TRAINING MANUAL ON SURVEILLANCE AND INTERNATIONAL REPORTING OF DISEASES IN WILD ANIMALS

Second Cycle

Workshop for OIE National Focal Points for Wildlife

WORLD ORGANISATION FOR ANIMAL HEALTH
Protecting animals, preserving our future

TRAINING MANUAL ON SURVEILLANCE AND INTERNATIONAL REPORTING OF DISEASES IN WILD ANIMALS

Workshop for OIE National Focal Points for Wildlife

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FOREWORD

Dr Bernard Vallat
Director General of the OIE

In 2009, the OIE launched a global programme of capacity building for OIE Delegates and OIE National Focal Points of the 180 Member Countries. The aim of this programme is to provide good governance concepts for the improvement of animal health, animal welfare and the safety of food products of animal origin at the national level, to explain and clarify the roles and responsibilities of the OIE National Delegates and Focal Points in this domain and to facilitate, when possible, consistency and harmonisation amongst OIE Member Countries when assigning responsibilities to these officials.

The first OIE Training Workshop for OIE National Focal Points on Wildlife, held in all OIE Regions in 2009-2010, provided an overview of the importance of pathogens and diseases in wild animals to domestic animal health, to trade in animals and animal products, to human health and to wild animal populations themselves, which have very high economic, social and cultural value. A Training Manual covering the topics of the first workshop is now available on the OIE website[1].

A second OIE Training Workshop for OIE National Focal Points on Wildlife was organised in all OIE Regions during 2011-2012. It provided practical information and exercises to help Member Countries to design surveillance programmes for pathogens and diseases in wild animals, including both general and targeted surveillance, diagnostic test performance and evaluation, data interpretation, survey design and sample size calculation for different purposes.

This Training Manual covers the topics of this second workshop and provides material that can be further used for training purposes at national and international levels.

I would like to sincerely thank Dr F.A. Leighton and Dr Jane Parmley from the Canadian Wildlife Health Cooperative (OIE Collaborating Centre for Research, Diagnosis and Surveillance of Wildlife Pathogens), who developed this Training Manual and contributed with other wildlife experts to the success and effectiveness of the second cycle of this OIE training objectives.

[1] http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/working-groups-reports/working-group-on-wildlife-diseases/

Introduction

The purpose of this Workbook is to provide OIE National Focal Points on Wildlife with some practical knowledge and skills associated with surveillance for, and reporting of, diseases and pathogens in wild animals.

A fundamental responsibility of each Focal Point on Wildlife is to gather information about the occurrence of pathogens and diseases in wild animals in his or her country, to assemble this information for the OIE Delegate and others in the country, and to assist the Delegate to report to the OIE on those occurrences when they meet the criteria established for reporting.

Disease surveillance is the process by which information about the occurrence of pathogens and diseases in a country is obtained. Thus, it is important that focal points for wildlife understand the process of pathogen and disease surveillance, and also understand the strengths and weaknesses of the information derived from surveillance. In this workbook, the various components of surveillance are presented and exercises are included to practice evaluating surveillance data, to identify within surveillance data the pathogens and disease events that must be reported internationally, , to consider the choice and performance of diagnostic tests, to practice estimating the number of samples required in targeted surveillance programs, and to gain experience calculating prevalence from surveillance data. More information about different types of surveillance systems and surveillance data is available in the OIE Guide to Terrestrial Animal Health Surveillance[2].

The Workbook is divided into two main sections, one on General Wildlife Disease Surveillance (sometimes called "passive" surveillance), and the other on Targeted Wildlife Disease Surveillance (sometimes called "active" surveillance). Both of these sections contain exercises that support the material presented. To complete the exercises, 4 datasets are available: 2 for general surveillance and 2 for targeted surveillance. These datasets are available as separate Microsoft Excel (2007) files. At the end of the workbook, there are 5 appendices: in Appendix A, the fictitious country of Atlantis is described; in Appendix B, charts and maps derived from the general surveillance datasets are shown; in Appendix C, the OIE listed and non-listed diseases are provided; in

[2] available at: http://web.oie.int/boutique/index.php?page=ficprod&id_produit=1418&PHPSESSID=20f118ecd4851daa92e7ccf8bc20ad36&lang=en

Appendix D, charts and maps derived from the targeted surveillance datasets are shown; and in Appendix E, appropriate responses to the exercises included in the General and Targeted Wildlife Disease Surveillance sections are provided.

Pathogen Surveillance or Disease Surveillance?

The term "disease" surveillance often is used in a very general way to mean surveillance activities to identify actual clinical disease or death in animals and to identify the causes of such disease and death. The same term often is used to refer to surveillance programs, such as serological surveys, in which evidence is gathered about the presence of a particular pathogen in a population of animals, but the animals themselves may be healthy when they are sampled. In this second example, no disease is detected; only the pathogen is detected. Thus, when one speaks of "wildlife disease surveillance", it is important to clarify whether the surveillance program is designed to detect actual disease and death, and their causes (disease surveillance), or only to determine whether or not a particular pathogen may be present even if it is not causing clinical disease in the species or population of interest (pathogen surveillance).

Introduction to the World Organisation for Animal Health (OIE)

An **overview of the OIE**, its organisation, mission and history, is available on the OIE website at http://www.oie.int/fileadmin/vademecum/OIE_A-Z_2015.html.

The **responsibilities of OIE National Focal Points for Wildlife** to their OIE Delegates are outlined in their Terms of Reference, as follow:

1. To establish a network of wildlife experts within his/her country or to communicate with the existing network;

2. To establish and maintain a dialogue with the Competent Authority for wildlife in his/her country, and to facilitate cooperation and communication among several authorities where responsibility is shared;

3. To support the optimal collection and submission of wildlife disease information to the OIE through WAHIS;

4. To act as a contact point with the OIE Animal Health Information Department and the Scientific and Technical Department on matters related to information on wildlife including wildlife diseases;

5. To receive from the OIE Headquarters

   – copies of the reports of the Working Group on Wildlife
   – selected reports of the Scientific Commission for Animal Diseases
   – other relevant reports on wildlife or related to the livestock–wildlife interface,

   and to conduct the in-country consultation process on such draft texts and of drafts of proposed changes to OIE Standards dealing with wildlife diseases;

6. To prepare comments for the Delegate

   • on relevant meeting reports
   • on the proposals for new OIE standards and guidelines related to wildlife
   • reflecting the scientific view and position of the individual OIE Member Country and/or the Region.

Recommendations from the **OIE Global Conference on Wildlife** – February 2011

The OIE Global Conference on Wildlife: Animal Health and Diversity-Preparing the Future took place in February 2011, in Paris (France). Over 400 people with relevant expertise and experience met there to review and discuss issues in animal health and biodiversity. At the conclusion of this 3-day conference, the participants made the following recommendations to the OIE as an organisation and to each of the Member Countries of the OIE. Several recommendations of particular relevance to the roles of focal points for wildlife are highlighted below in bold type.

CONSIDERING:

1. The emergence and re-emergence of diseases that are transmissible among wildlife, domestic animals and humans,

2. The societal, economic and ecological value of diverse and healthy wildlife populations,

3. The key contribution of biodiversity and ecosystems services to health and the need to encourage research and expand knowledge on its interactions,

4. The need to increase the capacity of all countries worldwide to conduct surveillance, early detection, and initiate appropriate response to outbreaks and spread of diseases in wildlife,

5. The fundamental responsibilities of Veterinary Services and their government partners to protect and improve animal health, including aspects related to wildlife and biodiversity,

6. That the OIE is continuously developing and updating standards and trade facilitating mechanisms such as disease free zoning, compartmentalisation and safe trade in animal origin commodities to harmonise national regulation contributing to address the ecosystem interface between wildlife and domestic species,

7. That organisations internationally and nationally responsible for the delivery of public health, veterinary services, wildlife and the environment may be accommodated in different institutional units,

8. The increased need for animal protein for growing populations worldwide,

9. The changes in land use and management that may lead to new or modified interfaces between humans, domestic animals and wildlife that could favour disease transmission and loss of biodiversity,

10. The need for a multidisciplinary commitment and cooperation by stakeholders including public and non-governmental organisations to achieve mutually beneficial outcomes within the wildlife/domestic animal and human ecosystem interface.

THE PARTICIPANTS OF THE OIE GLOBAL CONFERENCE ON WILDLIFE RECOMMEND TO THE OIE:

1. To continue developing science-based standards on disease detection, prevention, and control as well as safe trade measures to harmonise the policies related to disease risks at the interfaces between wildlife, domestic animals, and humans.

2. To continue supporting and updating the notification mechanisms of wildlife diseases through the global information systems OIE WAHIS and *WAHIS-Wild*, while carefully considering possible impact of such notification by Members on the trade in domestic animals and their products, and to further promote data sharing at the international level on the GLEWS platform.

3. To assist Members to strengthen their Veterinary Services to protect animal health including aspects related to wildlife and biodiversity using, if needed, the OIE PVS Pathway.

4. **To encourage OIE Delegates to utilise their OIE focal points for wildlife to identify needs for national capacity building.**

5. To support Members' ability to access and utilise appropriate sampling and diagnostic expertise, as well as validated tools for disease surveillance and management in domestic and wild animals.

6. To encourage research to expand the scientific basis for the protection of biodiversity and environment to promote animal health and public health.

7. To encourage systematic inclusion, in the curriculum for veterinary education, of the promotion, the protection and the improvement of animal health and animal welfare including aspects related to wildlife and biodiversity.

8. To explore opportunities for communication and establishing strong collaboration with relevant global public and private organisations working on wildlife and biodiversity such as FAO, WHO, UNEP, IUCN, CIC, CITES[3] and other relevant Multilateral Environmental Agreements and international organisations to strengthen support to existing regulations on trade in wildlife and wildlife products and advocate for the need for mobilisation of resources in this area.

9. To continue to develop and update OIE strategies and policies on wildlife and biodiversity through the work of the Scientific Commission and its Working Group on Wildlife Diseases as well as the network of OIE Reference Laboratories and Collaborating Centres.

RECOMMEND TO OIE MEMBERS:

10. To continue to implement international standards and guidelines on prevention and control of diseases including those transmissible among wildlife, domestic animals and humans.

11. To continue to implement international standards and guidelines to facilitate the acceptable, legal trade of wildlife animals and wildlife products and to help reducing the illegal trade in wildlife.

12. **To notify diseases in wildlife through WAHIS and *WAHIS-Wild*, including in quarantine facilities, while carefully acknowledging when the notifications should not impact on trade of domestic animals and their products with commercial partners according to the OIE standards on relevant diseases.**

[3] Food and Agriculture Organization of the United Nations, World Health Organization, United Nations Environment Program, International Union for Conservation of Nature, International Council for Game and Wildlife Conservation and Convention on International Trade in Endangered Species of Wild Fauna and Flora

13. To ensure that the national Veterinary Services and their partners fulfil their responsibilities on aspects of biodiversity conservation, animal health and animal welfare as they relate to wildlife and the environment, including appropriate legislation and regulation, and, where needed, seek assistance through the OIE PVS Pathway to improve their services.

14. **To nominate and support national OIE Focal Points for Wildlife in their tasks and encourage their collaboration with partner agencies and organizations.**

15. To seek and apply appropriate sampling and diagnostic expertise and validated disease management tools for wildlife diseases, including with the participation of private veterinarians, medical doctors, community workers, fishermen, hunters, rangers, and other stakeholders.

16. To support relevant research to expand the scientific basis for the protection of biodiversity and environment to promote animal health as well as public health.

17. To support systematic inclusion, in the curriculum for veterinary education, of the promotion, the protection and the improvement of animal health and animal welfare including aspects related to wildlife and biodiversity.

18. To encourage public and private components of Veterinary Services to play an active role in promoting biodiversity and protecting wildlife.

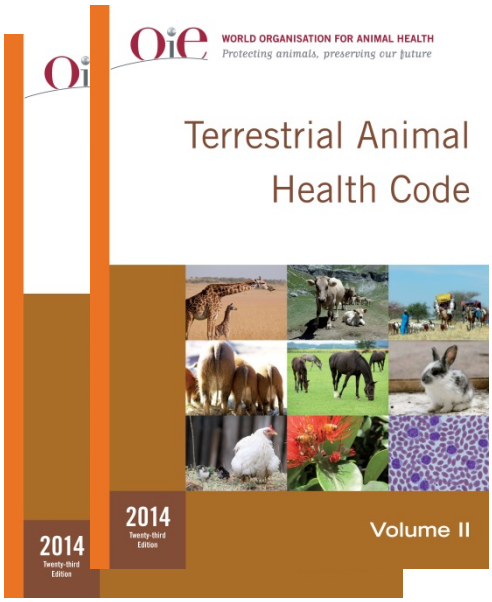
19. **To foster effective communication and collaboration at the national and regional level between different governmental agencies that share responsibilities for the environment and the health of wildlife, livestock and the public.**

20. **To explore and promote opportunities for communication, collaboration and partnerships with relevant public and private organisations having an interest in wildlife management and biodiversity including the tourism industry, private veterinarians and medical doctors, natural park and zoo managers, rangers, hunters, fishermen, conservation associations and local indigenous communities and stakeholders.**

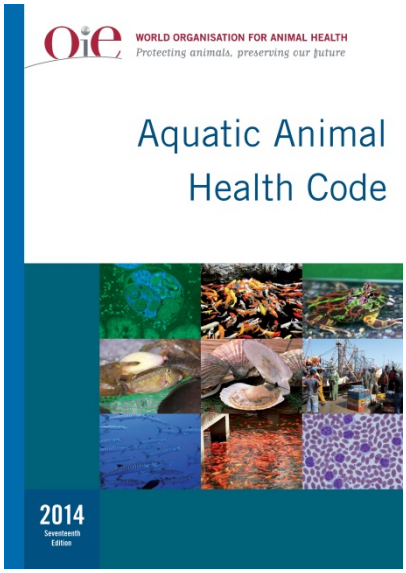
21. To promote the adoption of legislation to clarify or define ownership of wildlife by people and organisations.

OIE Terrestrial and Aquatic Animal Health Codes

These publications of the OIE provide fundamental information about surveillance and reporting of diseases to the OIE, and are important resources.



http://www.oie.int/en/international-standard-setting/terrestrial-code/access-online/



http://www.oie.int/international-standard-setting/aquatic-code/access-online/

General Wildlife Disease Surveillance

Introduction to General Wildlife Disease Surveillance

Definition and Purpose

Definition: General wildlife disease surveillance is a form of surveillance that identifies sick or dead wild animals in their native habitat and determines the causes of the illness and death. It is based on the diagnostic examination of wild animals found sick or dead in the wild. It is called "general" disease surveillance because the program includes a wide range of animal species and all causes of disease or death. It is not restricted to only one or a few species, or to only one or a few pathogens. Typically, national programs of general wildlife disease surveillance include all species of vertebrates (mammals, birds, reptiles, amphibians and fish), but some may also include some groups of invertebrates or there may be separate surveillance programs for terrestrial and for aquatic wild animal species.

There are many "official" definitions of "surveillance" and "monitoring" that overlap and differ in small ways. A common theme among these definitions is the concept that surveillance and monitoring are *on-going, continuous activities* and that the *information obtained* from these activities *is used in some way*, usually to help countries formulate their public policies and programs regarding animal and human health.

Definitions of "wildlife" also vary. Under different circumstances, different groups of animals may be considered "wildlife." This point is discussed from an OIE perspective on Page 4 of the OIE *Training Manual on Wildlife Diseases and Surveillance*, which is the training manual for Cycle One of this series of workshops and is available from the OIE Headquarters, Scientific and Technical Department, in English, French and Spanish.

The Purpose of General Wildlife Disease Surveillance: A surveillance program must always have a purpose, a reason why it is being done. Disease surveillance is difficult, time-consuming and expensive. It is undertaken because the information provided by surveillance is needed for one or more purposes. No disease surveillance program should be designed and initiated until its purpose is defined, because the purpose of the program will affect the design of the program in important ways.

Most often, national programs of general wildlife disease surveillance are undertaken for the following reasons:

- To learn what pathogens and diseases are present in wild animal populations in a country, their host species and their geographic distribution, including pathogens and diseases important to domestic animals, to public health and to wild animal populations themselves.
    - This information is an essential component of a country's capacity to manage animal and human health
- To detect new pathogens and diseases, or unusual epidemiological events that may indicate an emerging disease, as early as possible.
    - General disease surveillance can detect new, unusual or emerging pathogens and diseases while targeted surveillance detects only one or a small number of pathogens already known to exist.

- General disease surveillance is required in order for a country to be vigilant for emerging infectious diseases, many of which originate in wild animals.
- Early detection of new disease events permits an early management response to the event that is more likely to be successful, and likely to cost much less, than is the case when the disease event is detected later.

- To detect changes in patterns of disease occurrence over time.
  - General disease surveillance documents the current conditions of disease occurrence and distribution in a country, and provides a mechanism to detect changes in these conditions that may signal important changes in epidemiology and risk. Such risks can then be evaluated and responses made, if deemed necessary.

The Components of General Wildlife Disease Surveillance

Disease surveillance, whether general or targeted, consists of four very different activities, each one carried out by different groups of people and each one linked to the others to form a complete surveillance program.

- Detection of Pathogens and Diseases
- Identification of Pathogens and Diseases (diagnosis)
- Information Management
- Analysis and Communication

Disease surveillance thus depends most importantly on a network of people who know their capacities and responsibilities within the surveillance system and who communicate with each other easily and regularly.

Disease Surveillance in Wild Animals compared with Domestic Animals

Some aspects of disease surveillance in wild animals are the same or similar to disease surveillance in domestic animals. However, there also are important differences.

- Wild animals have no owners or attending veterinarians to recognize illness.
- The routine diagnostic tests for pathogens and diseases developed for domestic animals may or may not be valid for wild animal species. (This topic will be covered in greater depth later in the workbook)
- Wildlife biologists and ecologist are needed to provide data on populations and other aspects of wildlife biology and to analyse, interpret and communicate the results for a wildlife disease surveillance program.

Developing a Wildlife Disease Surveillance Program

To develop a surveillance program for pathogens and diseases in wild animals, it is necessary to establish the network of people and institutions needed to make the program function. This is one of the main tasks of OIE Focal Points for wildlife. The details of how best to do this will vary from country to country, but the general approach taken is likely to be similar among different countries.

Developing a Wildlife Disease Surveillance **Network**

We are briefly going to consider what kind of a network of people and institutions is needed for a general wildlife disease surveillance program in a country.

Step 1: Who can Detect Sickness or Death in Wild Animals?

The answer is "Anyone and everyone who observes wild animals or spends time in wild animal habitats." A wildlife disease surveillance program must recruit as many of these people as possible to participate in the program, to look for sick or dead wild animals and immediately to notify the coordinators of the surveillance program when they observe or find something.

The Objectives of *Detection of diseased wildlife*:

- To detect and record occurrences of death and disease in wild animals
- To obtain specimens of dead and diseased wildlife
- To preserve specimens for laboratory examination
- To transport those specimens to a diagnostic laboratory

Who can participate in Detection?

Network for Detection of Disease in Wildlife

Government Agency Staff	Non-government People
- Wildlife	- Hunters and trappers
- Environment	- Fish harvesters
- Fisheries	- Farm workers
- Forests	- Tourist guides
- Tourism	- Naturalist groups
- Agriculture	- Conservation organizations
- Nature parks and reserves	- University staff
- Pest control	- Private wildlife parks
- Border control	- Wildlife commodity merchants
- Military	- Industry:
- Veterinary service	- Forestry
- Public health	- Mining
- Economic development	- Fossil fuel
- Transportation	- Transport
- Regional/municipal government	- The General Public
- Aboriginal governments	

Note that the entire disease surveillance program depends on this first component: detection of dead and diseased wild animals. If this component is not working well and achieving its objectives, the surveillance program as a whole also will not achieve its objectives. This component also can be very challenging to organize and maintain. Considerable time, effort and resources will be required to achieve this component of wildlife disease surveillance.

When considering who can participate in disease detection, think about how the different groups and individuals may be involved. For example, will they collect and submit dead animals or do they simply have knowledge about how to report animals found dead?

Step 2: Who can Identify Pathogens and Diseases in Wild Animals?

People in the Field

Sometimes, it is possible for people in the field to correctly identify the disease they observe in wildlife.

- Some diseases can be identified by an animal's appearance, such as mange.
- Others have very characteristic lesions seen during autopsies that some field personnel may be competent to conduct.

People in Diagnostic Laboratories

Most of the time, *field identification is, at best, a tentative identification* and reliable identification of pathogens and diseases requires examination and testing of specimens in a diagnostic laboratory and, if needed, confirmation by a Reference Centre.

What laboratories might be able to do this in a country?

Potential Diagnostic Laboratories for Wildlife Pathogens and Diseases

- Government Veterinary Diagnostic Laboratories
- Veterinary Faculty/University Diagnostic Laboratories
- Private Veterinary Diagnostic Laboratories
- Government Medical Laboratories
- Private Medical Laboratories
- University Research Laboratories
- Hospital Laboratories
- Military Medical or Veterinary Laboratories
- International/Regional Laboratories
    - Veterinary & Medical Laboratories in Neighbouring Countries
    - OIE Reference Laboratories
    - OIE Collaborating Centres
    - FAO Reference Laboratories
    - WHO Reference Laboratories
    - NGO-affiliated Laboratories

- Countries will differ as to which kinds of laboratories can be recruited to participate in a wildlife disease surveillance program.

