveillance methods and types are most challenging for you or your community?

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Instructor Notes

SURVEILLANCE OBJECTIVES

Surveillance can be applied to different objectives, which change with time in the course of an outbreak within a geographical area (country, zone or compartment).

Objectives in chronological order:

1. Early detection

Early detection is a critical step, because it will provide an early warning of infection, thus increase the chances of controlling an outbreak when it is smallest.

- This is why surveillance needs a fast and reliable diagnostic service.

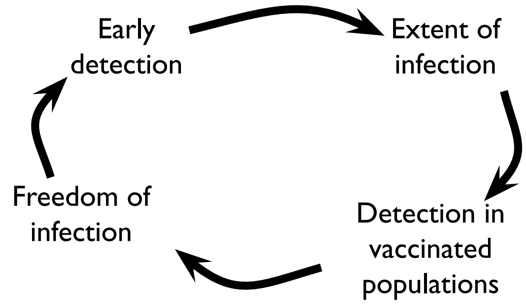
Risk is assessed by analyzing the likelihood that H5N1 HPAI will arrive in a country through known transmission paths.

The risk will be influenced by a nation's connections with infected areas:

- **Low risk**: Prioritize the investigation of unusual disease and mortality events (passive).
- **High risk**: i.e., when H5N1 HPAI is detected in neighboring countries or in countries connected through trade or waterfowl migratory routes.
    - Passive surveillance should continue.
    - Active surveillance should start in at risk populations

Course Material

SURVEILLANCE OBJECTIVES



1. EARLY DETECTION

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There are different early detection strategies depending on the estimated risk of H5N1 HPAI introduction:

- **Low risk**

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- **High risk:**

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    - Passive: ____________________
    - Active: ____________________

IMPORTANT POINT

It is important *not* to stop surveillance for other diseases that could also have devastating effects if introduced to a region.

Instructor Notes	Course Material

2. Extent of infection

Surveillance results are used to:

- assess progress in the control or eradication efforts
- act as an aid for decision making.

Once H5N1 HPAI has been detected, active surveillance efforts should begin in poultry populations and passive surveillance should continue as it was during early detection

2. EXTENT OF INFECTION

Later in an outbreak, it is important to determine the distribution and prevalence of infection in an area.

Surveillance results are used to:

3. Detection in vaccinated populations

- It is a very complex issue.
- Generally speaking, only domestic and captive birds are vaccinated.

3. DETECTION IN VACCINATED POPULATIONS

If the disease becomes established and vaccination is used, vaccinated populations must be tested to make sure they are not infected.

4. Freedom of infection

- It is defined by the OIE to allow nations to recover after an outbreak.
- It should start when there is confidence that the outbreak has been controlled.
- Finding evidence of infection in the target population invalidates any freedom from infection claim.
- A Nation's trading partners who may impose more strict requirements than

4. FREEDOM OF INFECTION

To prove freedom of infection in a zone, compartment or country, wide geographical surveys may be required.

Instructor Notes	Course Material

the OIE.

- After establishing freedom of infection, surveillance should move back to early detection strategies.

TRANSITION

Because it is very difficult to establish and maintain a disease-free status for an entire country, it is very beneficial to establish sub-populations with a freedom of infection status within a country

Two criteria:

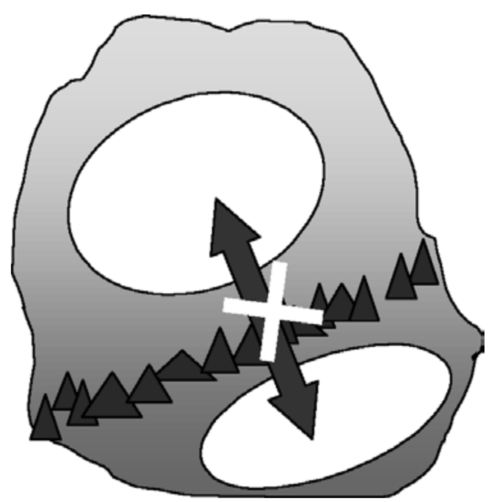
- Zoning: allows the separation of regions by natural or artificial geographical barriers, i.e. mountain ranges or deserts.

ZONING AND COMPARTMENTALIZATION

The OIE has established guidelines to allow nations to separate subpopulations using either zoning or compartmentalization.

There are two criteria to separate subpopulations:

- Zoning:



Zoning. If one region of a country is affected another can be free and thus continue to export.

Instructor Notes

- Compartmentalization: allows subpopulations in an area to be separated by management systems, such as biosecurity. It effectively separates commercial poultry from backyard poultry and/or wild birds.

Surveillance in other species

In animals

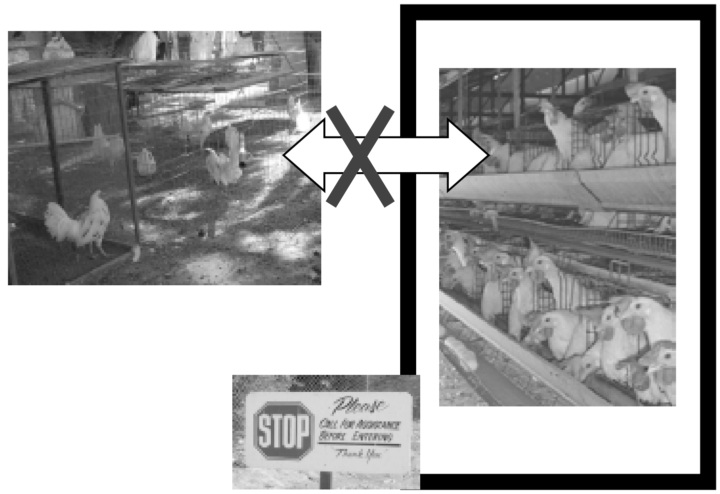
A growing list of mammals (cats, pigs, dogs, martens and humans) have been infected by H5N1 HPAI.

- Passive, clinical surveillance is a low cost and effective way to detect H5N1 HPAI.
- Surveillance of these species would increase in importance if a mutation occurred in the virus that allowed it to spread within these species. It is best to keep up to date with current information in order to determine whether this has occurred.

In humans

- Passive surveillance in patients reporting symptoms

Course Material



Compartmentalization. Commercial poultry compartments are separated from backyard poultry flocks with biosecurity allowing trade to continue from commercial flocks.

- Compartmentalization:

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Surveillance in other species

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In *humans*, suggested surveillance strategies are:

Passive:

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Instructor Notes	Course Material
• Active surveillance in at risk individuals: relatives of confirmed cases, members from the health services, workers involved in a poultry outbreak (farmers, vets, depopulation teams, etc.)	Active:
In other potential hosts Only if H5N1 HPAI becomes established in a species, should one consider an active surveillance program.	In other potential hosts:

LESSON 2
SAMPLE SIZE CALCULATION

Instructor Notes	Course Material

TIME: 20 MINUTES

START TIME: ________

END: ________

TRANSITION

Each surveillance objective requires the use of different approaches (calculations and/or tables) to calculate the sample size.

In order to calculate sample size, you will need to know or guess the prevalence of the disease and the confidence level that we want to use.

DEFINITIONS:

1) Prevalence is the rate of disease in a population.

2) Confidence level is the degree of certainty that a statistical prediction is accurate. Confidence levels from 95% to 99% are acceptable.

Because the prevalence of the disease is usually unknown, we can use a "best guess" based on published reports and/or expert opinion.

The lower the prevalence, the higher the sample size required.

Limitations:

- Most methods for sample size calculation, assume that the diagnostic test being used is perfect, and that is rarely the case. Often, the needed specificity and sensitivity information on tests is not available so, it is always best to be cautious and collect extra samples.
- Many times the required sample size is unaffordable and a targeted approach should be used.

Instructor Notes	Course Material

1. EARLY DETECTION

The sample size selected for testing will need to be large enough to detect infection at an accepted confidence level if infection occurred at a predetermined prevalence.

To calculate the sample size required for a particular prevalence, population size and confidence level two mathematical approaches can be followed: the *hypergeometric approach* or the *binomial approach.*

The hypergeometrical approach requires very complicated calculations, but there are tables that can be used (**Tables 1-3**).

The hypergeometrical is the most precise, but the binomial approach is a very good approximation for big populations (over 10,000).

When a particular combination of prevalence, population size and confidence level is not in the table, an interpolation can be done.

However, if a more precise sample size is required and the population is over 10,000 individuals, the binomial approach can be used by using the equation provided in the Appendix.

Calculation of Sample Sizes

Two examples:

Scenario A: The population size is 500, the prevalence of disease is estimated to be 0.5% and you would like to be 99% confident in your determination.

Scenario B: The population size is 500, the prevalence of disease is estimated to be 10% and you would like to be 99% confident in your determination.

Is it feasible to take that many samples, for example in each live bird market?

What alternative approaches could be taken?

Ask participants to read through the 2 scenarios in their guide and turn to Table 1 in their appendices for Module 2. Show them how to know that this is the correct table to use for the confidence level described in the scenarios. Point out how to find the population size (flock size column) and the expected prevalence (header row).

Ask participants to do the exercise with a partner but this time, use 90% confidence.

These exercises demonstrate that:

- the lower the prevalence, the more samples you need.
- the higher the confidence level, the more samples you need.

Instructor Notes	Course Material

Composite or Pooled Sampling:

- For virological surveillance, samples from more than one individual can be combined to make a composite or pooled sample.
- This is a very effective way to decrease testing costs.
- The number of samples that can be pooled together and still have a positive sample detected, is limited by the sensitivity of the test (usually, up to 5 samples).

Environmental sampling:

- Usually involves sampling of feces or feces-contaminated water
- It simplifies the collection of samples.
- It is especially useful for species that are difficult to capture, such as some free-flying birds.
- Environmental samples are always presumed to be pooled samples except under very special circumstances.

A Different Approach to Detect Infection: Composite or Pooled Sampling

Environmental sampling:

Tables 4 and 5 can be used to calculate the number of composites or samples per composite. The formula used to create these tables is in the appendix for your reference.

2. EXTENT OF INFECTION

Table 6 shows the required sample sizes, when you have determined:

- the hypothesized prevalence
- the precision (desired error limits for the estimated prevalence)
- the desired level of significance (equivalent to the confidence level).

Instructor Notes	Course Material

DETECTION IN VACCINATED POPULATIONS

There are two basic approaches, depending on the vaccination strategy that can be used: 1) sentinel placement or 2) DIVA strategy (differentiate infected from vaccinated animals).