- Rarely will a country have the resources to support a diagnostic laboratory exclusively for wild animal diseases. Wildlife disease identification most often will be done in laboratories established for veterinary or medical diagnosis.

Important role of Anatomic Pathology (autopsy and histology) in General Disease Surveillance

Autopsy and subsequent microscopic examination of tissues (histology) plays an especially important role in general disease surveillance. In general disease surveillance, all causes of all illness and death detected in wild animals are to be identified, if possible. The evaluation of organs and tissues at autopsy and subsequent histology are the main criteria for choosing what additional diagnostic tests need to be carried out, such as bacterial culture, PCR for certain pathogens or toxicological tests. Autopsy (necropsy) and histology are especially important for the identification of new or unexpected pathogens and diseases.

Thus, it is particularly important that a country have, or develop, adequate expertise and capacity in veterinary anatomical pathology that can be applied in its general wildlife disease surveillance program. It is not possible to achieve the usual objectives of a national general wildlife disease surveillance program without this capacity and competence in diagnostic anatomical pathology.

Step 3: Information Management

A disease surveillance program must have a system to record the information it generates about disease or pathogen occurrences so that the information can be used to achieve the objectives of the surveillance program. The most suitable approach to management of surveillance information is some form of computerised database or archive of surveillance data. While it is possible, initially, to manage such information with spreadsheets or database software available for personal computers, such software quickly becomes inadequate as the amount of data increases. Thus, it is better to **recruit to the wildlife disease surveillance program people with the required knowledge and skills in computer database design and management.**

Who can undertake disease surveillance information management?

Potential Sources of Information Management Expertise

- Government Information Management Systems:
  - Veterinary Services
  - Public Health
  - Wildlife
  - Environment
- Universities – Computer Science, Epidemiology
- Private companies
- International sources - for example*:
  - Canadian Wildlife Health Cooperative**
  - Wildlife Conservation Society
  - US National Wildlife Health Center**
  - Australian Registry of Wildlife Health

* These organizations have wildlife disease information management systems that can serve as models for system design by a country for its own surveillance information, or which may be available for direct use.

** OIE Collaborating Centre

Step 4: Analysis and Communication

To achieve the objectives of a disease surveillance program, the information acquired through detection of dead or sick animals and identification of their pathogens and diseases must be reviewed, analysed in various ways and communicated within the country and internationally.

Who will do the analysis and communication?

National veterinary services often employ people skilled in epidemiological analysis and also in communication, and who carry out similar work with respect to disease surveillance in domestic animals. However, for wildlife diseases, both analysis and communication nearly always require additional analytical expertise and have additional audiences requiring communications.

- Wildlife biologists and ecologists are required to properly analyse wildlife disease occurrence data.

- All of the participants in the wildlife disease surveillance program, including government and non-government groups, expect to receive the results of the surveillance program through various communications from the program coordinators.

Wildlife Disease Surveillance

Expertise Required for Analysis*

- Veterinary Medicine
- Epidemiology
- Wildlife Biology and Ecology
- Public Health

Audiences for Communications**

- Veterinary Services
- Public Health
- Wildlife and Environment
- ALL other surveillance program participants***
- OIE and other international organizations, as required

* Wildlife disease surveillance analysis needs input from a wide range of experts

** Wildlife disease surveillance information is needed by a wide range of agencies and groups.

*** People who participate in the surveillance program will expect to receive information about the results of their work. If they do not receive this kind of information, they may lose interest in participating in the program.

Making Surveillance Work – The Key Role of the Coordinator

The four components of a disease surveillance program must be closely and constantly coordinated. **It is coordination that turns these four independent components into surveillance.** Coordination of the program is challenging, full-time work for a small number of people. Their job is to ensure that all components of the program operate in a manner that achieves the objectives established for the surveillance program.

Who will coordinate a wildlife disease surveillance program?

Wildlife disease surveillance is challenging to coordinate for the following reasons:

- In nearly all countries, responsibility for wildlife health and disease management is poorly defined, and the responsibility is shared, uncertainly, by several different branches of government including wildlife, environment, public health, agriculture, veterinary services, tourism, economics, border services and international relations. Thus, no one branch or agency of government has unique authority over wildlife disease issues, and it is not clearly the responsibility of any one agency to coordinate wildlife disease surveillance.

- Wildlife disease surveillance requires a larger, and a different, network of people than do disease surveillance programs for people and domestic animals. Thus, organizational models for the coordination and operation of other disease surveillance programs may not work well for wildlife disease surveillance.

- Wildlife biologists and ecologists are essential participants in wildlife disease surveillance. Government agencies such as veterinary services and public health, which are familiar with their own forms of disease surveillance, often have no traditions or experience working with agencies responsible for wildlife and the environment where biological and ecological expertise is to be found.

- Non-government organizations, universities and other groups outside of government often are critically important participants in wildlife disease surveillance and the coordinator must understand and work closely with such groups as well as with government agencies.

There are several different ways in which coordination of wildlife disease surveillance can be organized. A few examples are given below.

1. Coordination by one government agency. It is common for a government agency that needs wildlife disease surveillance information, or is responsible for other aspects of animal health, to feel it must or should undertake coordination of the national wildlife disease surveillance program. This can work well, providing the coordinator is given the flexibility required to engage all of the different government and non-government groups needed for an effective program. Several countries have excellent surveillance programs based on this approach.

2. Coordination by a coalition of government agencies which manage the program together through a written agreement. This has the advantage that the surveillance program is not considered to be owned by a single agency, and other government agencies may be more willing to support the program.

3. Coordination by a non-government organization. This model facilitates the collaboration among government agencies from different ministries and among government and non-government participants in the surveillance program. Resources for the program can be pooled and managed by the coordinator, and the program is carried out under the authority of the participating government agencies, which also play an oversight and governance role.

No matter how or by whom coordination of wildlife disease surveillance is organized in a country, the OIE focal point for wildlife will want to play a key role in assuring and facilitating effective coordination.

Data Required for General Wildlife Disease Surveillance

What data should be collected in a program of general wildlife disease surveillance?

It is critically important to carefully consider and decide well before the surveillance program is initiated what data will be recorded, entered into the program database, analysed and used. It is a common impulse to try to capture too much data because there is so much that can be recorded. When planning a surveillance program, it is common that planners identify many, many pieces of information that could be recorded for each occurrence of wildlife disease detected, and which might be useful in some way.

In practice, however, attempts to collect very large amounts of information systematically for each disease occurrence often fail. The recording and entry into computers of data require a lot of time and effort. The personnel time required to record large amounts of information often is not available, and very soon the recording process breaks down, records become partial and incomplete, and some critically important data go unrecorded.

- Recording of data and entry into computer systems takes time and effort (resources)
- This can easily become too much work, that cannot be done sustainably
- The whole system fails if you try to record too much data

Thus, most often, the best practice is to define the minimum amount of information that is needed to achieve the objectives of the surveillance program, and to ensure that at least these data are always recorded and made part of the permanent record for every disease occurrence.

Minimum Essential Data to be Recorded

Data about the Incident (the disease occurrence event)

1. Unique Number to identify the disease Incident or Occurrence (An incident usually is defined as one or more sick and dead animals found at one location, on one day or very close together in time)
2. Date on which the incident occurred or was discovered
3. Geographic Location: Latitude and Longitude
4. Number of animals dead
5. Number of animals sick
6 Number of animals examined or submitted to a laboratory for diagnosis/disease identification

Data about the Animals in the Incident (Specimen data)
(For each animal examined or sampled)

1. Unique specimen number for each
2. Species of animal – Latin Name (*Genus species*)
3. Species of animal – Common Name
4. Laboratory Accession Number (if specimen was sent to a diagnostic laboratory)
5. Other Laboratory Accession Number (if sent to more than one diagnostic laboratory)
6. Cause of death or illness (name of pathogen, disease or other cause)
7. Method used to determine cause of death or illness

Example of a minimum set of data for general Wildlife Disease Surveillance:

Incident ID	Date Found	Latitude	Longitude	Num Dead	Num Sick	Num Submitted
0000035050	12/03/2015	-2.616582693	37.23678589	10	0	2
0000035050	12/03/2015	-2.616582693	37.23678589	10	0	2
0000035051	22/03/2015	54.73492899	-107.5211334	2000	50	10
0000035051	22/03/2015	54.73492899	-107.5211334	2000	50	10

Specimen ID	Latin Name	English Name	Lab Specimen Number
0000035050.1	Syncerus caffer	African Buffalo	ILRI2015.22
0000035050.2	Syncerus caffer	African Buffalo	
0000035051.1	Phalacrocorax auritus	Double-crested Cormorant	PDS20150341
0000035051.2	Phalacrocorax auritus	Double-crested Cormorant	PDS20150342

Primary Diagnosis	Basis for Diagnosis
Anthrax	Bacterial culture and PCR verification
Anthrax	Association with specimen 0000035050.1
Newcastle Disease	Autopsy, Histology, PCR
Newcastle Disease	PCR

Incident ID:

An "incident" generally means a disease occurrence at one location at one time, involving one or more animals. There should be an incident number for each such disease occurrence event for clarity of identification. Each specimen examined (see "specimen number" below) will be associated with a particular incident. This can be very important when there are multiple incidents and multiple specimens. Analysis and interpretation of the surveillance results requires that all the specimens associated with a particular incident can be identified. The incident ID should be designed so that the same format can continue to be used for decades to give sequential numbers to each new incident; that is the reason for all the zeros in the incident ID numbers listed above.

Date Found:

Usually you want to capture the date the animals died, or that the first animal died – the beginning of the event. Often, this date is not known and the first date that can be recorded with certainty is the date on which the incident was first discovered by someone. If this date is not known but a specimen was sent to a diagnostic laboratory, then the earliest date that is known may be the date the specimen is received in the laboratory. It is important to recognize that the date available may not be the same for each specimen; this can affect analysis and interpretation of surveillance data. A database can be set up with a space for any or all of these different potential dates so that it is clear to the people who use the data exactly what date has been recorded (date of first mortality, date dead animal was found, date specimen was received by a laboratory, etc…).

Latitude/Longitude – Location of the Incident:

Information about the location of the incident can be obtained from: GPS units carried by field personnel, GPS units available on mobile telephones, or from maps. Location coordinates can also be obtained from online sources such as: iTouch Map: http://itouchmap.com/latlong.html and Google Earth: https://www.google.com/earth/.

Number Dead, Sick, Submitted:

The point of this information is to give some sense of the scale of the incident. This approach does not capture the numbers of different species that may be dead or sick or submitted; this is a compromise: enough versus too much data. The species submitted to a diagnostic lab will be found in the specimen data. The number dead or sick can be VERY difficult to determine. If the affected animals are very large, and the landscape is flat and open, a total count may be possible. More often, most of the dead and sick animals are difficult to see, even if a systematic search is made. They will be hidden by forests, grass, rocks and other topography. Dead animal carcasses also disappear very quickly – in a few hours to a few days. Thus, for wildlife, the data recorded for number of dead and sick usually are very approximate, often represent a very minimum estimate, and must be interpreted in this way.

Specimen Identification Number:

A specimen is all or part of a dead/sick animal collected for laboratory examination and testing. A specimen number is needed to ensure that all information associated with one specimen is clearly identified as pertaining to that specimen. In the table above, the specimen number is the Incident number with sequential addition of a specimen number after a decimal point. This approach makes it easy to associate a specimen with an incident but it could also be a completely different number.

Latin Name & Common Name:

A Latin Name is essential because Common Names are totally unreliable. Often, there are many common names for a single species of animal and one common name can often be applied to more than one species of animal. It is essential to know the species precisely because a common objective of wildlife disease surveillance is to determine what species of animal carry what pathogens. Also, if a pathogen is found that is important to human or animal health, to trade, or to other things, knowing precisely what species are or can be infected with that pathogen is essential to managing the risks associated with that pathogen. The species is also required information when reporting disease occurrences in wild animals to the OIE. Correctly identifying wild animal species can be challenging and a wildlife disease surveillance program must include people who can correctly identify wildlife species, in the field and also in the laboratories where specimens are sent for examination.

Laboratory Specimen Number:

Most diagnostic laboratories will assign their own laboratory accession numbers to specimens sent to those labs for diagnosis. Thus it is essential to record these laboratory numbers in the surveillance database so that the identity of the specimen is maintained when it is sent to a diagnostic laboratory. The Laboratory probably will NOT record your incident or specimen number. Diagnostic laboratories keep their own records of the diagnostic procedures and tests they perform on specimens. With the laboratory number, it will be possible to go back to the laboratory for additional information about a particular specimen in the surveillance database.

Primary Diagnosis:

Often, a dead animal examined at necropsy and by further testing will be found to have more than one pathogen or disease. The diagnosis of interest to the general surveillance program is the cause of the disease incident. Other findings may be of interest but the main diagnosis of interest is the cause of the incident. Thus, an animal may be shot because it is behaving strangely, and diagnostic examination may find that the animal had rabies. In this case, rabies, not gunshot, is the diagnosis of interest to the surveillance program.

Basis for Diagnosis:

This field captures information about the reliability of the diagnosis. For example, a diagnosis that is made on the basis of clinical signs may be highly reliable for certain diseases in certain species, and very unreliable for other diseases in other species. All primary diagnoses should be recorded but it also is important to indicate the evidence that was used to make the diagnosis.

Additional Data:

There is a large amount of additional information that can be recorded for each incident and each specimen:

- Background information about the Incident
- Names, addresses and contact information for the people who discovered the disease occurrence, who obtained and submitted specimens, etc.
- Autopsy and histological observations
- Each laboratory test carried out, and the result
- Dates of each step in the diagnostic process

These additional data can be important to some surveillance programs and less so to others, and each program will have to decide on the total of information that is to be recorded.

Exercises:

General Wildlife Disease Surveillance in the "*Dominion of Atlantis*"

(Note: this country does not exist)

- ⇨ You will be designing a program of General wildlife disease surveillance for the fictitious country we are calling The Dominion of Atlantis.
- ⇨ General information about the Dominion of Atlantis is given in Appendix A.

Rationale for a Wildlife Disease Surveillance Program in *Atlantis*

Atlantis' reasons for organizing a program of wildlife pathogen and disease surveillance are:

1. To protect wildlife populations by detecting and managing important health issues
   - High economic value of wildlife to national economy
2. Identification of pathogens in wildlife potentially harmful to livestock
3. Identification of pathogens in wildlife potentially harmful to human health
4. Early recognition of new pathogens or diseases, and of unusual epidemiological events.

ACTIVITY 1

Review the information about the *Dominion of Atlantis* (Appendix A) and its reasons for wanting a surveillance program for wildlife diseases.

Outline, in the spaces provided below, how Atlantis could organize each of the four components of a disease surveillance program to meet its objectives.

1. **Detection of Diseases or Dead Wild Animals: (Who can do this? How could it be organized?)**

[write here]

2. **Identification of Pathogens and Diseases (Who can do this? How could it be organized?)**

[write here]

3. **Information Management (Who can do this? How could it be organized?)**

[write here]

4. **Analysis and Communication (Who can do this? How could it be organized?)**

[write here]

General Surveillance Scenarios for *Atlantis*, Data Review and Analysis

ACTIVITY 2

Review and Analysis of Surveillance Data.

(Note: Two different files of general wildlife disease surveillance data are available. Each provides a different set of data containing one year of data from the general wildlife disease surveillance program of the Dominion of Atlantis. Each dataset consists of just over 300 records of pathogens or diseases that were detected and identified in wild animals. The exercises based on these sets of general wildlife disease surveillance data can be carried out on just one of the datasets, or they can be carried out separately on each of the two sets of data.

The data are in separate Excel files entitled: Dataset General Surveillance 1 (G1) and Dataset General Surveillance 2 (G2). Download and save a copy of the spreadsheet on your computer. These files can be downloaded from the OIE website. If you introduce errors into the data, you can delete that file on your computer, and download another copy and start over.

To complete the exercises, you will also need to review the maps and charts that were created from the data provided in the Excel data files. These are available in Appendix B.1 for General Surveillance 1 and Appendix B.2 for General Surveillance 2.

1. Learn to sort the data on the spreadsheet so you can more easily review it.
(5 minutes)

To sort the data, use the following procedure:

- Highlight ALL of the rows and columns of the total set of data, all 300+ rows, *including* the row of column labels.
    - Note: If you leave out some rows or columns when you sort the data, those rows and columns will not be sorted, while the highlighted data will be reorganized. If this occurs, the data for each incident and specimen will be mixed up with data from other incidents and specimens and it will then be impossible review the surveillance results.
- Click on the "Sort & Filter" button at the top of the screen
- From the drop-down list, select "Custom Sort"
- A new window will appear in which you can choose the category of data to "sort by". Select the category you want from the drop-down list. The list of categories will be taken from the first row of the spreadsheet that you have highlighted. Choose the category of data by which you want to re-arrange the whole spreadsheet.
    - For example – you may choose to sort the data by "date found" or by "Latin Name" of the affected animal or by "primary diagnosis".
    - You can sort the data as many times as you want, but don't leave out any rows or columns when you perform each sort.

2. Review the full set of data and Map G1A or G2A, which shows the location of each disease occurrence for dataset G1 or G2.

What kinds of Errors can you find in the data? (Don't spend too much time on this)

- Missing Information:
    - Read the Column Headings to see the kinds of data recorded
    - Quickly look over the rows and columns and note where you see blank cells.
        - Note the kind of information that is sometimes missing
        - To see how commonly certain kinds of data were not recorded during the surveillance program of 2015, sort the whole data set by that category of data. For example, sort by Date Found, or Latin Name, or Primary Diagnosis, or Latitude and note how many records are missing this value.
        - List the categories of data you find are sometimes missing and note approximately the percent of records that are missing this information

- Can you find errors in location?
    - Look for Latitude with negative values and longitude with positive values – Atlantis is North of the equator (positive number) and west of 0 degrees longitude (negative number)
    - Look at Map G1 A or G2 A – this shows all of the locations at which dead animals were found. Do you see any potential errors on this map?
- Can you determine if there are any duplicate records? Cases that have been entered more than once and will seem to be different cases?
    - Try sorting by "Specimen ID" and look for duplicate entries

Make a list of the kinds of errors or omissions you have found. Why do you think these different kinds of errors have occurred in the survey data?

[write here]

Do you see any patterns in the Data?
(A "pattern" is an easily–seen difference from an even or random distribution of events)

- Patterns in the location of disease occurrence? (look at the Maps provided)
- Patterns according to time of year? (try sorting data by "date found")
    - Look at Chart G1A or G2A – this shows all dead animals found in 2015 according to the date on which they were found
- Patterns in the occurrence of certain diseases? (try sorting data by "primary diagnosis", and then by "date found" within the first sort)
    - Also Review Maps G1 B, C or G2 B, C, D, and the Charts provided in Appendix B

Make a list of the kinds of patterns you have found. Can you explain why some of these patterns may have occurred?

[write here]

Do you see any diseases or pathogens that strike you as particularly important for the *Dominion of Atlantis*?

- Review the economic and other relevant facts about Atlantis in Appendix A
- Sort the entire dataset by Primary Diagnosis and review the Primary Diagnosis column to see all the pathogens and diseases detected.
    - List the diseases you think might be important and note why you think they may be important.
- Do any of the pathogens or diseases you have identified as potentially important to Atlantis show any particular patterns of occurrence – for example, occurring close together or at the same time of year? To investigate this, you could try the following:
    - Sort the entire dataset by Latitude or by Longitude and then look at the Primary Diagnosis column to see if any of the pathogens or diseases you have identified as important seem to cluster at one location.
    - Sort the entire dataset by Date Found and then look at the Primary Diagnosis column to see if any of those pathogen or disease occurrences seem to all happen at a particular time of year.
        - If you think certain pathogens or diseases occur at a similar time of year, sort the data again by Primary Diagnosis and then look at the dates of occurrence for that pathogen or disease of interest.

List the pathogens and diseases you think may be important to Atlantis and explain your reasons for thinking each one might be important.

[write here]

Do you see any pathogens or disease occurrences that should be **reported to the OIE**?

- OIE-List Diseases? (See Appendix C for a list of these diseases)
    - Remember to look for and include any unusual epidemiological events that might become emerging diseases
- Non-List wildlife diseases of interest to the OIE? (see Appendix C for a list of Non-listed wildlife diseases of interest to OIE)
- Potential emerging diseases that may be of epidemiological significance to Atlantis and other countries?

OIE Terrestrial Animal Health Code, Article 1.1.3.
Veterinary Authorities shall, under the responsibility of the Delegate, send to the *Headquarters*:

1. in accordance with relevant provisions in the *disease* specific chapters, *notification* through the World Animal Health Information System (WAHIS) or by telegram, fax or e-mail, within 24 hours, of any of the following events:
   a) first occurrence of a *listed disease* and/or *infection* in a country, a *zone* or a *compartment*;
   b) re-occurrence of a *listed disease* and/or *infection* in a country, a *zone* or a *compartment* following a report declared the *outbreak* ended;
   c) first occurrence of a new strain of a pathogen of a *listed disease* in a country, a *zone* or a *compartment*;
   d) a sudden and unexpected increase in the distribution, incidence, morbidity or mortality of a *listed disease* prevalent within a country, a *zone* or a *compartment*;
   e) an *emerging disease* with significant morbidity or mortality, or zoonotic potential;
   f) evidence of change in the epidemiology of a *listed disease* (including host range, pathogenicity, strain) in particular if there is a zoonotic impact;

List the pathogens and disease occurrences you find that would fall under each of these categories: a) OIE Listed Diseases, b) non-OIE listed wildlife diseases of interest to the OIE, and c) potential emerging diseases.