1) Sentinels: There are no conclusive studies on what is the optimal number of sentinel birds that should be placed in a flock or how often or how many should be tested.

2) DIVA strategy: This involves the use of a vaccine that can be differentiated from infection with a diagnostic test. As with sentinel placement, there are no conclusive studies on how many samples to collect to detect an infection.

The level of infection in a vaccinated population will depend on the level of immunity in the flock and should be considered when designing a sampling strategy.

To estimate the sample size in vaccinated populations one should consider economic and convenience criteria as well as common sense.

FREEDOM OF DISEASE

Proving freedom of disease is determined by the OIE, but will also depend on trade agreements.

3. DETECTION IN VACCINATED POPULATIONS

The development of a sampling protocol to detect infection in a vaccinated population is a very complex issue.

1) Sentinels

2) DIVA strategy

4. FREEDOM OF DISEASE

Example: According to Animal Health Australia (2005), surveys should aim to demonstrate with a 95% confidence level of detecting a 5% infection rate in 1% of commercial flocks. Table 1 in the Appendix can be used for to determine sample sizes under these considerations.

LESSON 3
SURVEILLANCE IN POULTRY AND CAPTIVE POPULATIONS

Instructor Notes	Course Material

TIME: 45 MINUTES

START TIME:________

END: ________

TRANSITION

We have discussed the general concepts of surveillance. Now we'll focus on the aspects that are specific to poultry and captive populations.

SURVEILLANCE TYPES:

1. Clinical surveillance

- Poultry workers should know the H5N1 HPAI signs and lesions.
- Clinical surveillance can be used to target broad populations.
- It is impossible to distinguish clinically between HPAI and velogenic viscerotropic Newcastle disease.
- Suspect flocks can then be referred for further testing, thus using more sophisticated and expensive testing methods for likely infections.

The major impediments to effective clinical surveillance are:

- The people who are most likely to find cases, lack of information about what to look for and where to report
- A lack of education on the importance of surveillance and thus a lack of compliance
- Failure of poultry producers to keep good records. Without good records, subtle changes that may be early indications of illness may not be noticed.

SURVEILLANCE TYPES

1. Clinical or syndromic surveillance:

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The major impediments to effective clinical surveillance are:

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Instructor Notes | Course Material

2. Virological surveillance

There are three types of tests:

- RT-PCR is among the most rapid and most accurate tests available and are frequently used to screen commercial poultry populations.
- Virus isolation, although more time consuming, remains the gold standard method.
- Rapid on-site diagnostics (lateral flow antigen ELISA).

3. Serological Surveillance

Serosurveillance has limitations. Sometimes, infected birds don't develop antibodies:

- Most domestic fowl don't survive long enough after the infection with HPAI viruses.
- Some species, such as ducks, don't always seroconvert.

Non-infected birds may also have antibodies:

- Antibodies are produced in response to vaccination.
- Maternal antibodies can circulate in young birds for up to four weeks.

2. Virological surveillance

There are three types of tests:

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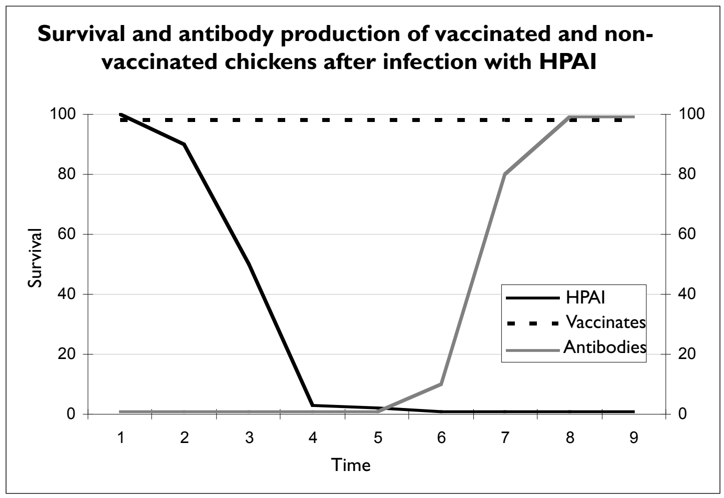
3. Serological surveillance



There are some limitations of serosurveillance:

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Despite its limitations, serosurveillance is very important for surveillance in vaccinated populations.

Instructor Notes	Course Material

Surveillance objectives

1. Early detection: The strategy employed will depend on the risk of infection in poultry.

Low risk: Passive surveillance.

High risk: High risk populations should be actively targeted:

- Those with the greatest connections to infected regions or populations, i.e. birds being traded or poultry close to migratory wild birds or borders.
- The main aim of active surveillance of chickens and turkeys is to proof safety to consumers.
- Sentinel flocks may be a great aid to detect infection.

Ask the participants about the specific at-risk populations in their region or country.

2. **Extent of infection:** Surveillance should focus on the same populations as in early detection under high risk.

It is of special importance to investigate all dangerous contacts after an outbreak occurs, i.e. farms connected by movement of birds, people and vehicles.

SURVEILLANCE OBJECTIVES

1. Early detection

The strategy employed will depend on the risk of infection in poultry.

Low risk:____

High risk:____

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Sentinel flocks should consist of free range, unprotected, domestic poultry located in or near places where there are high risk populations such as wetlands or live bird markets. Sentinel flocks should have the opportunity to intermingle or share water sources with the birds for which they are sentinels.

2. Extent of infection:

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Surveillance in zoos and pet shops: *Only when an outbreak has perpetuated in a country should the focus of surveillance efforts shift. For example, if pet stores are spreading disease, then clearly targeted surveillance should shift to focus on them.*

Instructor Notes	Course Material

Live bird markets bring together different species of birds from many different providers. Birds may be slaughtered on site or at home.

- They are an excellent place to conduct surveillance, because birds from many sources are brought together in one place making testing very convenient.
- It is important to test birds from every source, understanding that the source that is positive in the market is probably not positive on the farm.
- If possible, targeted surveillance of sick/dead birds should be conducted

Explain the graphs:

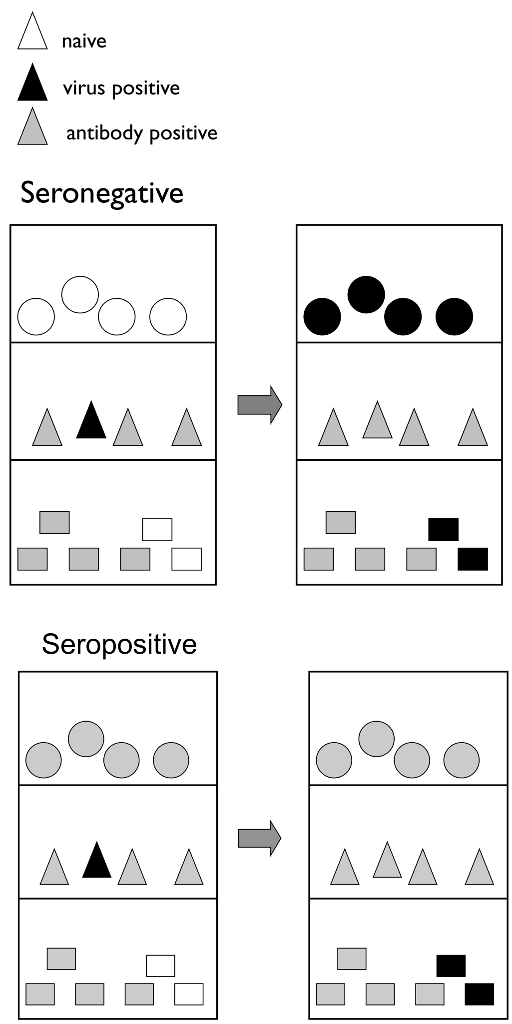
Seronegative:

- Infection is introduced into a mostly seronegative population.
- Many birds from seronegative flocks will get infected.
- Shedding starts 6 to 24 hours post-exposure.
- It will be easy to detect shedding birds, because the prevalence will be high after a few days.
- The negative source flocks act as sentinels.

Seropositive:

- If most birds are seropositive, individuals are resistant to infection and few will become infected and shed virus.
- It will be much more difficult to detect shedding birds, because the prevalence is low.
- In this scenario, the use of sentinels might be more cost and time effective.

Live bird markets (*or wet markets*)



Instructor Notes	Course Material

3. Detection in vaccinated populations

- Only a few individuals will be infected (the prevalence will be much lower than that of an infected non-vaccinated flock).
- Infected vaccinated birds may have very low virus titers

Sentinel poultry should:

- Be healthy, unvaccinated and antibody negative birds.
- Be clearly & permanently marked (e.g leg bands or wing bands)
- Be monitored with virological, serological, and clinical surveillance.
- Remain negative by all tests if the flock is not infected.

Problems with the use of sentinel birds:

- Sentinel birds may not become infected even if the flock is infected.
  - All birds around sentinel birds are vaccinated and therefore protected.
  - Therefore, the few birds that may become infected will shed very low amounts of virus.
- There are no conclusive studies on how many should be placed in a flock, how often they should be tested, andhow many of them should be tested.

3. Detection in vaccinated populations

When trying to detect infection in a vaccinated flock, there are two main complications:

1. ____________________

2. ____________________

If **sentinel birds are used** to provide further confidence of the absence of virus circulation. They should be:

____

____

____

____

____

The vaccinated flocks in a region should be tested at least every 6 months or at shorter intervals according to the risk in the country, zone or compartment to determine that the virus is not present (OIE 2005).

There are some problems with the use of sentinel birds.

- Sentinel birds may not become infected even if the flock is infected:

____

____

____

- Knowledge gaps:

____

____

Instructor Notes	Course Material

If sentinel birds are not used:

It is key to know what type of vaccine was used in order to develop a serological based strategy to differentiate infected from vaccinated animals. This has been explained in the *Overview*.

In any vaccinated population, there will be birds that were not vaccinated, or that are immunosuppressed and thus have not been immunized. These birds will die if infected, so testing only the birds that die, is a great way to skew your sampling strategy towards those that may have detectable virus. This is called ***mortality monitoring.***

4. Freedom of infection

It is not possible to be 100% confident that a population is free from infection (unless every member of the population is examined simultaneously with a perfect test with both sensitivity and specificity equal to 100%)

4. Freedom of infection:

NOTE: A country, zone or compartment is considered free from HPAI when it has been shown free of infection for the past 12 months, although its LPAI status may be unknown.