[write here]

*Based on your review of the data, **what improvements do you think are needed in the surveillance program** of the Dominion of Atlantis? What improvements would you suggest? How could Atlantis achieve the improvements you think are needed?*

[write here]

Targeted Wildlife Disease Surveillance

Introduction to Targeted Wildlife Disease Surveillance

Definition and Purpose

Definition: **Targeted** wildlife disease surveillance (also called 'active' surveillance) focuses surveillance efforts on one or more particular pathogens (viruses, bacteria, fungi, protozoa) in one or more wild animal species. Different from General wildlife disease surveillance, Targeted surveillance programs usually focus on detection of the target pathogen(s) or infection, not disease (sick animals).

It is not practical to have targeted surveillance programs for every disease or pathogen. Priorities and criteria for the inclusion of pathogens for targeted surveillance vary from country to country and between different regions of the world. The surveillance system should generate information that is needed to improve the current understanding of a certain pathogen or infection, where it occurs and does not occur, how frequent it is and whether it is becoming more or less common, so that appropriate management decisions can be made. Most often, the decision to include a pathogen or infection in a Targeted wildlife disease surveillance program is based on the importance of the pathogen to public health and human wellbeing, either directly (e.g. zoonotic pathogens) or indirectly (e.g. pathogens that can have important effects on livestock production or trade).

The Purpose of Targeted Wildlife Disease Surveillance: Like General wildlife disease surveillance programs, Targeted wildlife surveillance programs need a clearly stated purpose, a clear reason why the surveillance program is needed. Targeted surveillance programs typically are developed when new information about a particular pathogen is received from other surveillance programs, from research projects within a country or from a neighbouring country. This information may be specific to a wild animal species or may arise from surveillance carried out in domestic animals or people. Information from other sources does not only identify the pathogen of concern (the target), but can also provide critical insight about which wild animal species should be included in the program and which diagnostic tests would be most appropriate to use.

Targeted wildlife disease surveillance programs are usually developed and implemented for one of the following reasons:

- To demonstrate freedom from a particular pathogen or infection
- To determine if particular pathogens of concern are present
- To identify trends/patterns in the distribution and occurrence of the pathogen

Most targeted disease surveillance programs that aim to demonstrate freedom from infection are also able to detect the presence of a pathogen should it spread to the country or region. In the first case (to demonstrate freedom from infection), analysis of the data collected will provide evidence that the pathogen is **not** present above a certain level of prevalence, assuming that no, or very few, animals test positive. In the second case (to detect the pathogen), analysis of the data will provide evidence that the pathogen **is** present at or above a certain level of prevalence if one or more animals do test positive.

When a Targeted wildlife surveillance program is designed to identify trends and patterns in the distribution and occurrence of a particular pathogen, usually the intent is to measure prevalence. Prevalence is the proportion of affected animals in the population (these can be new/active infections and older/chronic/recovered cases, depending on the tests used).

Some Targeted disease surveillance programs report incidence rather than prevalence. Incidence is the number of new cases observed in a group of animals in a given time period. Incidence is not the same as prevalence. The difference between incidence and prevalence is most important when investigating infections that can lead to chronic diseases; the difference is less important when doing surveillance on infections that cause only acute disease. Because of the challenges involved in getting accurate population data for wild animal populations, prevalence often can be estimated but incidence usually cannot.

Data collected through Targeted surveillance programs designed to identify trends and patterns in infection are often compared across years (to look for temporal trends) or across regions (to identify higher and lower risk areas). This information can then be used to inform and enhance sanitary practices for domestic animals, and wildlife management plans and activities.

Components of Targeted Wildlife Disease Surveillance

General and Targeted wildlife disease surveillance programs share the same 4 essential components: detection of disease by finding sick or dead wild animals is replaced by a planned collection of samples from wild animals; identification of the target pathogen or infection, management and analysis of the data collected, and interpretation and communication of the findings are all the same. Similarly, both types of surveillance programs rely on a network of people and organisations that work together and communicate well.

Differences between General and Targeted Wildlife Disease Surveillance Programs

Although General and Targeted surveillance programs are designed with the same 4 essential components, there are important differences between the two.

The most important differences are in how samples are collected. While General wildlife disease surveillance programs rely on samples that are obtained opportunistically, effective Targeted surveillance programs carefully plan which animal species will be sampled, from which locations, at what time of year, how many animals should be sampled, whether or not the surveillance will be done with live or dead animals, and whether it will detect the pathogen or only antibodies against the pathogen. Because of these differences, there are sometimes different people involved in sample collection.

In Targeted surveillance, there may not be any need to transport whole carcasses to a laboratory; a sample of feces, blood, or other tissues may be adequate for the purpose of the program.

Targeted surveillance programs also carefully select what tests will be used to determine whether or not a pathogen or antibodies are present in the specimen collected. The characteristics of the tests used can have important impacts on the design of a Targeted surveillance program, particularly on the kinds of samples needed and the number of samples required.

Diagnostic Tests

General Principles

Earlier in the Workshop, the importance of diagnostic laboratories was discussed. Here, we will consider a few more details about the laboratories and the tests that they perform. These concepts are important considerations for interpretation of both General and Targeted wildlife surveillance data.

Many things can be considered diagnostic tests, ranging from clinical observations of animals to complex laboratory analyses. In the case of Targeted surveillance, we typically consider a diagnostic test to be a laboratory test performed on tissues, feces, serum or other samples that evaluates current or past exposure to a particular pathogen. No diagnostic test works perfectly all of the time. Some tests perform better than others and test results also are greatly affected by the quality of the samples collected and how they are handled between collection and testing. Even when all the specimens have been handled correctly, a diagnostic test can yield false positive or false negative results. The biology of each individual animal is slightly different as is their response to infection, and these small differences can have important effects on the behaviour of a diagnostic test.

Selection of which test(s) to use is based on the purpose of the Targeted wildlife disease surveillance program, what the test is intended to measure (i.e. current versus past exposure to a pathogen), the characteristics of the test (i.e. how well the test works), and whether or not the test has been validated for the host animal species for which it is to be used. There are also some practical issues to consider, such as how much the test costs, what specimens are needed, how the specimens need to be handled, what equipment and supplies are needed, and where the testing can be done (i.e. what laboratories can do the testing).

The OIE publishes information about diagnostic tests and their use for diagnosing particular OIE-listed pathogens in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*[4] and the *Manual of Diagnostic Tests and Vaccines for Aquatic Animals*[5]. The focus of these Manuals is on domestic and farmed animals, and consequently there is limited information about diagnostic tests for use in wild animal populations. Information specifically about testing wildlife is being added as chapters are revised. The OIE also designates certain laboratories around the world as OIE Reference Laboratories[6] to recognize their expertise in diagnosis of selected pathogens. These laboratories can provide advice and expertise that are invaluable in the design of Targeted surveillance programs and interpretation of the data. OIE Collaborating Centres[7] can also provide advice and expertise.

Diagnostic Tests for Use on Wild Animal Species

A diagnostic test is only useful if it will correctly identify the pathogens and diseases of interest. To know if a diagnostic test is reliable in this way, it must be thoroughly assessed by a process referred to as "validation." If a diagnostic test is validated, most often the validation is for use on samples taken from only one or a few animal species and for specified samples from that species: e.g. serum from horses. Validation of a diagnostic test requires time, money and expertise, and seldom are diagnostic tests validated for use on more than a small number of different species. Since there are many different species of wild animals, a major concern in wildlife disease surveillance is the selection of diagnostic tests that are likely to provide correct information when used on a range of different species, or even for a single species, for which the test has not been properly validated.

This is a very serious concern. Some diagnostic tests, such as standard ELISA tests to detect antibodies to particular pathogens, simply do not work when applied to species other than the one for which the test was developed. The results from such tests applied to other species are completely invalid and without value. They cannot be interpreted or used in any way.

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4 Available at: http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/
5 Available at: http://www.oie.int/international-standard-setting/aquatic-manual/access-online/
6 Reference Laboratories: http://www.oie.int/our-scientific-expertise/reference-laboratories/introduction/
7 Collaborating Centres: http://www.oie.int/our-scientific-expertise/collaborating-centres/introduction/

More detailed information about specific issues related to the validation of diagnostic assays is provided in a series of OIE Validation Guidelines[8] that are tailored for several fundamentally different types of assay (e.g. detection of nucleic acids, antibodies, or antigens). For specific information for wildlife species, refer to OIE Validation Guideline 3.6.7[9] Principles and methods for the validation of diagnostic tests for infectious diseases applicable to wildlife.

Selecting a Diagnostic Test

In wildlife disease surveillance, it is best to use diagnostic tests which are unlikely to be affected significantly by the host animal species from which the samples to be tested have been taken. To a large extent, this can be predicted based on the technical details of a diagnostic test. For example, a test that detects a pathogen directly, such as culture of bacteria from a tissue, is less likely to be affected by the host species than is a test that is based on the response of the host animal to infection, such as a test for antibodies or a test for another immune response (e.g. TB skin test).

The following table provides some very general guidelines regarding choice of diagnostic tests for wildlife disease surveillance.

Choosing Diagnostic Tests for Wild Animal Pathogens

	LESS likely to be affected by host animal species	**Intermediate**	**MORE likely to be affected by host animal species**
Tests for pathogens	• Direct identification: e.g. parasites • Culture of bacteria, fungi, protozoa • PCR[10] • Immunohistochemistry • Chemical analysis (toxicology)	• Culture for viruses[11]	
Tests for antibodies or immune response	• Virus neutralization • Blocking (competitive) ELISA		• Most standard serology tests: o ELISA • Antigen skin tests (TB)
Other	Newcastle Disease	Brain Cholinesterase[12]	

The OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* contains much information on choice of diagnostic tests for particular pathogens. Some chapters also consider the application of these tests to a range of host animal species.

8 Available at: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/GUIDELINE_3.6.0_INTRODUCTION.pdf - Chapter 1.1.5. — Principles and methods of validation of diagnostic assays for infectious diseases

9 Available at: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/GUIDELINE_3.6.7_WILDLIFE.pdf

10 There can be problems using PCR on some samples from some species, for example ruminant feces.

11 Some viruses can be cultured in standard cell cultures or chicken embryos, but others grow only in cells derived from their natural host species.

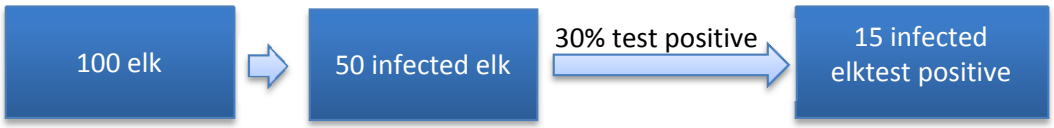
12 This is used as a screening test for poisoning by organophosphate and carbamate insecticides. However, animal species vary greatly in normal background levels.

Attributes and Characteristics

Here you will explore the issue of false test results and how they can affect how you interpret and act on the data generated from Targeted surveillance programs. Start with some examples of tests that have been validated, so you know in advance how likely the results are to be correct or incorrect.

Example 1: Baermann technique to detect larvae of the meningeal worm *Parelaphostrongylus tenuis* in fecal samples from elk (*Cervus elaphus* – a North American ungulate)

Consider a population of 100 elk – 50 that are infected with meningeal worm and 50 that are not. The Baermann technique has been validated for elk and approximately 30% of animals that are infected test positive. That means that 30% of the infected elk will test positive; in other words, only 15 out of the 50 infected elk will test positive for *P. tenuis* using this diagnostic test. This isn't a great diagnostic test!



Example 2: Tuberculin skin test to detect exposure to bovine tuberculosis (*M. bovis*) in cattle

Consider a herd of 1000 cattle in which 500 have tuberculosis and 500 are not infected. The tuberculin skin test has been validated for cattle several times and in several countries. On average, 80% of infected animals test positive. So, of the 500 infected cattle, we can expect 400 of them to test positive. That means that 100 infected animals will test negative (false negatives).

For this particular test, you also know something about how it works in uninfected animals. From the test validation process, it has been determined that, on average, 99.5% of uninfected cattle will test negative. In this example, 498 out of the 500 uninfected animals will test negative; 2 uninfected animals will test positive for tuberculosis (false positives).

		True pathogen status		Total
		+	–	
Diagnostic test result	+	400	2	402
	–	100	498	598
Total		500	500	1000

These two examples introduce the critical characteristics of any diagnostic test – the proportion of truly infected animals that test positive and the proportion of truly pathogen-free animals that test negative. These two measures are called **sensitivity** and **specificity** of the test:

- Sensitivity = the proportion of truly infected animals that test positive
- Specificity = the proportion of truly infection-free animals that test negative

Another way to think about these test characteristics is to ask, "How likely is the test to produce false negative or false positive results?" If the sensitivity of a test is 80%, then 20% of test results will be false negative (100% - 80% = 20%); if the specificity of a test is 90%, then 10% of test results will be false positives (100% - 90% = 10%).

The key concept to grasp here is that a positive diagnostic test result does not always mean that the targeted pathogen is present or that the animal was exposed. Additionally, a negative test result does not always mean that the pathogen is absent. No matter how common the pathogen or infection is in the population of interest, the sensitivity and specificity of a diagnostic test do not change. But in the case of interpreting diagnostic tests applied to wildlife, keep in mind that a test validated for a domestic animal species may behave very differently in a different species. You cannot assume that the sensitivity and specificity of a test will be the same when applied to samples from a different species. Almost certainly they will be different.

Note: Epidemiologists and laboratory-based researchers both use the terms sensitivity and specificity but each means something different. The definitions above refer to epidemiological sensitivity and specificity. To the laboratory-based researcher, (analytic) sensitivity refers to the lowest concentration the test can detect in a specimen while (analytic) specificity refers the ability of the test to react to only one compound.

Interpretation of Test Results

The Influence of True Prevalence

Although sensitivity and specificity of diagnostic tests are independent of the pathogen prevalence, **interpretation of the test result depends on the proportion of animals that are infected in the population of interest**. To explore this further, you are going to work through the example below.

Example 3: Consider a validated diagnostic test with sensitivity = 99% and specificity = 90%, and apply this test to a population of 1000 animals in which 10% of the animals are truly infected.

		True pathogen status		Total	Predictive values
		+	–		
Diagnostic test result	+	99	90	402	PPV=99/189=52%
	–	1	810	598	NPV=810/811=99.9%
Total		100	1000	1000	

To complete the table above, start with a population of 1000 animals. Assume you know that 10% of the animals in this population are infected. This measure (10%) is often referred to as the **true prevalence**. (You rarely know this for wildlife, but assume you do know it for this example) If the true prevalence is 10%, then 100 animals in this population are infected and 900 are not.

You also know that the test sensitivity is 99%. This means that 99 out of the 100 infected animals will test positive, whereas 1 infected animal will test negative. With a test specificity of 90%, 810 of the uninfected animals will test negative and 90 will test positive.

The test characteristics (sensitivity and specificity) tell you about how the test works in the population. But remember, when you are doing real surveillance, you don't know ahead of time which animals are truly infected and which ones are not. The test results will only indicate which animals *tested* positive and which ones *tested* negative. In this example, 189 animals tested positive and 811 tested negative. These *test results* suggest that the prevalence of infection is 19% (189/1000). To distinguish the prevalence estimated from the test results from the true prevalence (10%), the prevalence estimated from the test results is called the **apparent prevalence**. In this example, it is almost twice as high as the true prevalence.

Using Test Performance Characteristics to Interpret Surveillance Results

In the example above, 99 infected animals tested positive and 90 uninfected animals tested positive; in total, 189 animals tested positive. Because you know the true prevalence of infection in the population and the sensitivity and specificity of the diagnostic test, you can calculate how likely it is that a test-positive animal is truly infected. In this case, 99 out of 189 (52%) test-positive animals are truly infected. This is called the **positive predictive value** of the test. In this case, there is a 52% chance that an animal that tests positive truly is infected. Another way to look at this is that 90 out of 189 (48%) test-positive animals are actually not infected and that 48% of the test results are false positives.

By doing a similar calculation for the test-negative animals, you can determine that, out of 811 test-negative animals, 810 (99.9%) are truly not infected; just one animal falsely tested positive. In this example the **negative predictive value** of the test is 99.9%. In other words, if an animal tested negative, there is a 99.9% chance that the animal is not infected.

- The positive predictive value indicates the probability that a test-positive animal is truly positive
- The negative predictive value indicates the probability that a test negative animal is truly negative.
- These two calculated values indicate how confident you can be in the positive and in the negative test results
- The predictive values of a diagnostic test, and the confidence you can have in test results, depend on the sensitivity and specificity of the diagnostic test and they also depend on the true pathogen prevalence in the population.

To show how the predictive values of a test can be changed by the actual, true prevalence of infection in the population, fill out the two tables below. In both tables, apply the imaginary diagnostic test to a population of 1000 animals and use a test sensitivity of 99% and a specificity of 90%.

Exercises:

Targeted Wildlife Disease Surveillance in the "*Dominion of Atlantis*"

(Note: this country does not exist)

Diagnostic Tests Exercises (part 1): Prevalence

ACTIVITY 3

1. Complete the tables below with the information provided.
2. Calculate the predictive values for the different prevalence levels.

A. Low pathogen prevalence – 2%

		True disease/pathogen status		Total	Predictive values
		+	–		
Diagnostic test result	+				
	–				
Total				1000	

True prevalence = 2%
Sensitivity = 99%
Specificity = 90%

B. High pathogen prevalence – 40%

		True disease/pathogen status		Total	Predictive values
		+	–		
Diagnostic test result	+				
	–				
Total				1000	

True prevalence = 40%
Sensitivity = 99%
Specificity = 90%

C. Describe the effect of true prevalence on the interpretation of the test results.

What happened to the predictive values of the test when true prevalence was changed?
What happened to the apparent prevalence?

[write here]

Diagnostic Tests Exercises (part 2): Sensitivity and Specificity

ACTIVITY 4

1. Complete the tables below with the information provided.
2. Calculate the predictive values for the different tests characteristics.
3. Describe how the interpretation of a test changes as sensitivity and specificity increase and decrease

Now that you have seen how the prevalence can affect interpretation of test results, examine what happens when test sensitivity and specificity increase and decrease. Use the first example as a starting point – a population with a true prevalence of 10% and a diagnostic test with sensitivity = 99% and specificity = 90%. Now, what would happen if you used a test with a different sensitivity and specificity to detect infection in the same population (where 10% of the animals are infected)? Work through the next 2 examples.

A. Low Sensitivity

		True disease/pathogen status		Total	Predictive values
		+	–		
Diagnostic test result	+				
	–				
Total				1000	

True prevalence = 10%
Sensitivity = 80%
Specificity = 90%

B. High Specificity

		True disease/pathogen status		Total	Predictive values
		+	–		
Diagnostic test result	+				
	–				
Total				1000	

True prevalence = 10%
Sensitivity = 80%
Specificity = 99%

C. What happened to the predictive values of the test when sensitivity or specificity was changed?

[write here]

D. There are times when tests with higher sensitivity are preferred and other times when higher specificity is needed

Propose some examples of surveillance objectives for which tests with higher sensitivity, or with higher specificity, would be advantageous.

[write here]

These examples are all theoretical. Remember that you rarely know the test characteristics (sensitivity and specificity) for tests applied to wildlife and usually don't know even the approximate true prevalence in the population of interest, particularly in the case of an emerging or new disease.

How can you make practical use of this information?

- When you know nothing about test performance or prevalence, you can do a preliminary survey with one or more diagnostic tests and use those results as approximate information about test performance and prevalence. This can provide a baseline for future surveillance, a starting point for further work.
- If a test is applied to a wild population and unexpected results are obtained, further investigation is warranted before management actions are taken. For example, additional testing procedures may be available to confirm that the test-positive animals truly are positive.

How can the predictive values of a test be increased?

There are several ways to increase the predictive value of a test:

- Apply the test to a subgroup of animals with a higher pathogen prevalence (e.g. only test clinically sick animals, or animals in which the pathogen is more frequently observed – e.g. older or younger animals; males versus females)
    - Effectively you are increasing the pathogen prevalence in the surveillance sample.
- Use more than one diagnostic test. If this option is chosen, there are 2 ways of interpreting the test results:
    - Series – animals are tested with one test first; those that test positive are then tested again using a second test (the tests are applied one after the other)
        - Positive animals test positive to both (all) tests applied; this effectively increases the test specificity and decreases the test sensitivity
        - If this option is chosen, usually all animals are tested with the cheaper/easier test first and then, if they test positive, the more expensive or complex test is run
    - Parallel – all animals are tested with both tests and the tests are applied at the same time

- Positive animals test positive to one or more tests; this effectively increases the test sensitivity and decreases the test specificity)

It is important to emphasize that the sensitivity and specificity of diagnostic tests reflect how the test behaves in an individual animal. How a particular diagnostic test works in the population at large (known as herd sensitivity and herd specificity) depends on what proportion of animals in the herd is infected (i.e. prevalence), how many animals are tested (the sample size) and the cut-off value for the number of animals that may test positive before the herd is considered to be positive. This topic of herd-level specificity and sensitivity is beyond the scope of this workbook.

Calculating sample size

How many animals need to be included in a surveillance program?

You will now turn your attention to determining how many samples are needed for Targeted wildlife disease surveillance, and consider how diagnostic test performance can affect requirements for sample size.

Except in very rare situations, you will not be able to test every animal in the population of interest. Consequently, you usually select a subset of the whole population. Typically, the number of individual animals that are sampled in a targeted surveillance program will be decided on the basis of what you want to measure, how confident you want to be in the results, and what can practically be achieved with the available money, time and resources.