If infection has occurred, free status can be regained 3 months after a stamping-out policy is applied (OIE, 2005).

LESSON 3
SURVEILLANCE IN POULTRY AND CAPTIVE POPULATIONS

EXERCISE 2-3: CHECK YOUR UNDERSTANDING

Purpose: This exercise allows you to assess your current knowledge of the topics to be included in this module and identify topics of particular interest.

Instructions: Review the list of surveillance concepts that you saw at the outset of this module and determine whether you believe them to be True or False. Check one True or False box after each statement. Compare your responses with your responses on p. 8.

	Surveillance Component	**True**	**False**
1	The use of targeted surveillance for H5N1 HPAI optimizes the budget spent on surveillance	o	o
2	Active surveillance of chickens is key for the early detection of H5N1 HPAI	o	o
3	Surveillance plans for H5N1 HPAI are no different from the plans for any other type of AI virus	o	o
4	Clinical surveillance may fail because people don't know where to report cases	o	o
5	Serosurveillance is key to the detection of H5N1 HPAI in unvaccinated commercial chickens	o	o
6	According to the OIE, a country is considered free from H5N1 HPAI infection if it tests negative for the previous 12 months, although its LPAI status may be unknown	o	o

LESSON 4
SURVEILLANCE IN WILD BIRDS

Instructor Notes	Course Material

TIME: 30 MINUTES

START TIME:________

END: ________

TRANSITION

Surveillance in wild birds may be an important component of an overall H5N1 HPAI surveillance plan for a given region.

Although the steps are the same as for poultry, designing an effective surveillance strategy presents additional challenges in wild birds:

- **Locating** susceptible populations.
  - Identify information sources in country.
- **Detection**, reporting and assessment of morbidity and mortality events.
  - Dead wild birds may be scavenged or drown.
  - People don't own them, so there is less interest in reporting.
  - Remoteness
- Rapid **collection and transport** of samples.
  - Remoteness, thus longer transport times will become an issue.
  - Samples may be very deteriorated.
- Rapid **diagnosis and reporting**.
  - Wild birds are not reportable to OIE.
- **Traceability** of the samples.
  - Lack of data about when, where and what species. Need to collect good field data.
- Recording, managing and **analyzing** data.

WILD BIRD SURVEILLANCE NEEDS AND CHALLENGES

NOTE: Wild birds refer to those that are not in captivity (free-ranging). OIE guidelines do not apply to wild bird species in the wild or held in captivity.

1. Locate:

____

____

2. Detect:

____

____

3. Collect and transport:

____

____

4. Diagnose and Report:

____

____

5. Trace back:

____

____

Instructor Notes

- Data may be from many species, some of whom are carrying many AI virus subtypes.
- Data may be from multiple locations and be in different formats.
- **Dissemination** of information

SURVEILLANCE OBJECTIVES

1. Early detection

Although wild birds infected with H5N1 HPAI tend not to show clinical signs, the systematic investigation of dead wild birds has proved to be a valuable strategy for early detection i.e. swans in Europe.

Low risk: Passive surveillance (investigating disease and mortality events).

High risk: In addition to passive surveillance, there are active surveillance strategies that could be implemented.

Active surveillance options

A. **Sample live and apparently healthy birds:** Efforts should be targeted at the species or populations most likely to be infected, i.e. waterfowl, birds migrating from HPAI H5N1-infected areas, birds in large water bodies, etc.
B. **Hunter-killed wild birds:** Some of the most common carrier species of AIV, i.e. ducks or geese, may be killed by hunters.
Sampling stations may be established in hunting areas.

Course Material

6. Analyze:

____

7. Disseminate:

____

____

SURVEILLANCE OBJECTIVES

1. Early detection:

____

____

____

____

Similar to poultry, the surveillance strategy for wild bird populations will depend on the risk of H5N1 HPAI introduction:

- Low risk:
- High risk:

Active surveillance options

A. Sample live and apparently healthy birds

____

____

____

B. Hunter-killed wild birds

____

____

Instructor Notes	Course Material

C. **Sentinel birds:** Identifiable sentinel bird flocks can be placed in strategic locations, such as wildlife reserves (where they can intermingle with wild waterfowl).

D. **Environmental sampling:** Fecal or environmental sampling may be used to avoid the cost and effort needed to capture wild birds.
However:
- It may not be possible to know which species or how many individuals are being tested.
- Fresh samples (ideally <12 hours old) are needed because the virus only remains infective for a short period.

C. Sentinel birds

D. Environmental sampling

2. Extent of infection

Once H5N1 HPAI has been detected in either poultry or wild birds, it may become important to determine the extent of infection in wild birds.

- The primary purpose is to inform decision makers regarding actions that could be taken to reduce the chance of poultry or humans from being exposed.
- Other reasons could include:
  - prediction the virus spread
  - assess risk to endangered species
  - prioritize target surveillance species
  - study the evolution of the virus

2. Extent of infection:

The same strategies applied to high risk early detection (passive surveillance plus a targeted active surveillance effort) can be used.

Why might it become important to determine the extent of H5N1 HPAI infection in wild bird populations?

a)

b)

c)

d)

3. Detection in vaccinated populations

4. Freedom of infection

3. Detection in vaccinated populations

Generally speaking, wild free living birds are not vaccinated.