The choice of what population of animals to sample will depend on the purpose of the surveillance program. If the intent is to estimate true prevalence, then the sample should be as representative of the population of interest as possible. If the purpose is just to detect infection or demonstrate freedom, it may be more appropriate to focus surveillance efforts on animals at higher risk of infection, recognizing that these data will not represent the population at large.

The number of animals that need to be tested as part of a targeted surveillance program (sample size) depends on four main factors:

1. The purpose of the surveillance program and whether you are seeking information about prevalence or an indication that the targeted pathogen or infection is present or not in the population of interest.

2. How confident you want to be in the estimates generated by the surveillance data

    - Most surveillance programs, regardless of their purpose, aim to achieve 95% or 99% confidence

        - If the purpose of the surveillance program is to demonstrate freedom from infection and none of the sampled animals test positive, then the confidence level is a measure of how certain you can be that the pathogen is not present in the population

        - If the purpose of the surveillance program is to estimate pathogen prevalence in the population, then the level of confidence is a measure of how certain you can be that the true prevalence is within the range of the apparent prevalence that you have calculated.

3. The size of the population of interest

    - Most wild populations are fairly large and so the size of the population of interest does not have a large impact on how many animals need to be included in the surveillance program. However, in the case of species at risk or other small populations, occasionally the normally required sample size represents a large proportion of all the animals in the population or even a number greater than the total population. In these situations, the sample size can and should be recalculated in consideration of the small total population (see below).

4. The characteristics of the diagnostic tests used

    - As discussed earlier, diagnostic tests are rarely perfect and may over- or under-estimate the number of animals infected or not infected. This is particularly the case for wild animal populations for which there are few validated diagnostic tests. The sensitivity and specificity of diagnostic tests can increase OR decrease the sample size needed.

There are some basic equations that can be used to calculate a sample size for targeted surveillance programs. The equations are a bit different, depending on what the purpose of the program is.

Sampling to detect infection or to demonstrate that the pathogen is not present at or below a specified value:

The simplest equation has been described as the "rule of three" (Hanley and Lippmann-Hand, 1983). According to this "rule", if no animals test positive for the target pathogen, you can be 95% confident that, at most, 3 of the sampled animals were truly positive. If 47 bison were tested for tuberculosis and none tested positive, then you can be 95% confident that at most 3/47 bison are truly positive (i.e. the apparent prevalence of infection is 6% or less). This rule was developed using more exact methods, such as the equation below.

$$n=[1-(1-\alpha)^{1/D}][N-(D-1)/2]$$

α= desired level of confidence
N=Number of animals in the population of interest
D=Number of infected animals in the population of interest

Minimum sample sizes needed to be 95% confident that the pathogen is present at/or below specified prevalence, if no infected animals are observed

Population size	Estimated pathogen prevalence			
	1%	5%	10%	50%
100	95	45	25	5
1000	258	58	29*	5
10000	294	59	29*	5

* If you sample 29 animals and all of them test negative then you can be 95% confident that, if the pathogen is present, the prevalence is less than 10%

Two take home messages:

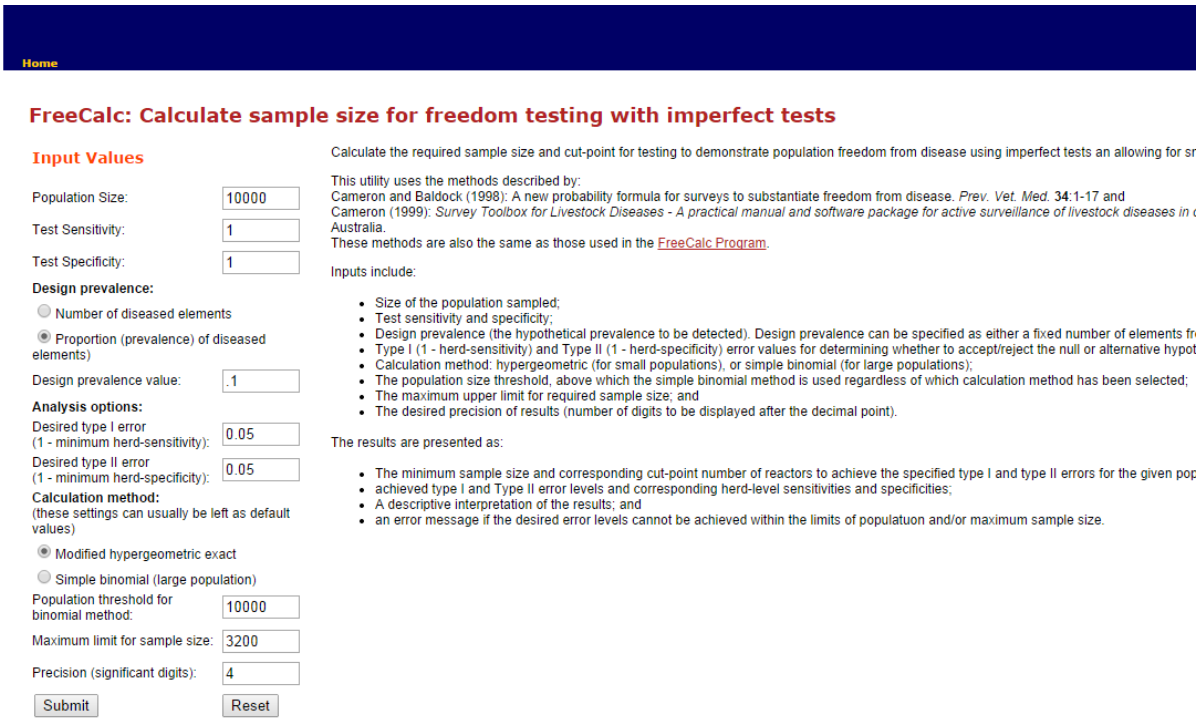
1) **The rarer you expect the infection to be, the greater the number of animals that will need to be tested** and
2) **The bigger the population of interest, the greater the number (but the smaller the proportion) of animals that will need to be tested**

What has this equation ignored? Consider the 4 main factors that affect sample size calculations (provided above). The characteristics of the diagnostic tests used have not been considered.

Computer Tools to Calculate Required Sample Size

There are ways to include the sensitivity and specificity of the diagnostic test in the sample size calculations, but it makes the calculation quite a bit more complicated. Luckily, there are several website applications and down-loadable software that will do the calculations for you; all you need to do is plug in the numbers. Examples from Epi Tools (AusVet Animal Health Services - http://www.ausvet.com.au/content.php?page=epitools) are shown below. The same numbers from the table above were entered into FreeCalc (http://epitools.ausvet.com.au/content.php?page=FreeCalc2). Below are some screen shots to demonstrate how this tool works.

If you first assume that the tests are perfect, you get the same answer as you did in Table above. To do this yourself, enter the information in the first window, press "submit" and the FreeCalc sample size estimation results are generated: the required sample size is 29 animals, the same as in Table above. The desired type I and type II error level was set at 5% (shown in the screen shots below).



FreeCalc sample size estimation

Analysed: Fri Sep 12 2014 @ 03:27

Inputs

Test sensitivity	1
Test specificity	1
Population size	10000
Design prevalence	0.1
Diseased elements	1000
Analysis method	Modified hypergeometric exact
Target Type I error	0.05
Target Type II error	0.05
Population threshold for infinite probability formula	10000
Maximum sample size	3200

Results

Required sample size:	29
Cut-point number of reactors:	0
Type I error:	0.0469
Type II error:	0
Herd-level sensitivity:	0.9531
Herd-level specificity:	1
Interpretation:	If a random sample of **29** units is taken from a population of **10000** and **0** or fewer reactors are found, the probability that the population is diseased at a prevalence of **0.1** is **0.0469.**
Method:	Modified hypergeometric exact

Download excel file of results

Detailed results

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AusVet Animal Health Services

This site was created by AusVet Animal Health Services with funding from the Australian Biosecurity Cooperative Research Centre. It provides a range of epidemiological tools for the use of epidemiologists, particularly in animal health. Please send any comments, questions or suggestions to Evan Sergeant

Now, adjust the test characteristics to a sensitivity value of 99% and specificity value of 90% and see what happens.

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FreeCalc: Calculate sample size for freedom testing with imperfect tests

Input Values

Population Size: 10000

Test Sensitivity: .99

Test Specificity: .9

Design prevalence:

- Number of diseased elements
- Proportion (prevalence) of diseased elements)

Design prevalence value: .1

Analysis options:

Desired type I error (1 - minimum herd-sensitivity): 0.05

Desired type II error (1 - minimum herd-specificity): 0.05

Calculation method:
(these settings can usually be left as default values)

- Modified hypergeometric exact
- Simple binomial (large population)

Population threshold for binomial method: 10000

Maximum limit for sample size: 3200

Precision (significant digits): 4

Submit Reset

Calculate the required sample size and cut-point for testing to demonstrate population freedom from disease using imperfect tests an allowi

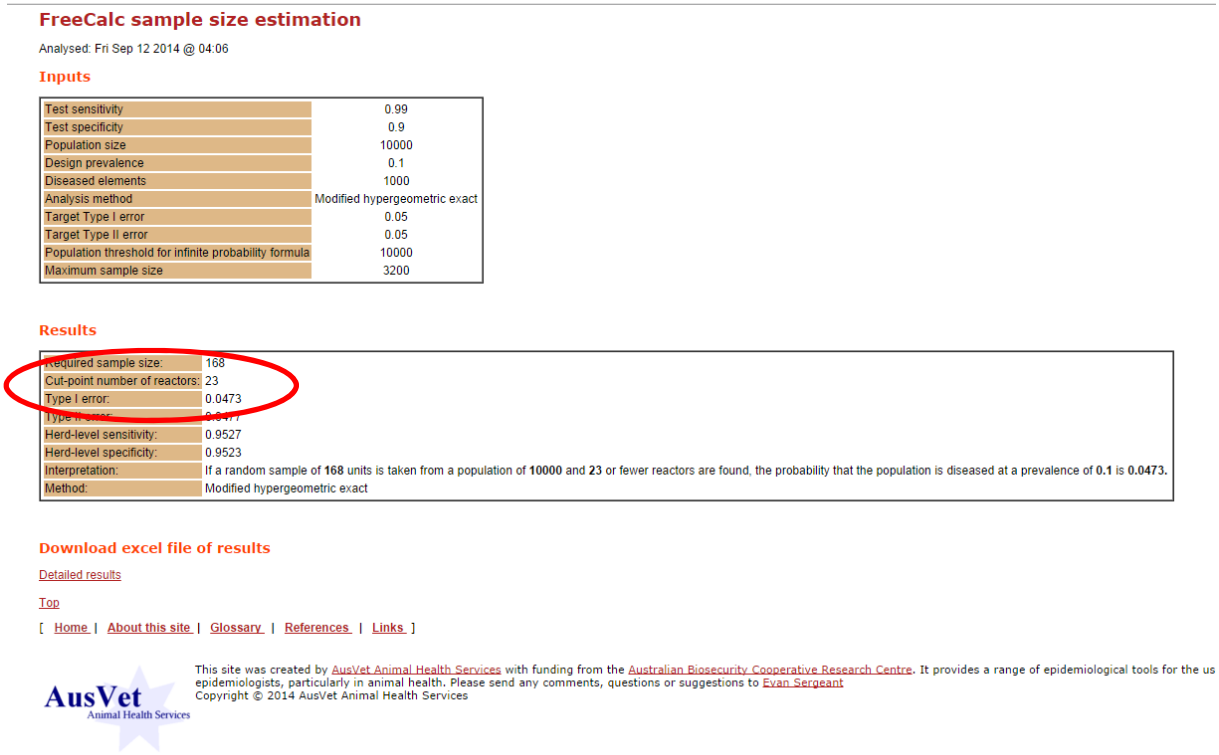
This utility uses the methods described by:
Cameron and Baldock (1998): A new probability formula for surveys to substantiate freedom from disease. *Prev. Vet. Med.* **34**:1-17 and
Cameron (1999): *Survey Toolbox for Livestock Diseases - A practical manual and software package for active surveillance of livestock dise*
Australia.
These methods are also the same as those used in the FreeCalc Program.

Inputs include:

- Size of the population sampled;
- Test sensitivity and specificity;
- Design prevalence (the hypothetical prevalence to be detected). Design prevalence can be specified as either a fixed number of ele
- Type I (1 - herd-sensitivity) and Type II (1 - herd-specificity) error values for determining whether to accept/reject the null or alternati
- Calculation method: hypergeometric (for small populations), or simple binomial (for large populations);
- The population size threshold, above which the simple binomial method is used regardless of which calculation method has been se
- The maximum upper limit for required sample size; and
- The desired precision of results (number of digits to be displayed after the decimal point).

The results are presented as:

- The minimum sample size and corresponding cut-point number of reactors to achieve the specified type I and type II errors for the g
- achieved type I and Type II error levels and corresponding herd-level sensitivities and specificities;
- A descriptive interpretation of the results; and
- an error message if the desired error levels cannot be achieved within the limits of populatuon and/or maximum sample size.



FreeCalc sample size estimation

Analysed: Fri Sep 12 2014 @ 04:06

Inputs

Test sensitivity	0.99
Test specificity	0.9
Population size	10000
Design prevalence	0.1
Diseased elements	1000
Analysis method	Modified hypergeometric exact
Target Type I error	0.05
Target Type II error	0.05
Population threshold for infinite probability formula	10000
Maximum sample size	3200

Results

Required sample size:	168
Cut-point number of reactors:	23
Type I error:	0.0473
Type II error:	0.0477
Herd-level sensitivity:	0.9527
Herd-level specificity:	0.9523
Interpretation:	If a random sample of **168** units is taken from a population of **10000** and **23** or fewer reactors are found, the probability that the population is diseased at a prevalence of **0.1** is **0.0473**.
Method:	Modified hypergeometric exact

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Detailed results

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AusVet Animal Health Services

This site was created by AusVet Animal Health Services with funding from the Australian Biosecurity Cooperative Research Centre. It provides a range of epidemiological tools for the us epidemiologists, particularly in animal health. Please send any comments, questions or suggestions to Evan Sergeant

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From the screen shots above, the minimum required sample size is 168 and out of those 168 animals, up to 23 animals could test positive and you would still be 95% confident that the prevalence of infection in the population of interest was less than 10%.

Sample size calculation to estimate prevalence

Now that you have detected the pathogen, how many animals do you need to test to determine how prevalent the pathogen is in the animal population of interest? To calculate this, you use different equations, but basically the same information is needed: an estimate of the true prevalence and the level of confidence you want or require. You will also need to decide how closely you want the sample prevalence estimate (apparent prevalence) to be to the true prevalence. When good information about pathogen prevalence in the population is not available, you may wish to calculate a sample size based on several different but possible prevalence estimates and balance the resulting range of sample sizes with the resources available.

Equations:

a) For 95% confidence: $n=4P(1-P)/L^2$

b) For 99% confidence: $n=6.6P(1-P)/L^2$

P = estimate of the true pathogen prevalence in the population of interest
L = allowable error – this is a measure of how close you want the apparent prevalence to be to the true prevalence

Calculated sample sizes you will need for the given allowable error and estimated pathogen prevalence (with 95% confidence)

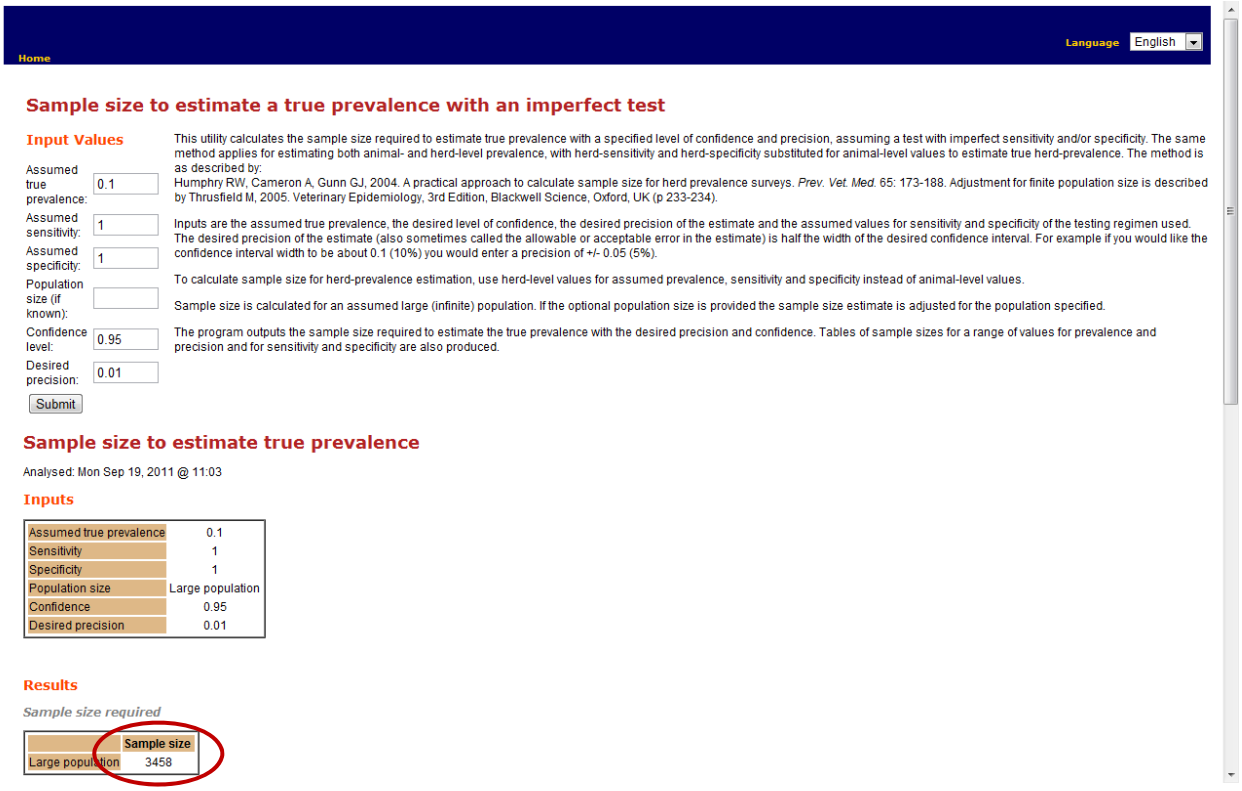
Allowable error (closeness to true prevalence)	**Estimated pathogen prevalence**		
	2%	**10%**	**45%**
0.1%	78400	360000	990000
1%	784	3600	9900
10%	8	36	99

Table (above) lists a few sample sizes needed to estimate prevalence. Try using equation a) above, for 95% confidence, to calculate the sample size needed to determine prevalence if the estimated true prevalence (P) is 10%, the allowable error (L) is 1%.

In general, **the more extreme the prevalence estimate is** (i.e. if nearly all of the animals in the population are believed to be infected or virtually no animals are thought to be infected), **the smaller the number of samples needed to attain the desired level of confidence**.

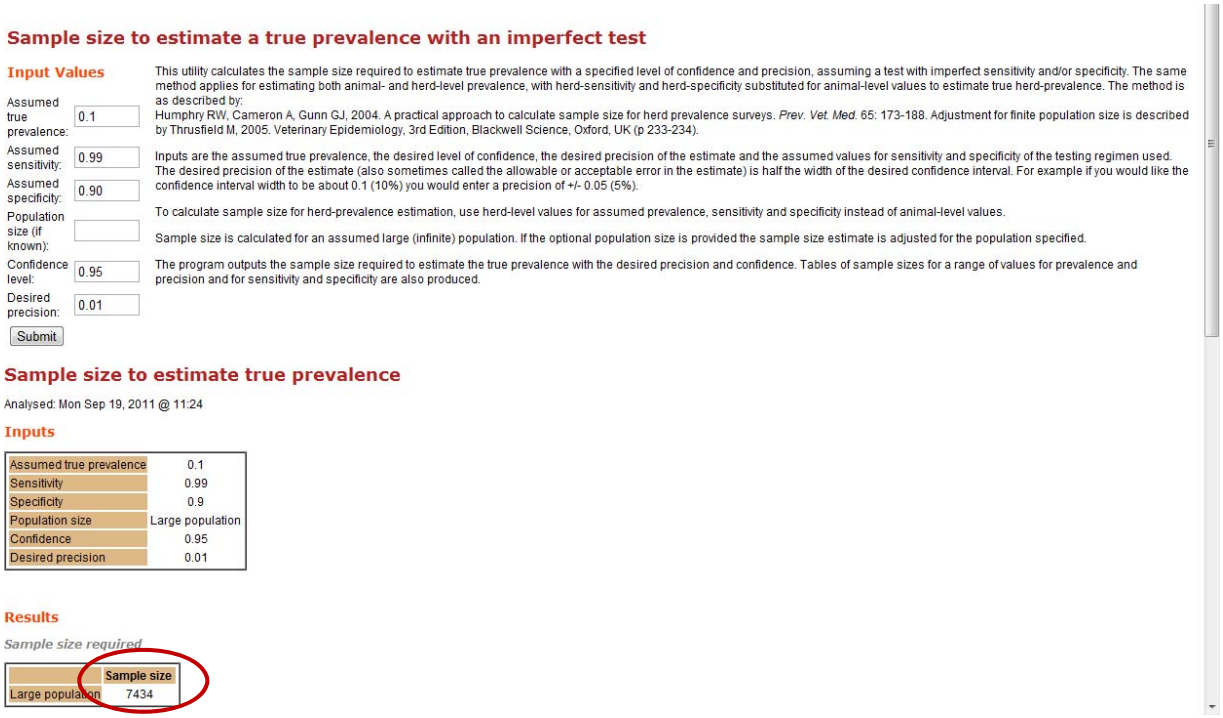
Again, there are programs available online that will calculate sample size needed to estimate prevalence and account for imperfect tests. As an example, the same numbers above have been entered into the prevalence sample size calculation page developed by Ausvet (http://epitools.ausvet.com.au/content.php?page=PrevalenceSS). Below are some screen shots to demonstrate how this tool works.

Sample size calculation using a <u>perfect diagnostic test</u> (sensitivity and specificity are 100%)



From the screen shot above, the minimum required sample size is 3458. This is similar to what you calculated using the equation provided; estimating the true prevalence to be 10% and an allowable error of 1%, the equation (with 95% confidence) determined that a sample size of 3600 animals was needed (see Table above).

Sample size calculation using an <u>imperfect diagnostic test</u> (sensitivity=99% and specificity=90%)



Sample size to estimate a true prevalence with an imperfect test

Input Values

Assumed true prevalence: 0.1
Assumed sensitivity: 0.99
Assumed specificity: 0.90
Population size (if known):
Confidence level: 0.95
Desired precision: 0.01
Submit

This utility calculates the sample size required to estimate true prevalence with a specified level of confidence and precision, assuming a test with imperfect sensitivity and/or specificity. The same method applies for estimating both animal- and herd-level prevalence, with herd-sensitivity and herd-specificity substituted for animal-level values to estimate true herd-prevalence. The method is as described by:
Humphry RW, Cameron A, Gunn GJ, 2004. A practical approach to calculate sample size for herd prevalence surveys. *Prev. Vet. Med.* 65: 173-188. Adjustment for finite population size is described by Thrusfield M, 2005. Veterinary Epidemiology, 3rd Edition, Blackwell Science, Oxford, UK (p 233-234).

Inputs are the assumed true prevalence, the desired level of confidence, the desired precision of the estimate and the assumed values for sensitivity and specificity of the testing regimen used. The desired precision of the estimate (also sometimes called the allowable or acceptable error in the estimate) is half the width of the desired confidence interval. For example if you would like the confidence interval width to be about 0.1 (10%) you would enter a precision of +/- 0.05 (5%).

To calculate sample size for herd-prevalence estimation, use herd-level values for assumed prevalence, sensitivity and specificity instead of animal-level values.

Sample size is calculated for an assumed large (infinite) population. If the optional population size is provided the sample size estimate is adjusted for the population specified.

The program outputs the sample size required to estimate the true prevalence with the desired precision and confidence. Tables of sample sizes for a range of values for prevalence and precision and for sensitivity and specificity are also produced.

Sample size to estimate true prevalence

Analysed: Mon Sep 19, 2011 @ 11:24

Inputs

Assumed true prevalence	0.1
Sensitivity	0.99
Specificity	0.9
Population size	Large population
Confidence	0.95
Desired precision	0.01

Results

Sample size required

	Sample size
Large population	7434

From the screen shot above, the minimum required sample size is 7434. As the diagnostic test used was not perfect, more than twice as many animals have to be tested to be able to estimate the prevalence with the desired level of confidence (95%) and precision (1%).

Sample Size for small populations of animals

If you are doing surveillance in a small population (e.g. rare species), then you may need to correct the sample size estimate for small populations. From the examples above, you first determined (assuming a perfect test) that you needed to test 3458 animals. But what if there were only about 5000 animals in total in the population of interest? There are enough animals but it might be very hard to obtain samples from that many. **If the calculated required sample size represents 10% or more of the total population, you can adjust the sample size using the following equation**:

$$1/n^* = 1/n + 1/N$$

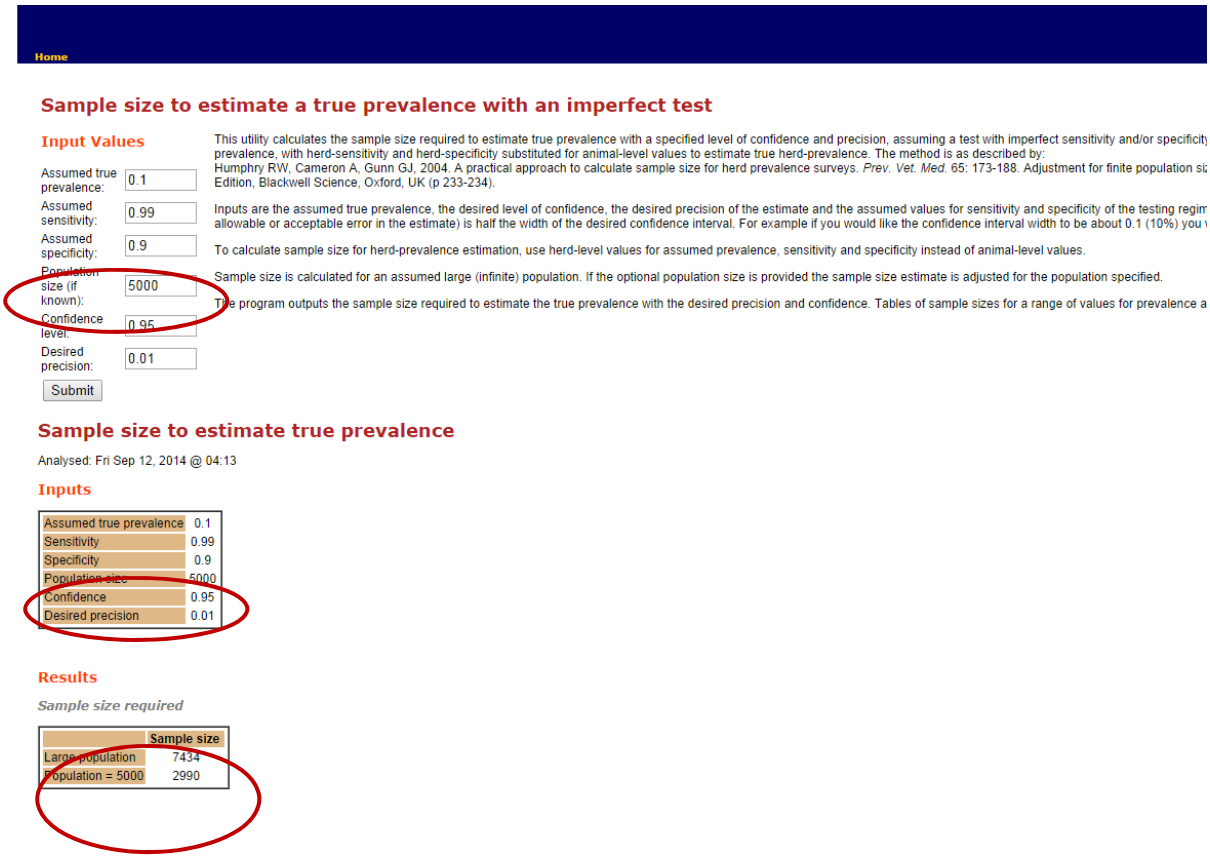
n*= the corrected sample size
n=the estimated sample size before correction
N=the population size

So, for the examples above:

1/n*=1/3458 + 1/5000 and so n*=2044 animals instead of 3458.

Alternatively, you can add information to the online program and it will account for the small population for you, as well as test performance parameters.

Sample size calculation using an imperfect diagnostic test (sensitivity=99% and specificity=90%) <u>for a small population</u>



From the screen shot above, when accounting for the small population size, the sample size changed from 7434 to 2990.

All sample size calculations provide estimates of minimum sample size. It is a good idea to increase the number of samples above the minimum value, even when the characteristics of the diagnostic tests have been accounted for, in case some samples are mishandled, there is a problem at the laboratory or for other unforeseen issues that might arise.

Designing a Targeted Wildlife Disease Surveillance Program

In which wild species will you look for the pathogen or disease?

Pre-existing knowledge about the target pathogen or infection is critical for design of effective Targeted wildlife surveillance programs. This preliminary knowledge comes, most often, from General wildlife disease surveillance. The wild species that are competent hosts for the pathogen of interest in the country must be known, along with the approximate size of their population(s) and geographic distribution(s). Identification of the wild animal species to be sampled will inform how animals will be caught or trapped, from where and by whom.

The choice of which competent host species are most appropriate to include in a Targeted surveillance program depends on how the targeted pathogen behaves in the various host species and on the purpose of the surveillance program. For instance, if the purpose is to determine *if* a particular disease or pathogen is present in wild animals in a country, you might select a species that is known to develop actual disease (i.e. show clinical symptoms characteristic of the disease caused by the target pathogen) rather than a species that does not develop disease following infection. However, if the purpose of the targeted surveillance program is to estimate prevalence of an infection for which the wild animal may be a carrier of the pathogen, but may not develop clinical disease, then you may choose to sample both apparently healthy and sick animals.

In some situations, certain wild animal species are selected for inclusion in Targeted surveillance programs because they are easier to find and to sample than others.

If the purpose of the Targeted surveillance program is to detect the presence of a targeted pathogen, or demonstrate freedom, it might be appropriate to target certain subsets of the host populations. For example, for some pathogens, animals of a certain age or sex may be more susceptible to infection than others and therefore may be targeted to maximise the chances of detecting the pathogen. In other situations, it might be appropriate to sample domestic animals to determine whether or not a pathogen of wildlife is present or absent from an area. For example, farm dogs have been used to determine whether or not plague (*Yersinia pestis*) was present in local rodents[13]. The dogs, which hunted the rodents, were easier to catch and handle than the wild rodents themselves.

Where should you look and when?

When sampling wild animals, you need to consider where the host animals of interest are located and whether or not their location changes over time. This is particularly important when migratory species are the target of the surveillance program. For example, avian influenza surveillance in wild waterfowl in the northern hemisphere is often conducted in the late summer and early fall when the birds congregate together at staging grounds before their southward migration. At this time and place, there are many birds together in a smaller area so trapping is easier and many of the birds are young of the year and they tend to be more susceptible to infection with avian influenza than older birds.

If a Targeted surveillance program is set up to look for a pathogen that is known to affect animals in a neighbouring jurisdiction, surveillance activities may be focused along the border region.

[13] Leighton FA, Artsob HA, Chu MC, & Olson JG. 2001. A serological survey of rural dogs and cats on the southwestern Canadian prairie for zoonotic pathogens. *Canadian Journal of Public Health*, **92**(1):67-71.

The decision about where to look for a pathogen also depends on the purpose of the surveillance program. If the intent is to detect a zoonotic disease agent of public health importance, surveillance of sentinel wildlife species might be done primarily in urban areas where the greatest numbers of people live. Similarly, if the purpose is to detect a pathogen of importance to domestic animals, then wildlife may be targeted in and around important agricultural areas.

What specific animals should be included?

The choice regarding which animals to include in a targeted surveillance program depends on the objectives of that program. For example, if the purpose of the targeted surveillance program is to detect the potential arrival of a new pathogen, you may want to target animals that show clinical signs of the pathogen or infection. In this case, you simply want to know if the pathogen is present in the population or not. The subgroup of animals in the population that is showing typical clinical signs is more likely to be infected with the pathogen of interest than is the much larger subgroup of animals that are apparently healthy. If you relate this back to the earlier discussion of diagnostic tests, you are effectively increasing the true prevalence in the population of interest, and therefore the laboratory test being used in the surveillance program is better able to predict infection (better positive predictive values).

If the purpose of the Targeted wildlife disease surveillance program is to determine pathogen prevalence for the population, then the sampled animals should be as similar as possible to the whole population i.e. if the population is 60% female, then ideally 60% of the sampled animals would also be female. It is not easy to get a representative sample of a wild animal population.

Ideally, to ensure that the sampled animals are representative of the whole population, a random sampling approach should be used. In a random sample, every animal or group of animals has an equal chance of being selected. Unfortunately, truly random sampling is rarely possible in the case of wild animal surveillance. Also, typically there is limited information about the population of interest, including the accurate estimates of the number of animals, sex, age or where they are located.

Random Sampling

There are several ways in which a random sample can be obtained. Three of the most common are:

- Simple random sampling - e.g. flipping a coin
- Stratified sampling– particular habitats or groups of animals are identified (e.g. hedgerows, woodlands or known herds) and then a simple random sample is taken from each group
- Cluster sampling – particular groups are randomly selected and then all animals within the selected groups are sampled.

For many reasons, however, in surveillance of wild animals, non-random (non-probability) sampling often is used. Non-probability sampling is not necessarily bad; it is simply important to recognise that such samples may not be representative of the overall population of interest and that this will affect how the data can be interpreted.

How will the required samples be acquired?

In Targeted surveillance programs, usually a specific animal species (or group of species) is targeted because those species are known or believed to be able to carry the pathogen of concern. In some situations, samples (e.g. blood/serum, feces) are collected from live or live trapped animals. In other cases, you rely on dead animals for targeted surveillance, such specimens may be available from hunters.

The particular pathogen of interest will influence what samples are appropriate for testing (e.g. brain for chronic wasting disease, feces for avian influenza), or whether or not samples can be collected from live animals. The particular pathogen will also determine how the sample should be handled and who and what facilities exist that can handle the sample and carry out the testing. The quality of the samples collected and how they are handled will influence the reliability of any laboratory results obtained.

All of the considerations described above need to be balanced against what resources are available to carry out the surveillance program.

Bias

Anytime a sample is taken from a population, there is a possibility that bias will be introduced. A biased sample is one that is systematically different from the population as a whole. For example, if samples are collected from hunters, the animals that they kill may not be representative of the whole population of interest. Hunters may preferentially select for larger, healthier animals from a population. The samples may be from older animals or be more of one sex than another. If animals are live trapped and samples are obtained, the animals trapped may be different from the overall population in some manner. All of these differences introduce bias into the surveillance findings and need to be considered in the interpretation and communication of the results. The best way to minimise bias is to take a random sample of the population. Unfortunately this is often not possible in wildlife surveillance programs.

Targeted Surveillance Scenarios for Atlantis: Data Review and Analysis

ACTIVITY 5

Review and Analysis of Targeted Surveillance Data

Data from two different Targeted wildlife surveillance programs in Atlantis are provided for use in the following exercises. The two Targeted surveillance programs and the data sets for each are very different and explore different aspects of Targeted wildlife disease surveillance; you may choose just one or you may carry out the exercise twice, once with each set of data. One program was designed to determine the prevalence of a new strain of rabies virus (Scenario TS1), in order to determine whether or not a control strategy needs to be implemented; the other was designed to demonstrate that Atlantis is free of foot and mouth disease (Scenario TS2).

The data are in separate Excel files entitled: Data Targeted Surveillance 1 and Data Targeted Surveillance 2. Download and save a copy of the spreadsheet(s) on your computer. These files can be downloaded from the OIE website. If you introduce errors into the data on your computer, you can delete that file, and download another copy and start over.

To complete the exercises, you will also need to review the background information about each Targeted surveillance program, as well as the maps and charts that were created from the data provided in the Excel data files. All of the required information is provided in Appendix D.1 for Targeted Surveillance 1 and Appendix D.2 for Targeted Surveillance 2.
To begin, review and describe the data provided (i.e. what animal species were tested, how were they caught, where and when were they sampled, etc...) and then answer the following questions:

1. What errors or inconsistencies did you find in the datasets?

[write here]

2. What patterns are present in the data? (Spatial, temporal, other...)

[write here]

3. Do you have all the data that you need to interpret the information provided and meet the stated objectives of the targeted surveillance program? If not, make a list of the additional information that is needed.

[write here]

4. Are there any important biases that would affect how you interpret the targeted surveillance data results? Describe how the data might be biased and whether or not this is a problem.

[write here]

5. What are the important surveillance findings? How should these findings be reported to the OIE? Who else should be informed about the surveillance findings?

Review Chapter 1.1 of the OIE Terrestrial Animal Health Code (http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_notification.htm)

[write here]

Targeted Surveillance in Atlantis: Capacity to meet its objectives?

ACTIVITY 6

Is the Targeted Surveillance Program able to meet its Stated Objectives?

Review the one or both of targeted surveillance datasets provided. Are the data provided adequate to meet the stated objectives for each program? (see Appendix D for descriptions of the two Targeted surveillance programs)

Consider what each program was designed to do.

- Are the data that have been gathered in each of the two programs adequate to meet the stated objectives?
- How would you improve the program?

[write here]

Appendix A

The *Dominion of Atlantis,* General Information

(This country does **not** exist)

The *Dominion of Atlantis* is a parliamentary democracy with a capitalist economy.

Economy

- Mostly self-sufficient in food production, 10% export balanced by 10% import
- Main sources of wealth:
    - Agricultural products
        - Major exports of poultry, cheese, farmed mink pelts and wine
    - Forest products for export
    - Tourism
        - Wildlife viewing, seaside and forest natural environments, hunting and fishing
        - Important wildlife populations for tourism include
            - White-tailed Deer – 30,000
            - Moose – 2,000
            - Bald Eagles – 800
            - Black Bears – 3000
    - Commercial seafood harvest for export (finfish & shellfish)
    - Wind and tide-generated electricity
    - Banking (tax haven)

Location: An island in the North Hibernian Ocean (see map on last page)

Human Population:

- 946,000 people
    - 40% rural
    - 60% in urban centres,
        - 43% in the capital city of Bigtown
- Wealth: Median Annual Family Income: US$30,000

Size: ~ 56,000 km^2 (~ 130 km x 560 km)

Climate: North Temperate

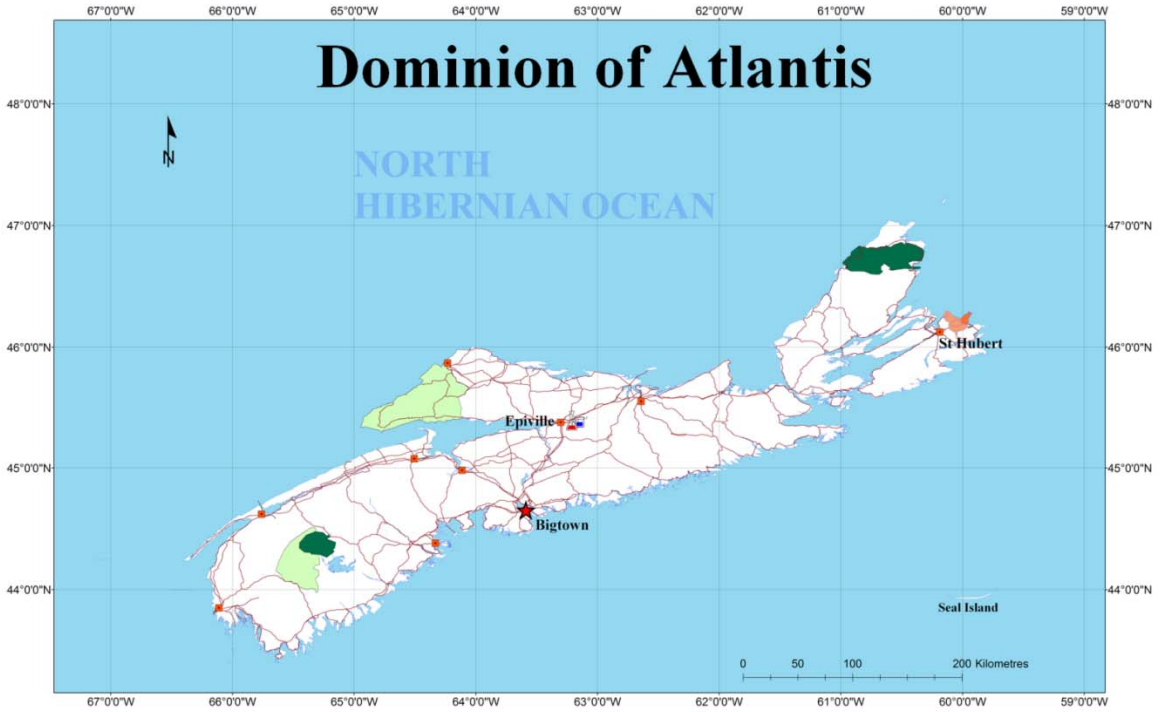
- Average summer temperature = +21C
- Average annual extreme temperatures: -10C to +28C
- Annual precipitation: 1,500 mm, (300mm as snow in winter)

Social Infrastructure:

- Relevant National Ministries/Departments:
    - Ministry of Health
        - Medical laboratory in Bigtown
        - 16 Regional Hospitals

- Ministry of Agriculture & Aquaculture
  - Veterinary diagnostic lab in Epiville
  - 10 Regional Offices
- Ministry of Natural Resources (Fish and Wildlife Department)
  - 18 Regional Offices
- Ministry of Environment (Jurisdiction over Wilderness Areas and National Parks
  - 6 Regional Offices
- Ministry of Ocean Resources (jurisdiction over ocean fish and marine mammals)
  - 18 Regional offices
- Ministry of Tourism
  - Fishing, Hunting & Ecotourism Guide License Department

- Aboriginal Government
  - Anguille Original People's Council – Government for 20,000 aboriginal people which controls all resources on 5,000 km^2 of Atlantis, mostly adjacent to parks and wilderness areas. Special hunting and fishing rights extend to the whole country.
- Universities:
  - Harrison Lewis National University (20,000 students, Bigtown)
    - Includes Atlantis Veterinary College
  - 6 small (500 to 4000 students) regional universities distributed across country
- Non-Government Organizations:
  - National Farmers Association
  - Atlantis Natural History Club (naturalists)
  - National Fish and Game Association (recreational hunters and fishermen)
  - National Fishermen's Union (commercial ocean fisheries)
- Calliope International (animal rights and welfare association)