Instructor Notes	Course Material

- It is unknown whether or not H5N1 HPAI will become endemic in wild bird populations.
- Elimination of the virus may not be possible or necessary in wild birds.
- The only solution may be compart-mentalization.

4. Freedom of infection

IMPORTANT POINT

Passive surveillance of wild birds should continue after poultry have been declared free of infection. This will allow for a rational assessment of the risk posed by wild birds.

CONCLUSION AND FINAL EXERCISE

Instructor Notes	Course Material

TIME: 45 MINUTES

START TIME:________

END: ________

EXERCISE 2-3: SURVEILLANCE PLANNING

See Exercise Next Page

Purpose: This exercise allows participants to apply surveillance principles to a real-world setting by creating a sample surveillance plan, given the following criteria.

Instructions: Follow the steps below to conduct this exercise:

- **Scenario A**: A country is free of H5N1 HPAI, but migratory birds are about to return from breeding areas in infected countries. You are asked to develop a **national H5N1 HPAI surveillance plan** that will allow early detection.
- **Scenario B**: A site with backyard chickens has just been diagnosed with H5N1 HPAI in a region. You are asked to develop a **modified national H5N1 HPAI surveillance plan.**
- **Scenario C**: H5N1 has become established in a country. Wild birds have been extensively affected in terms of distribution and species affected. There is a geographical cluster of commercial poultry farms that has experienced high mortalities and two fatal human cases have been diagnosed in poultry workers. Also a cat has just been diagnosed. You are asked to develop a **modified HPAI surveillance plan for the most affected region.**

Note: Budget is limited. Please prioritize the most effective and economical actions.

EXERCISE 2-4: SURVEILLANCE PLANNING

Purpose: This exercise allows participants to apply acquired surveillance knowledge to a real-world setting by creating a sample surveillance plan, given the following criteria.

Instructions: In your small groups follow the steps below to conduct this exercise:

- **Scenario A**: A national H5N1 HPAI surveillance plan for early detection
- **Scenario B:** A modified national surveillance plan when HPAI has been detected in one region within a country
- **Scenario C:** A modified surveillance plan surveillance plan for the region where HPAI has been detected

Step	Action
1	Describe Given the ONE Scenario from above that you were assigned, identify the surveillance plan and approach you would use. Describe specifically o what is the main objective of your surveillance o what method of data collection and what type of surveillance approach would you use on which species o when and where would you do it o describe how would you distribute and prioritize economic and human resources to each aspect of the surveillance plan ____ ____ ____ ____ ____ ____

EXERCISE 2-4: SURVEILLANCE PLANNING, CONTINUED

<table>
<tr><td>2</td><td colspan="2">Define

List three steps you take to implement your plan.

1.

2.

3.</td></tr>
<tr><td rowspan="2">3</td><td colspan="2">Analyze
Analyze the relative importance and impact of each step.</td></tr>
<tr><td>Importance</td><td>Impact</td></tr>
<tr><td rowspan="2">4</td><td colspan="2">Evaluate
Evaluate potential challenges or obstacles you might face and what measures you would take to manage them.</td></tr>
<tr><td>Potential Obstacle</td><td>Countermeasure</td></tr>
<tr><td>5</td><td colspan="2">Be prepared to share your answers with the larger group.</td></tr>
</table>

Instructor Notes	Course Material

Summarize the key points from this module:

Allow 2-3 minutes for individuals to record their planned action on the Planned Action section of their guides.

Next, ask participants to pair up with another group member and discuss planned actions. Allow 5-10 minutes for discussion. Invite participants to share examples of planned actions with the larger group, as time allows.

Encourage participants to preview materials for the next scheduledprior to attendance.

THANK EVERYONE FOR ATTENDING THIS SESSION.

MODULE REVIEW

- An effective surveillance plan for H5N1 HPAI may not be the right strategy for general AI surveillance, because of the unique characteristics of H5N1 HPAI virus.
- Surveillance approaches for captive and free living populations are different.
- Surveillance efforts require resources and, as such, should be prioritized and targeted.
- There are two main methods used for data collection: passive and active surveillance.
- Surveillance can be classified according to the objective, which changes with time through the course of an outbreak within a geographical area.
- The right number of samples should be collected to find out if H5N1 HPAI is present.

MODULE OBJECTIVES

- Design a national H5N1 HPAI surveillance plan (for domestic and wild birds), based on guidelines
- Modify the surveillance plan as the disease situation changes
- Identify types of surveillance for inclusion in a surveillance plan
- Identify appropriate sampling procedures
- Understand the importance of collecting appropriate sample sizes

Instructor Notes	Course Material

Planned Action

Identify one action you plan to take with regard to what you learned from this Module:

By when:

With whom will you share your planned action?

REFERENCES AND RESOURCES

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APPENDIX A

SAMPLE SIZE CALCULATION

As mentioned, each phase of surveillance requires its own calculation of sample size (and the use of different calculations and/or tables). This is mostly because the goals of surveillance will be different during each phase.

When using most methods for sample size calculation, remember that they assume that the diagnostic test being used is perfect, and that is rarely the case. Often, the needed specificity and sensitivity information on tests is not available, so it is always best to be cautious and collect at least the calculated number of samples.