Appendix B

Appendix B.1: Charts and maps for General Surveillance 1

Dataset G1 Charts:

Chart G1 A: Number of dead animals by date, Atlantis 2015

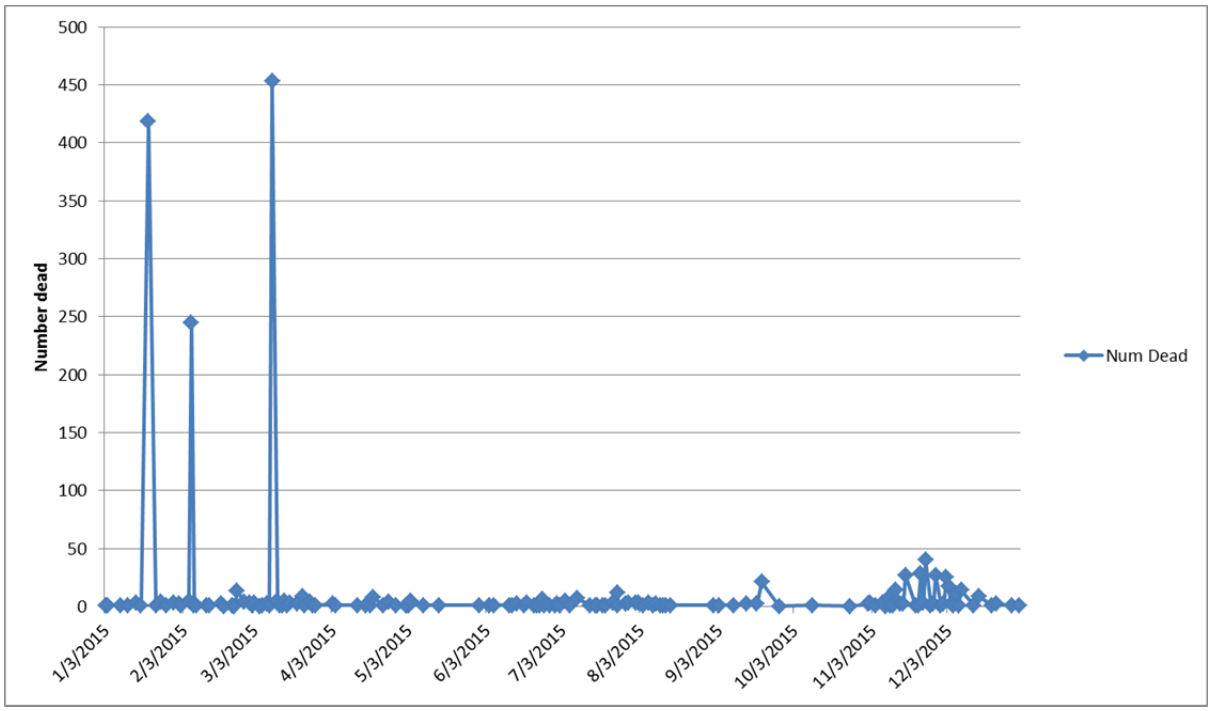


Chart G1 B: Number of animals that died of Avian Influenza H5N1 (Highly Pathogenic Strain), Atlantis November 1-December 15, 2015

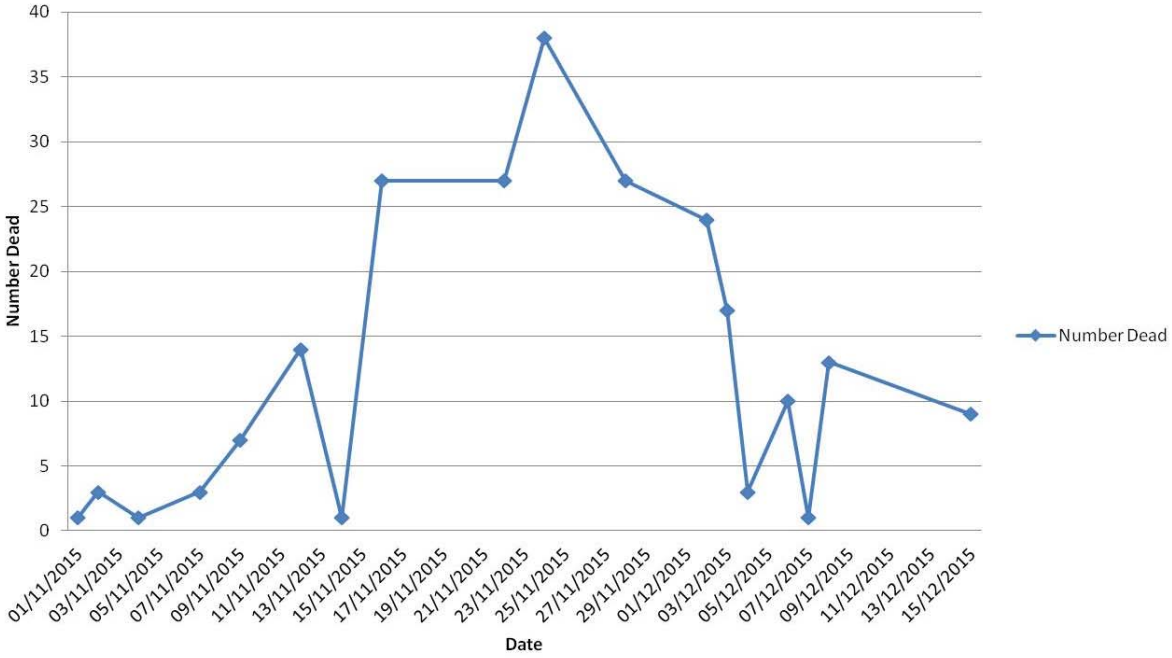


Chart G1 C: Number of crows that died of Reovirus, Atlantis January 15-February 26, 2015

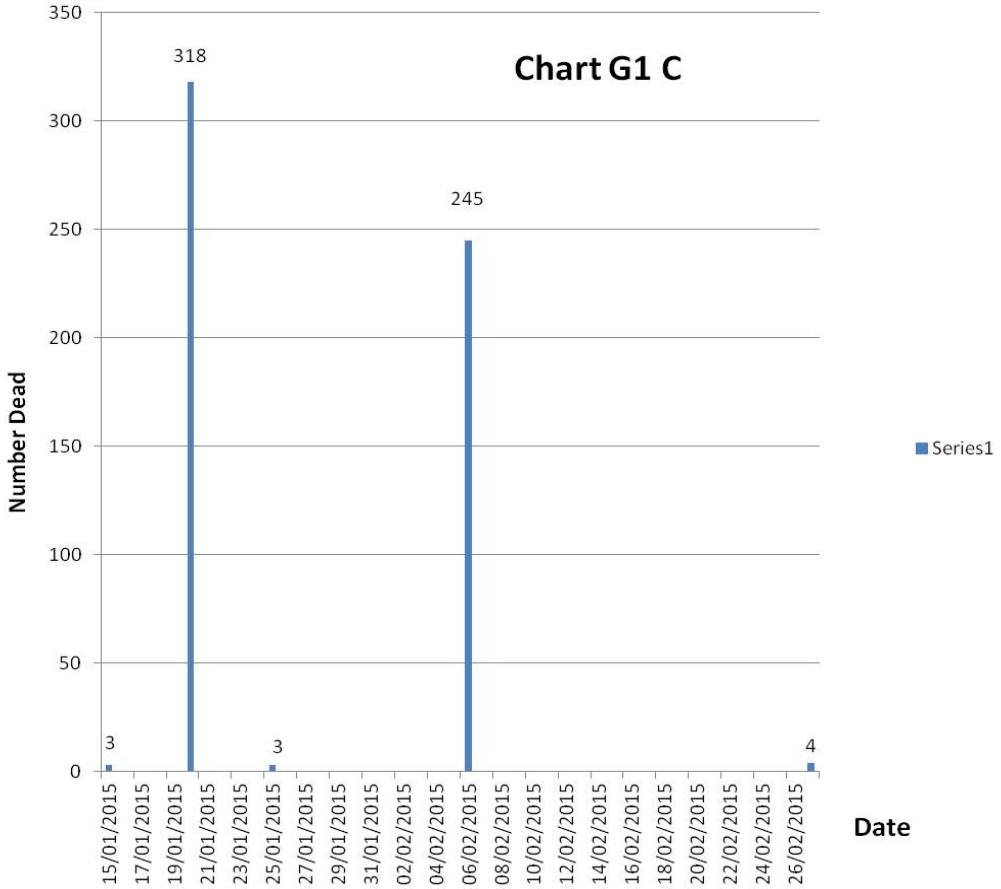
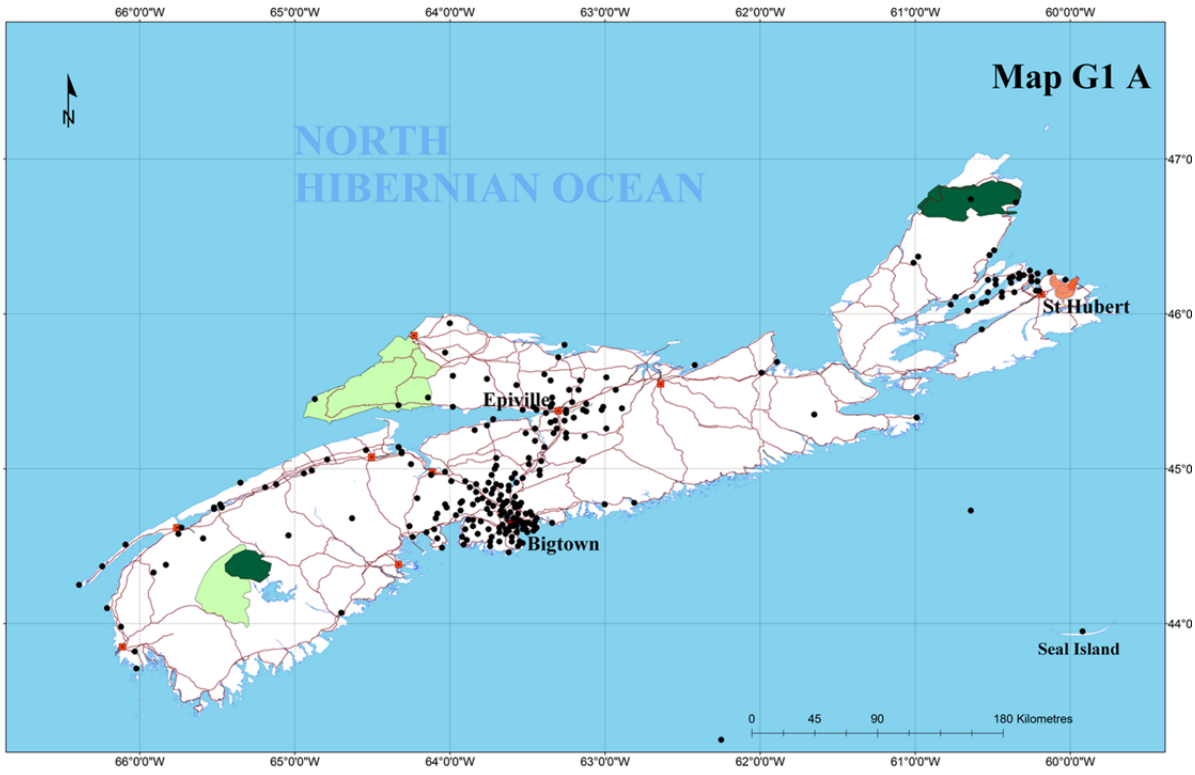
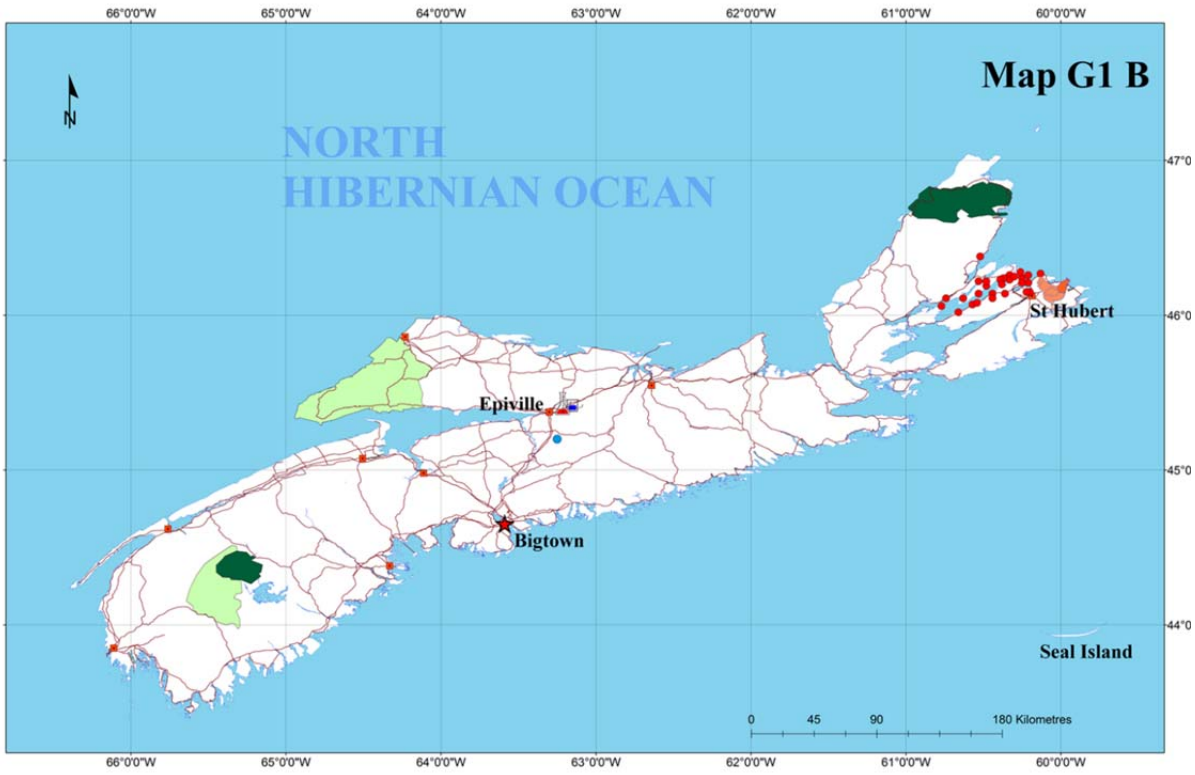
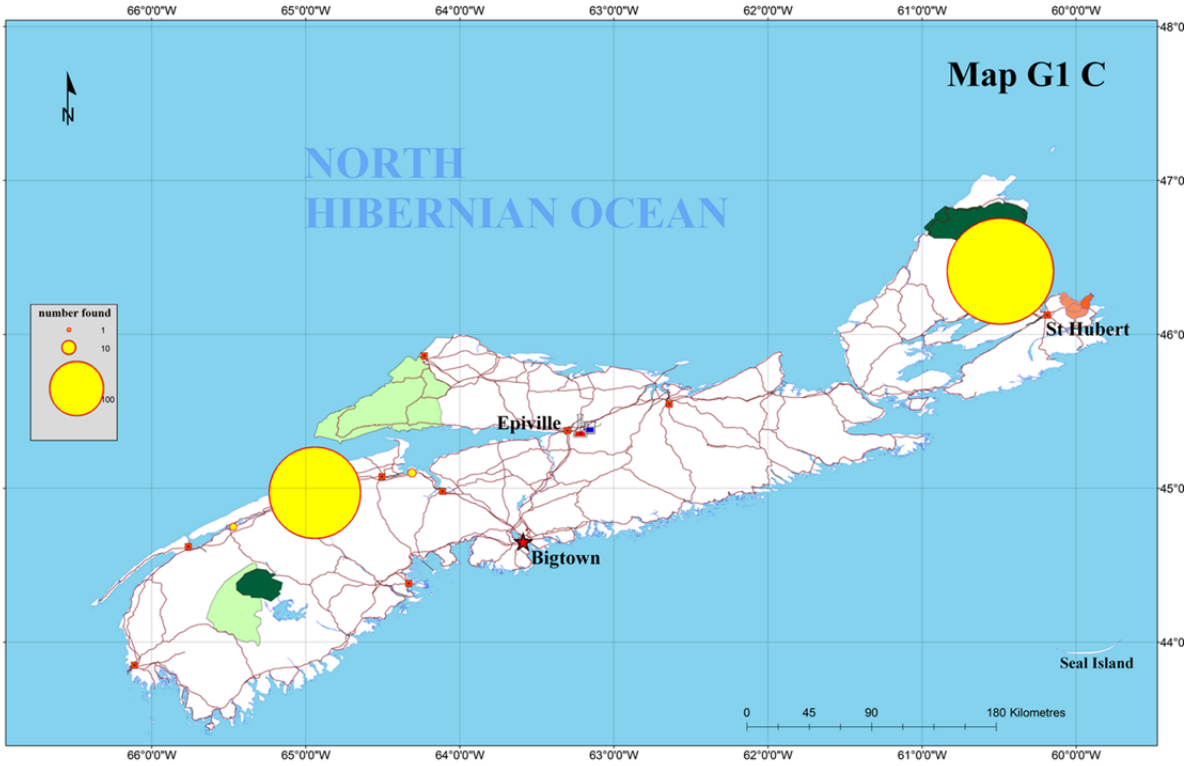


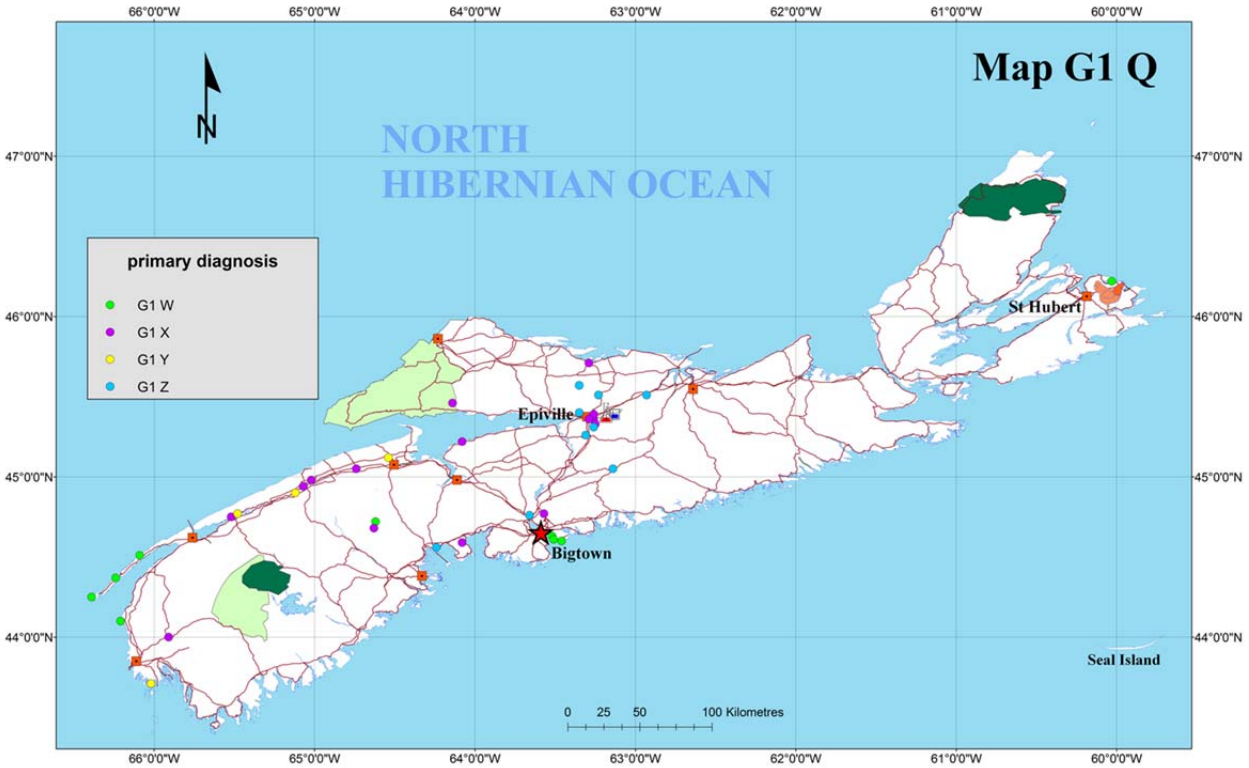
Chart G1 D: Number of animals that died of *Salmonella*, Atlantis March 3-March 27, 2015

Dataset G1 Maps:






Map G1 C
N
NORTH
HIBERNIAN OCEAN
number found
1
10
100
Epiville
Bigtown
St Hubert
Seal Island
0 45 90 180 Kilometres
66°0'0"W
65°0'0"W
64°0'0"W
63°0'0"W
62°0'0"W
61°0'0"W
60°0'0"W
48°0
47°0
46°0
45°0
44°0


Map G1 Q
N
NORTH
HIBERNIAN OCEAN
primary diagnosis
G1 W
G1 X
G1 Y
G1 Z
Epiville
Bigtown
St Hubert
Seal Island
0 25 50 100 Kilometres
66°0'0"W
65°0'0"W
64°0'0"W
63°0'0"W
62°0'0"W
61°0'0"W
60°0'0"W
47°0'0"N
46°0'0"N
45°0'0"N
44°0'0"N

Appendix B.2: Charts and maps for General Surveillance 2

Dataset G2 Charts:

Chart G2 A: Number of dead animals by date, Atlantis 2015

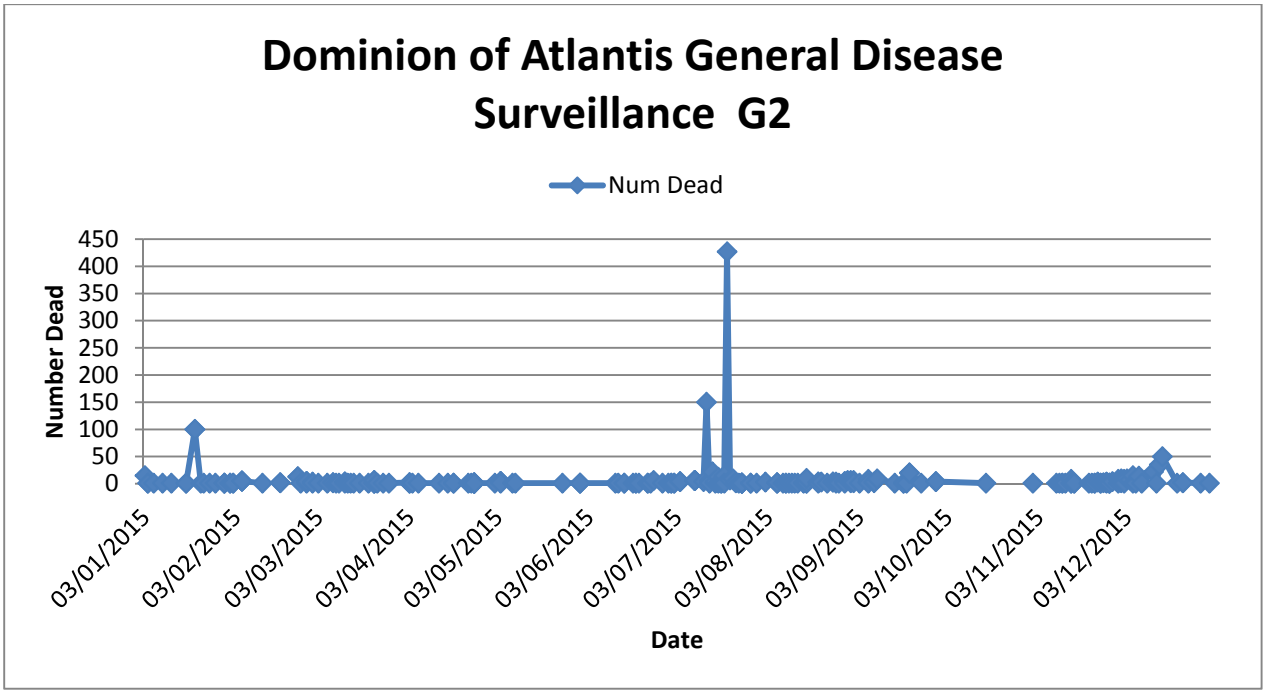


Chart G2 B: Number of dead animals from Saxitoxin poisoning (Harmful algal bloom), Atlantis August 15-September 9, 2015

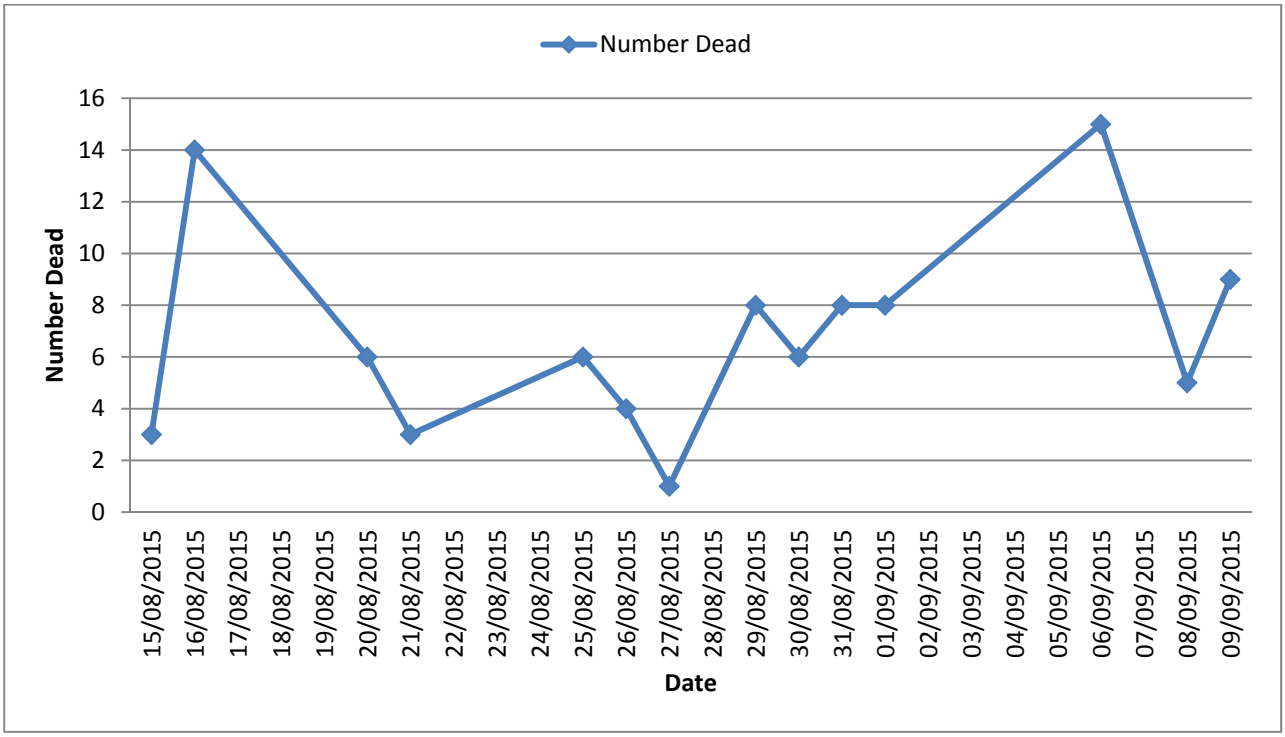


Chart G2 C: Number of animals dead of Newcastle disease (Highly pathogenic APMV-1), Atlantis June 23-August 22, 2015

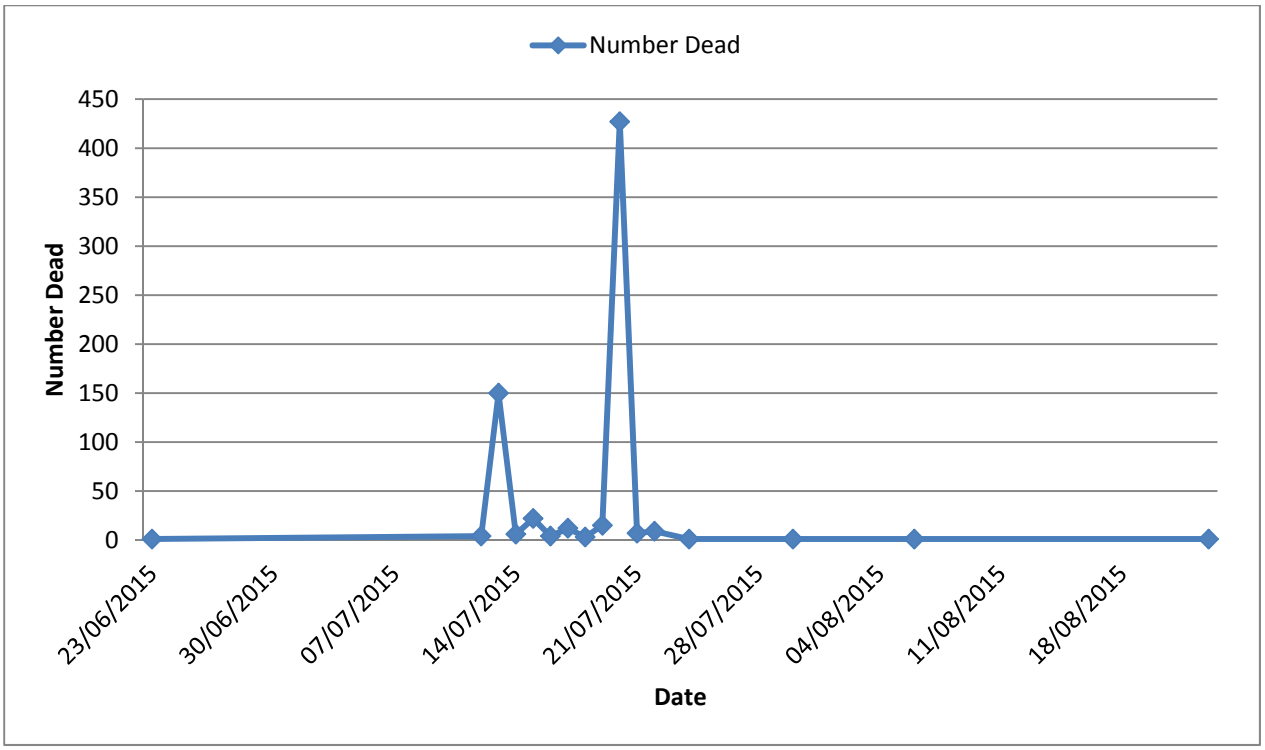
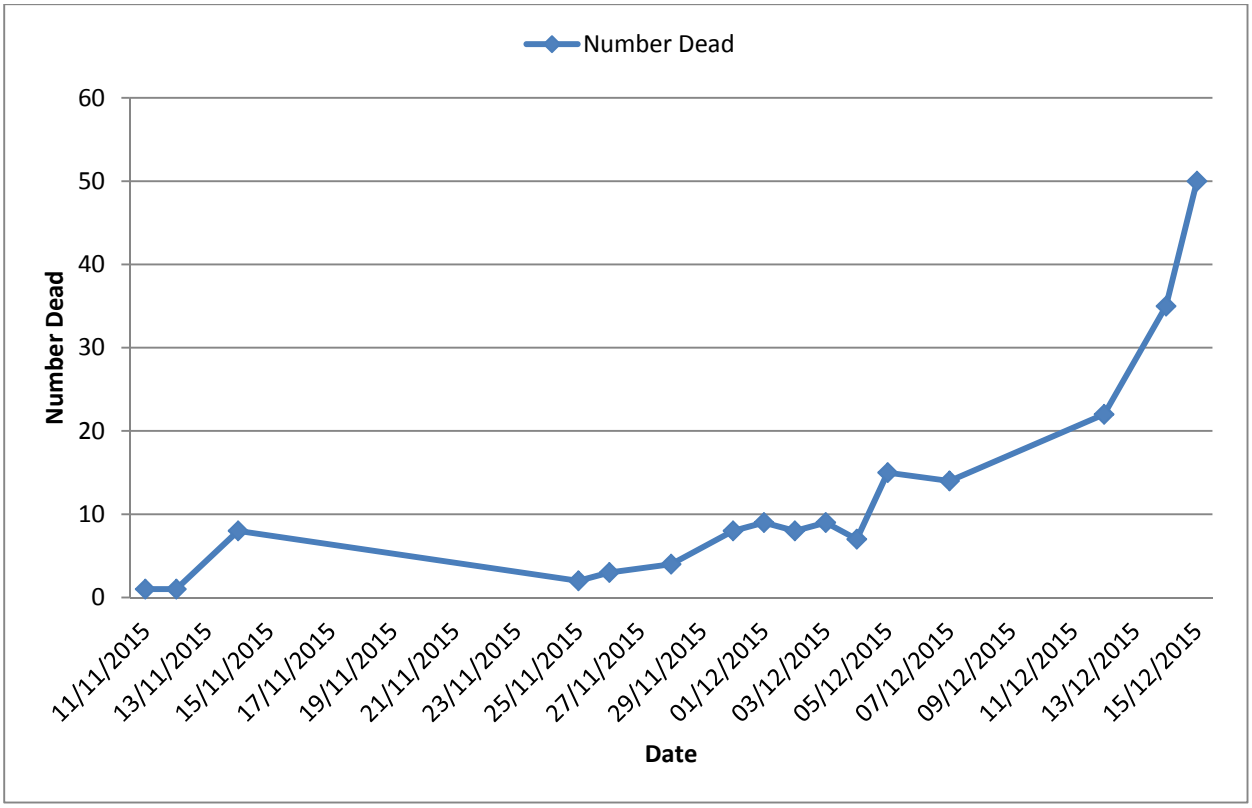
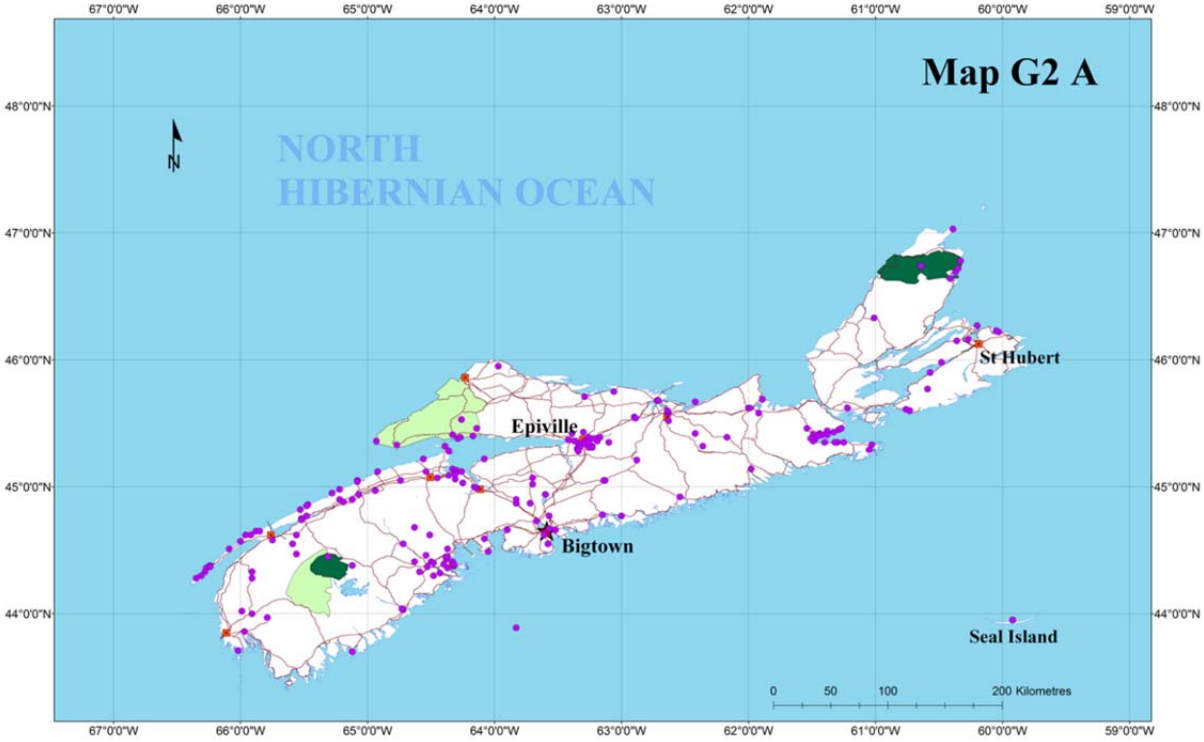
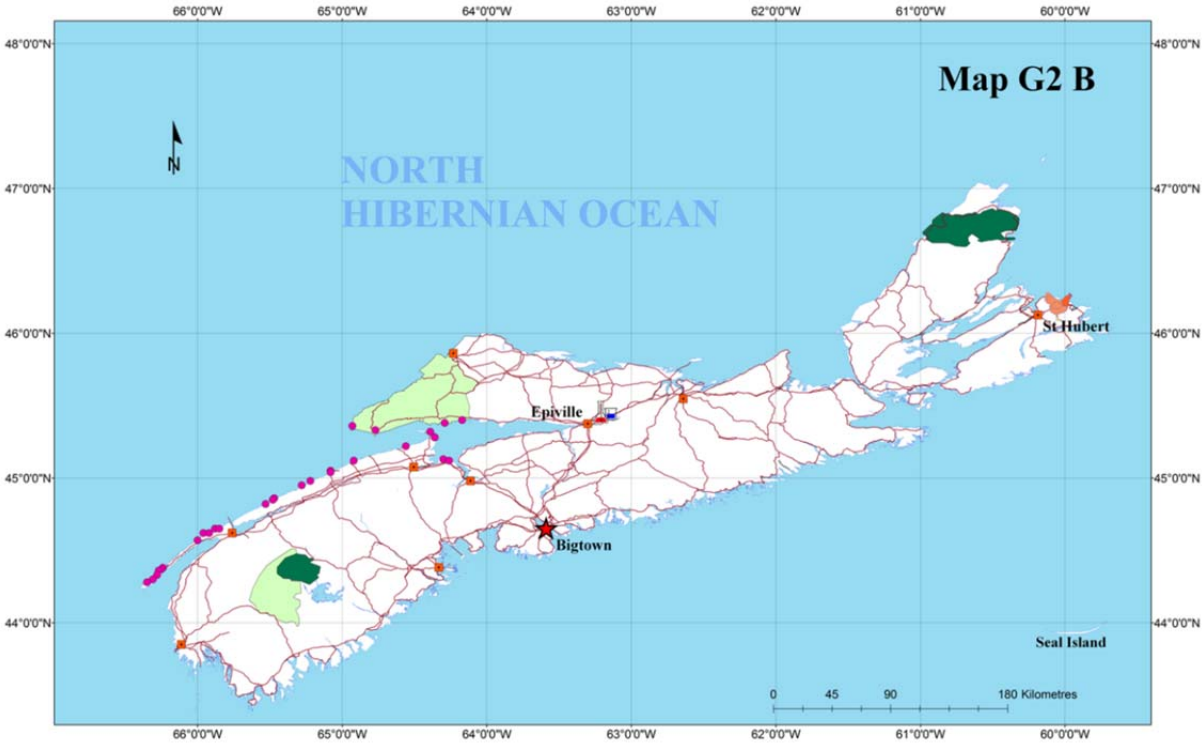


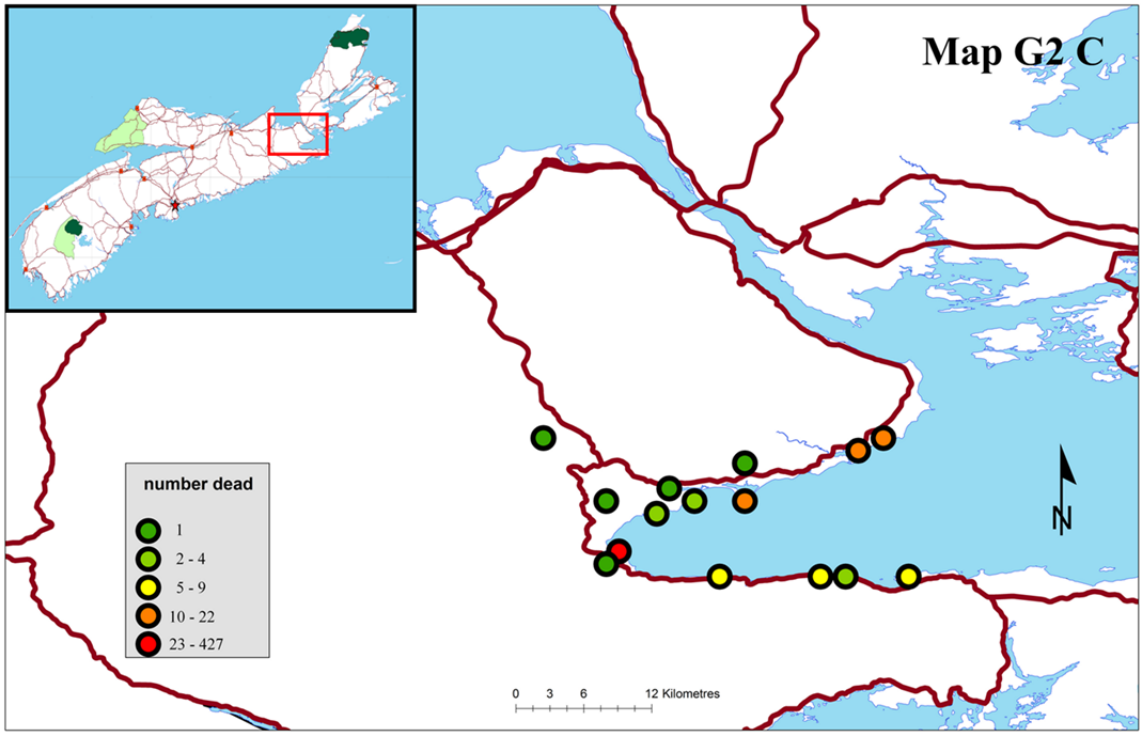
Chart G2 D: Number of animals that died of Morbillivirus infection, Atlantis November 11-December 15, 2015