1. Early detection

According to the OIE (2005), the sample size selected for testing will need to be large enough to detect infection if it were to occur at a predetermined minimum rate. The sample size and expected disease prevalence determine the level of confidence in the results of the survey.

The applicant country must justify the choice of design prevalence and confidence level based on the objectives of surveillance and the epidemiological situation.

Review **Mathematical approaches to calculate the sample size** required for a particular prevalence and confidence level:

- **Hypergeometric approach** considers sampling without replacement. This means that a bird's chance of being selected increases after each sample is taken.
- The **binomial approach** considers sampling with replacement, is a very good approximation to the hypergeometric distribution when the sample size is small relative to the total population size. If the population is infinitely large, there is no difference between the hypergeometric distribution and the binomial.

Mathematical approaches to calculate the sample size required for a particular prevalence, population size and confidence level can follow either the **hypergeometric** or **binomial approach.**

The hypergeometric approach is always more precise, but it requires very complicated calculations. There are, however, tables that can be used (Tables 1-3).

When a particular combination of prevalence, population size and confidence level is not in the table, an interpolation can be done. However, if a more precise sample size is required and the population is over 10,000 individuals, the binomial approach can be used.

Binomial approach

For the calculation of the recommended sample size following the binomial distribution, the following equation should be used (U.S. Interagency Strategic Plan 2006):

$n = \log(1 - c) / \log(1 - P)$

where n is the sample size, c is the desired level of confidence, and P is the prevalence of positive samples in the population. An adequate sample size should allow for over 95% confidence that AI is detected.

The selection of the design prevalence needs to be based on the prevailing or historical epidemiological situation. Prevalence will vary depending on species, age, geographic area, season, etc.

Example: to detect AI at or below 1.5% prevalence with a >95% level of confidence, 200 individual birds from the population of interest should be sampled (U.S. Interagency Strategic Plan 2006).

$n = \log(1 - 0.95) / \log(1 - 0.015) = 198.2 = 199$

Note: The sample size should always be rounded up to the nearest integer.

However, if the prevalence decreases to 0.1%, then nearly 3000 birds should be sampled. Since the prevalence cannot be known prior to testing, the larger the sample size, the better the chance of detecting HPAI at low levels. However, the cost of the analysis is a crucial factor and the number of samples should be minimized. This is why targeted sampling is the most effective approach.

NOTE:

One limitation about the formula above is that it is based on assumptions that may not apply to H5N1 virus, namely that the agent is homogeneously distributed within a host population that also is homogenously distributed (U.S. Interagency Strategic Plan 2006).

A Different Approach to Detect Infection – Composite or Pooled Sampling

For virological surveillance, samples from individuals can be combined to make a composite or pooled sample. This is a very effective way to decrease testing costs. The number of samples that can be pooled together, and still have a positive sample detected, is limited by the sensitivity of the test. Usually, 5 samples can be effectively pooled without altering detection capabilities.

Environmental sampling, such as the sampling of feces or feces-contaminated water, can be a very useful approach to sampling because it makes the collection of samples simple. It is especially useful for species that are difficult to capture such as some free-flying birds. Environmental samples are always presumed to be pooled samples except under very special circumstances.

Tables 4 and 5 can be used to calculate sample sizes for pooled or composite samples.

The following equation can be used to calculate the number of composites and number of samples per composite:

Equation 1: $n = \ln(1 - r / m) / \ln(1 - p)$

where p is the proportion of infected individual samples across all composite samples, r is the number of composite samples that test positive for the presence of highly pathogenic H5N1 avian influenza virus, m is the total number of composite samples tested, and n is the number of individual samples in each composite sample (e.g., fecal count or volume).

2. Extent of Infection

The sample size for estimating population prevalence is as follows:

$$n = \frac{P \times (1 - P) \times (Z_{1-\alpha/2})^2}{e^2}$$

where P is the hypothesized prevalence, e is the precision (desired error limits for estimated P), α is the desired level of significance (equivalent to the confidence level), and n is the uncorrected sample size for each group. The sample size estimates, assumes that the sample is small compared to overall population size. If the sample size is relatively large then a finite population correction factor should be applied to the calculated sample size. As a rule of thumb, if $n/N > 0.05$, then a population correction factor should be applied:

$$n_{adj} = \frac{n}{1+((n-1)/N}$$

where n_{adj} is the adjusted sample size for each group and N is the population size.

For example, if the hypothesized prevalence was 0.25, the precision 0.05 and the level of significance was 0.05, then:

$$Z_{1-\alpha/2} = Z_{0.975} = 1.96$$

$$n = \frac{(0.25)\ (0.75)\ (1.96)^2}{(0.05)^2} = 288.12$$

Table 1. Number needed to test to be 99% confident that the disease will be detected if present at or above five levels of prevalence present at or above five levels of prevalence (hypergeometric).

Flock size (*N*)	Prevalence (*P*)				
	P = 0.10	*P* = 0.05	*P* = 0.02	*P* = 0.01	*P* = 0.005
	Sample size (*n*)				
N	*n*	*n*	*n*	*n*	*n*
20	18	20	20	20	20
50	29	41	50	50	50
100	35	59	90	99	100
150	38	67	117	143	150
200	39	72	136	180	198
250	40	75	149	210	244
300	41	77	159	235	286
350	41	79	167	255	324
400	41	80	174	272	360
450	42	81	179	287	391
500	42	82	183	300	420
600	42	83	189	320	470
700	42	84	194	336	511
800	43	85	198	349	546
1,000	43	86	204	367	601
1,200	43	86	208	381	642
1,400	43	87	210	391	673
1,600	43	87	212	398	699
1,800	43	88	214	404	719
2,000	43	88	215	409	736
3,000	43	88	219	425	791
4,000	44	89	222	433	821
5,000	44	89	223	438	839
6,000	44	89	224	441	852
10,000	44	89	225	443	888
100,000	44	90	228	457	915
∞	44	90	228	458	919

Adapted from Beal, 1983

Table 2. Number needed to test to be 95% confident that the disease will be detected if present at or above five levels of prevalence (hypergeometric).

Flock size (*N*)	Prevalence (*P*)				
	P = 0.10	*P* = 0.05	*P* = 0.02	*P* = 0.01	*P* = 0.005
	Sample size (*n*)				
N	*n*	*n*	*n*	*n*	*n*
20	15	19	20	20	20
50	22	34	48	50	50
100	25	44	77	95	100
150	26	48	94	129	150
200	27	51	105	155	190
250	27	52	112	174	-
300	27	53	117	189	-
350	27	54	121	201	-
400	27	54	124	210	310
450	28	55	126	218	-
500	28	55	128	224	-
600	28	56	131	235	378
700	28	56	134	243	-
800	28	56	135	249	421
1,000	28	57	138	258	450
1,200	28	57	139	264	471
1,400	28	57	141	268	486
1,600	28	57	142	272	500
1,800	28	57	142	275	509
2,000	28	58	143	278	517
3,000	28	58	145	284	542
4,000	28	58	146	287	555
5,000	28	58	146	289	563
6,000	28	58	146	291	569
10,000	28	58	147	294	580
100,000	28	58	148	298	596
∞	28	58	148	298	598

Adapted from Beal, 1983

Table 3. Number needed to test to be 90% confident that the disease will be detected if present at or above five levels of prevalence (hypergeometric).

Flock size (*N*)	Prevalence (*P*)				
	P = 0.10	*P* = 0.05	*P* = 0.02	*P* = 0.01	*P* = 0.005
	Sample size (*n*)				
N	*n*	*n*	*n*	*n*	*n*
20	13	18	20	20	20
50	18	30	45	50	50
100	20	36	68	90	100
150	20	39	80	118	150
200	21	40	87	136	180
250	21	41	92	150	210
300	21	42	95	160	235
350	21	42	97	168	256
400	21	42	99	174	273
450	21	43	101	180	288
500	21	43	102	184	300
600	21	43	104	190	321
700	22	43	105	195	337
800	22	44	106	199	359
1,000	22	44	108	205	368
1,200	22	44	109	209	382
1,400	22	44	109	211	392
1,600	22	44	110	213	399
1,800	22	44	110	215	405
2,000	22	44	111	216	410
3,000	22	45	112	221	426
4,000	22	45	112	223	434
5,000	22	45	113	224	439
6,000	22	45	113	225	442
10,000	22	45	113	227	449
100,000	22	45	114	229	458
∞	22	45	114	229	459

Adapted from Beal, 1983

Table 4. Expected number of tests needed for a single positive reaction for each composite sample containing 100 individual fecal samples, *n*, as a function of expected prevalence of HPAI.

Prevalence in waterfowl (*p*)	Number of positive composites (*r*)	Individual fecal samples/ composite (*n*)	Number of composite samples to test (*m*)
10^{-3}	1	100	10
10^{-4}	1	100	100
10^{-5}	1	100	1,000
10^{-6}	1	100	10,000
10^{-7}	1	100	100,000

Table 5. Number of individual fecal samples *n*, for a fixed prevalence *p*, needed to detect the presence of HPAI in 1 out of 100 composite samples. Calculation is based on the probability model given by equation 2.

Prevalence in waterfowl (*p*)	Number of positive composites (*r*)	Total number of com-posites samples (*m*)	Individual fecal samples per composite (*n*)
10^{-3}	1	100	10
10^{-4}	1	100	100
10^{-5}	1	100	1005
10^{-6}	1	100	10050
10^{-7}	1	100	10050

Table 6. Sample size required to estimate prevalence with the desired fixed width confidence limits

Level of confidence									
	90%			95%			99%		
Expected prevalence	Desired absolute precision			Desired absolute precision			Desired absolute precision		
	10%	5%	1%	10%	5%	1%	10%	5%	1%
0.5%	1	5	135	2	8	191	3	13	330
1%	3	11	268	4	15	380	7	26	657
2%	5	21	530	8	30	753	13	52	1,301
5%	13	51	1,285	18	73	1,825	32	126	3,152
10%	24	97	2,435	35	138	3,457	60	239	5,972
20%	43	173	4,330	61	246	6,147	106	425	10,617
30%	57	227	5,683	81	323	8,067	139	557	13,935
40%	65	260	6,494	92	369	9,220	159	637	15,926
50%	68	271	6,765	96	384	9,604	166	664	16,589
60%	65	260	6,494	92	369	9,220	159	637	15,926
70%	57	227	5,683	81	323	8,067	139	557	13,935
80%	43	173	4,330	61	246	6,147	106	425	10,617
90%	24	97	2,435	35	138	3,457	60	239	5,972