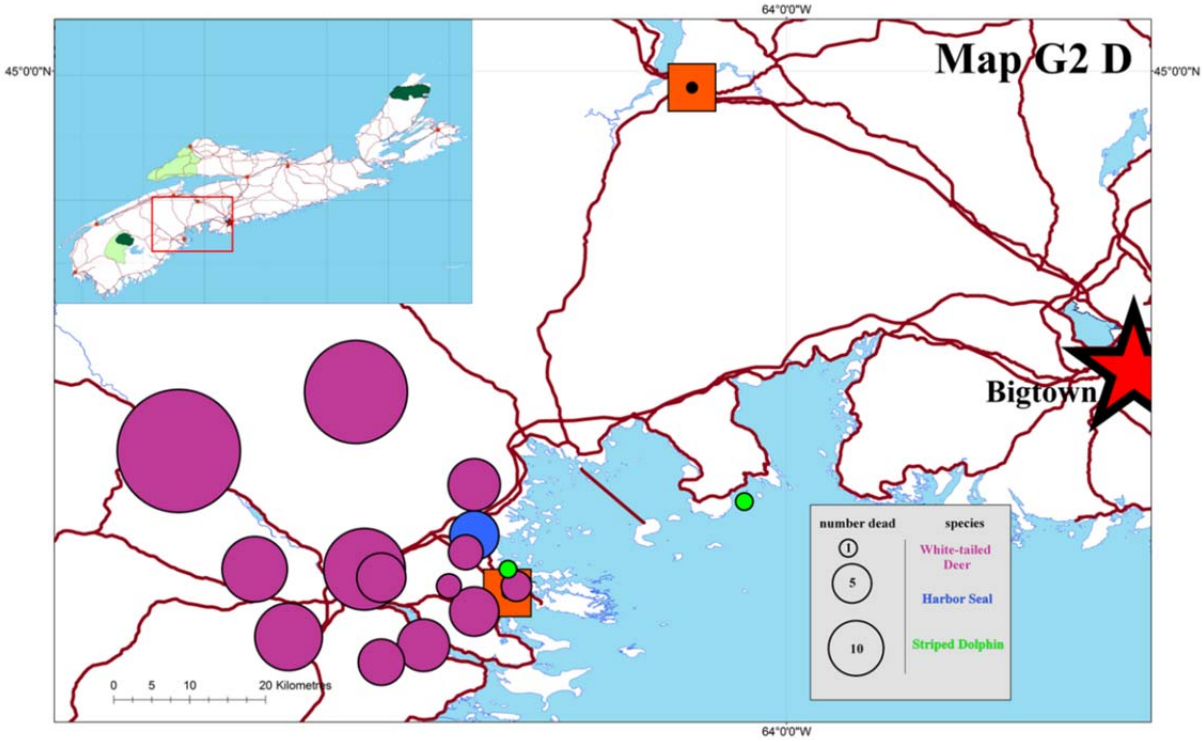


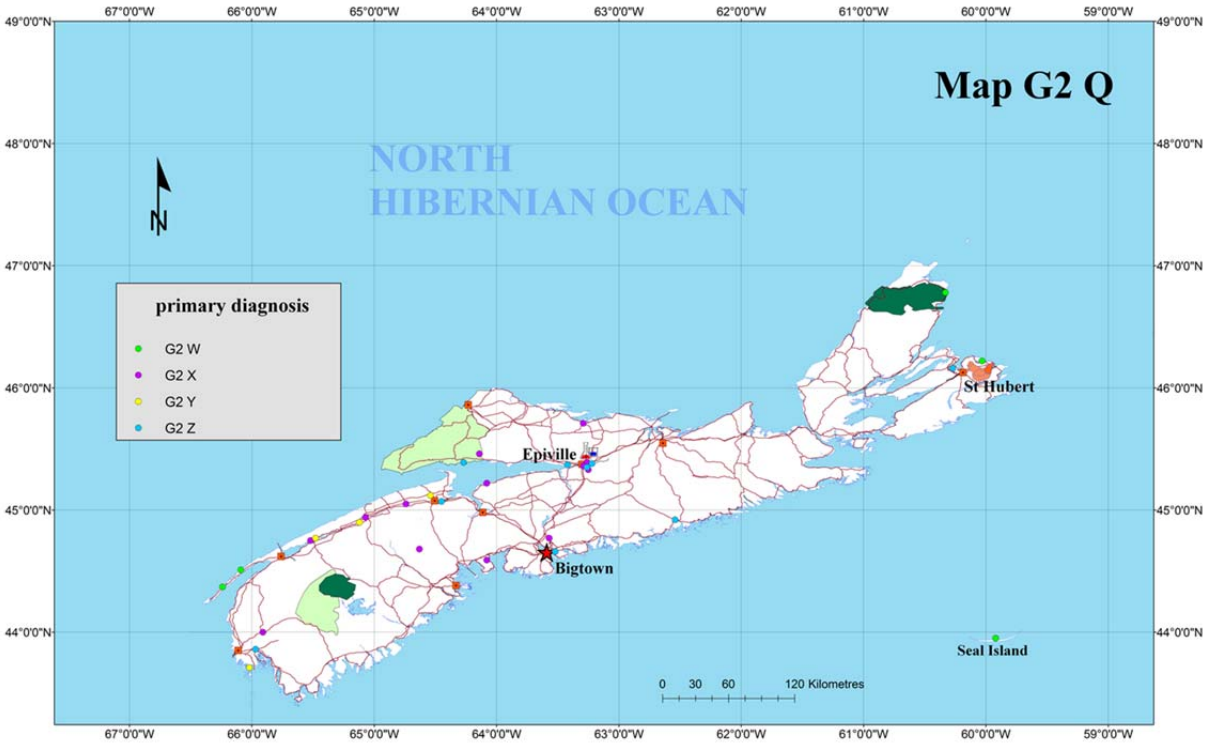
Dataset G2 Maps:






Map G2 C
number dead
1
2 - 4
5 - 9
10 - 22
23 - 427
N
0 3 6 12 Kilometres


64°0'0"W
45°0'0"N
Map G2 D
45°0'0"N
Bigtown
number dead
species
1
5
10
White-tailed Deer
Harbor Seal
Striped Dolphin
0 5 10 20 Kilometres
64°0'0"W


Map G2 Q
NORTH
HIBERNIAN OCEAN
N
primary diagnosis
G2 W
G2 X
G2 Y
G2 Z
Epiville
Bigtown
St Hubert
Seal Island
0 30 60 120 Kilometres
67°0'0"W
66°0'0"W
65°0'0"W
64°0'0"W
63°0'0"W
62°0'0"W
61°0'0"W
60°0'0"W
59°0'0"W
49°0'0"N
48°0'0"N
47°0'0"N
46°0'0"N
45°0'0"N
44°0'0"N

Appendix C

OIE Listed Diseases affecting wild animals

- Anthrax
- African horse sickness
- African swine fever
- Aujeszky's disease
- Avian chlamydiosis
- Avian infectious bronchitis
- Avian infectious laryngotracheitis
- Avian mycoplasmosis (*Mycoplasma gallisepticum*)
- Avian mycoplasmosis (*Mycoplasma synoviae*)
- Bluetongue
- Bovine anaplasmosis
- Bovine babesiosis
- Bovine genital campylobacteriosis
- Bovine spongiform encephalopathy
- Bovine tuberculosis
- Bovine viral diarrhoea
- Brucellosis due to *Brucella abortus*
- Brucellosis due to *Brucella melitensis*
- Brucellosis due to *Brucella suis*
- Caprine arthritis/encephalitis
- Classical swine fever
- Contagious agalactia
- Contagious bovine pleuropneumonia
- Contagious caprine pleuropneumonia
- Contagious equine metritis
- Crimean Congo haemorrhagic fever
- Dourine
- Echinococcosis/hydatidosis
- Enzootic abortion of ewes (ovine chlamydiosis)
- Enzootic bovine leukosis
- Epizootic haemorrhagic disease
- Equine encephalomyelitis (Eastern)
- Equine encephalomyelitis (Western)
- Equine infectious anaemia
- Equine influenza
- Equine piroplasmosis
- Equine rhinopneumonitis
- Equine viral arteritis
- Foot and mouth disease
- Fowl cholera
- Fowl typhoid
- Glanders
- Haemorrhagic septicaemia
- Heartwater
- Highly pathogenic avian influenza
- Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis
- Infectious bursal disease (Gumboro disease)
- Japanese encephalitis
- Leishmaniosis
- Leptospirosis
- Lumpy skin disease
- Maedi–visna
- Marek's disease
- Myxomatosis
- Nairobi sheep disease
- New world screwworm due to *Cochliomyia hominivorax*
- Newcastle disease
- Nipah virus encephalitis
- Old world screwworm due to *Chrysomya bezziana*
- Ovine epididymitis due to *Brucella ovis*
- Paratuberculosis
- Peste des petits ruminants
- Porcine cysticercosis
- Porcine reproductive and respiratory syndrome
- Pullorum disease
- Q fever
- Rabbit haemorrhagic disease
- Rabies
- Rift Valley fever
- Rinderpest
- Salmonellosis due to S. abortusovis
- Scrapie
- Sheep pox and goat pox
- Surra (*Trypanosoma evansi*)
- Swine vesicular disease
- Theileriosis
- Transmissible gastroenteritis
- Trichinellosis
- Trichomonosis
- Trypanosomosis (tsetse-transmitted)
- Tularemia
- Venezuelan equine encephalomyelitis
- Vesicular stomatitis
- West Nile fever

Amphibians (Aquatic OIE listed diseases)

- Infection with *Batrachochytrium dendrobatidis*
- Infection with ranavirus

Non-OIE listed diseases affecting wildlife

Arboviruses	Measles
Avian Malaria	Meningeal worms of cervids
Avian Pox	Morbillivirus infection in aquatic mammals
Avian Vacuolar Myelinopathy	Paramyxoviruses
Babesiosis	Pasteurellosis
Baylisascaris procyonis	Pestiviruses
Besnoitiosis	Pseudotuberculosis
Calicivirus Marine Mammals	Psoroptic Mange
Canine distemper	Salmonellosis (*S. enterica*)
Chronic wasting disease (CWD)	Sarcoptic Mange
Circoviruses	Plague
Contagious Ecthyma	Tick Borne Encephalitis
Ebola Virus Hemorrhagic Fever (EVHF)	Toxoplasmosis
Elephant Herpesvirus	Trasmissible Mink Encephalopathy (TME)
European Brown Hare Syndrome (EBHS)	Trichomonas sp.
Feline Leukaemia (FLV)	Tyzzer´s Disease
Feline morbillivirus infection	White-nose syndrome in bats
Feline Panleucopenia	**Reptiles**
Hantaviruses	Inclusion Body Disease
Histomoniasis	Fibropapillomatosis in sea turtles
Immunodeficiency viruses (Feline, Simian)	Papillomatosis in crocodiles
Inclusion Body Hepatitis	Trichinellosis
Large Liver Flukes	**Non-Infectious Diseases**
Listeriosis	Algal toxicosis
Louping ill	Botulism
Low pathogenic avian influenza	Chemical poisons
Lyme borreliosis	Mycotoxins
Malignant catarrhal fever	**Diseases of Unknown Cause**
Marburg virus	Unknown disease

Appendix D

Appendix D.1:
Targeted Surveillance Scenario TS1 - Rabies in Atlantis

Background and Design

Early in the summer of 2014, a coyote (*Canis latrans*) in Kejimkujik National Park was found dead and submitted to the General wildlife surveillance program. The cause of death was determined to be rabies and the virus detected appeared to be a new coyote-adapted strain that had not been detected in the country before. Within a few weeks of the first diagnosis, 4 more coyotes tested positive for the new rabies strain and there were multiple reports of coyotes across south-western Atlantis exhibiting abnormal behaviour. Additionally, 2 dogs in Bigtown were euthanized because they were suspected of being infected with rabies and neither had been vaccinated. Both had traveled recently to Kejimkujik National Park. Both dogs were confirmed positive for the same new rabies strain and 22 people in Bigtown required post exposure prophylaxis.

Because of the public health concern posed by rabies, particularly a new virus strain, an inter-agency group was formed to investigate the occurrence. The group determined that they needed more information about the virus, how wide-spread it is (prevalence, geographic distribution) and which species were primarily affected. A targeted surveillance program for rabies was initiated in 2015. Based on the data generated through targeted surveillance, the country could then decide whether or not a control program was needed. It was decided that the surveillance program should be designed so that, if a control program was implemented, the data could also be used to evaluate the success of the control program.

Purpose of the Targeted rabies surveillance in Atlantis:

1) To determine how wide-spread the new coyote-adapted strain of rabies is in Atlantis
2) To identify what species are involved
3) To generate the information needed to determine whether or not a control program is needed

Which wildlife species were included in the Targeted wildlife disease surveillance program?

Because the new rabies strain was first identified in coyotes, they were targeted for inclusion in the surveillance program. However, the main reservoir for rabies in Atlantis has traditionally been the raccoon (*Procyon lotor)* and so a decision was made to include raccoons as well.

Where and when was Rabies virus surveillance carried out?

Because of the need to gather as much data as possible about this new strain of rabies, coyotes and raccoons were trapped and submitted for testing all year. As the original case was identified in Kejimkujak, this was considered to be the centre of the outbreak and coyotes and raccoons within 150km of the park were included in the surveillance program.

Which wildlife sub-groups were targeted?

All coyotes and raccoons, regardless of age or sex, were included in the targeted surveillance program.

How were the animals caught and samples collected?

Commercial hunters and trappers, and recreational hunters were recruited to assist in sample collection. Participating hunters and trappers brought the animals to the nearest offices of the Ministry of Natural Resources and trained biologists removed the heads and submitted them to the Epiville lab for virus identification. The name and contact details of the hunters and trappers were recorded along with the specimen ID code so that they could be informed of the laboratory results and seek out appropriate treatment should the animal be confirmed to be rabid (data not provided).

A public education campaign was also launched warning people about the risk to humans and domestic animals posed by rabies in wildlife. At the same time, the public was asked to report coyotes and raccoons they found dead to their nearest Ministry of Natural Resources office. A technician then picked up the animal and submitted its head to the lab for testing.

Based on the results of General surveillance, the Atlantis epidemiologists estimated that the prevalence of rabies in coyotes was 10%; they further estimated that the prevalence in raccoons could be as high as 2%.

Test characteristics:

At the Epiville lab, the fluorescent antibody test is used to determine whether or not an animal is infected with rabies virus. This test is reported to have a sensitivity of 96.3% and specificity of 98.4%. All positive brain samples are then tested by real time RT-PCR to determine the strain-type. All the positive animals detected in Atlantis in 2015-2016 were determined to have the new coyote-adapted strain (data not shown).

Scenario TS1 Charts:

Chart 1: Number of coyotes and raccoons submitted for rabies testing in Atlantis by year

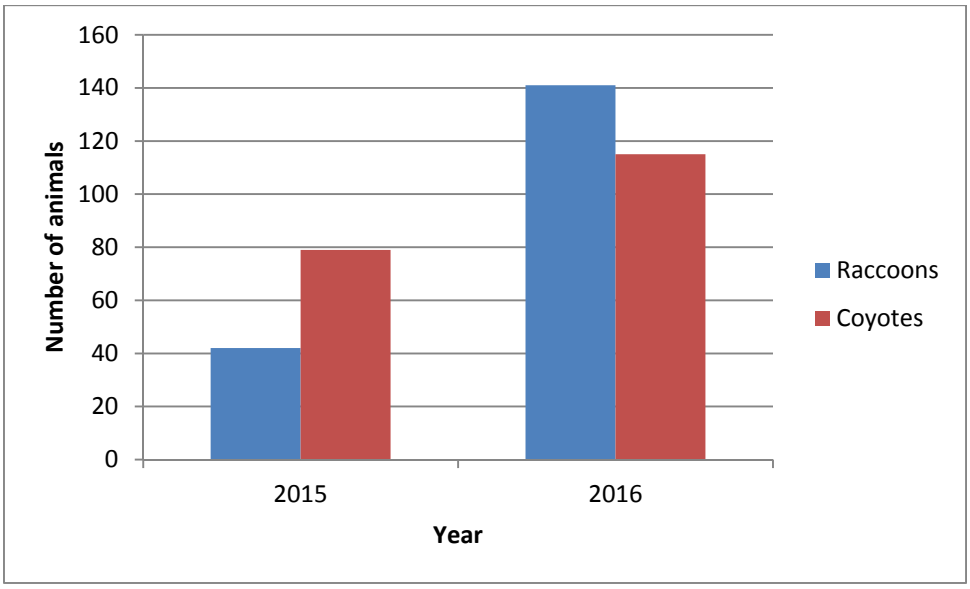


Chart 2: Number of coyotes and raccoons tested for rabies by month (2015-2016)

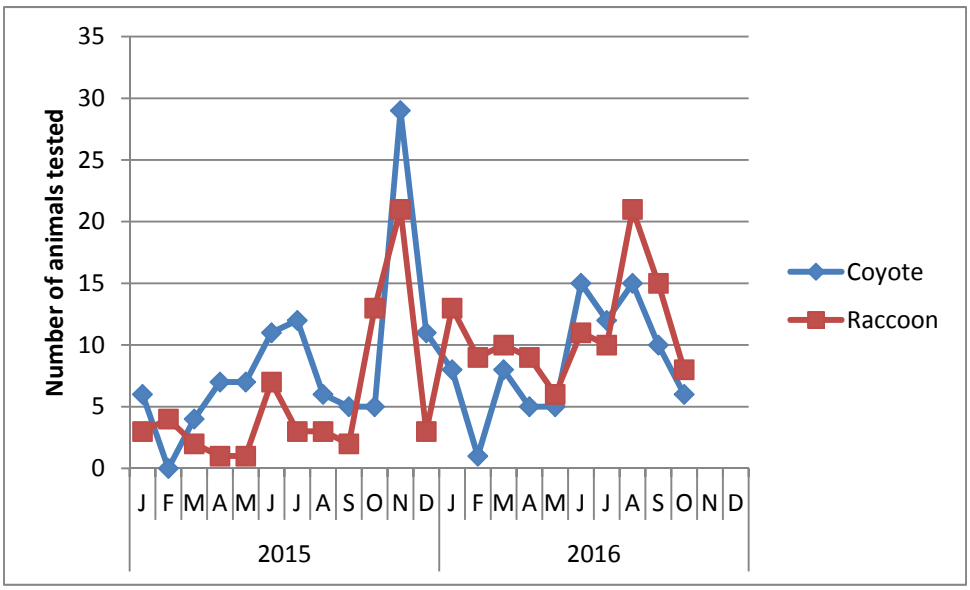


Chart 3: Proportion of raccoons and coyotes tested for rabies that were male, female or of unknown sex, 2015-2016

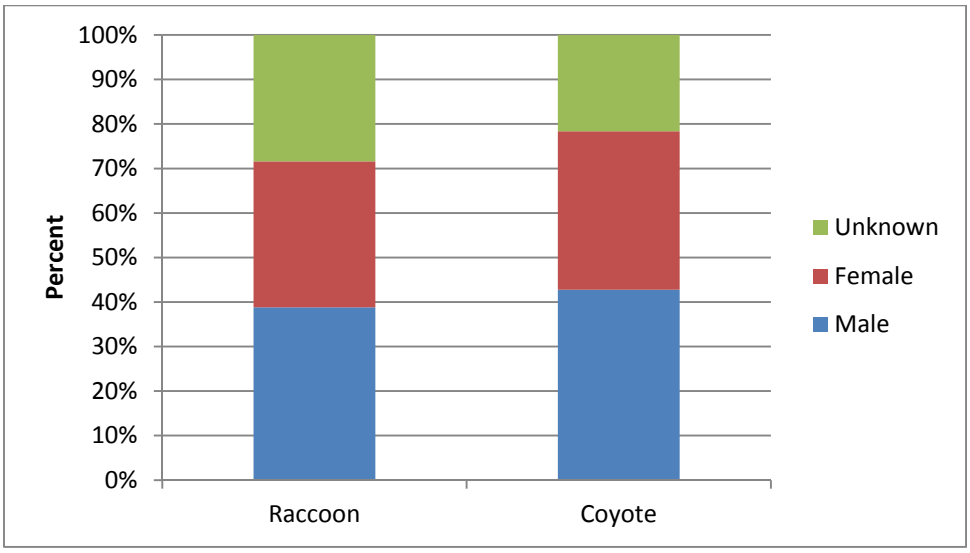


Chart 4: Number of coyotes and raccoons tested for rabies by age category, 2015-2016

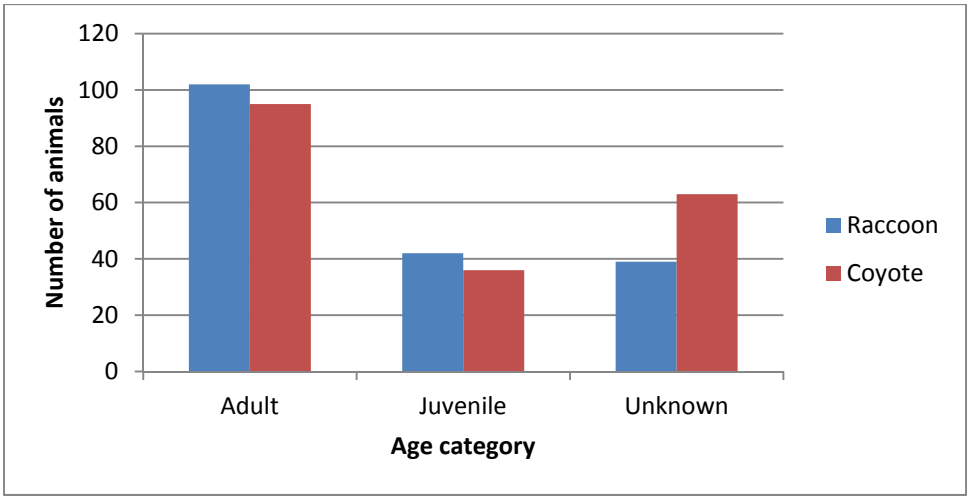
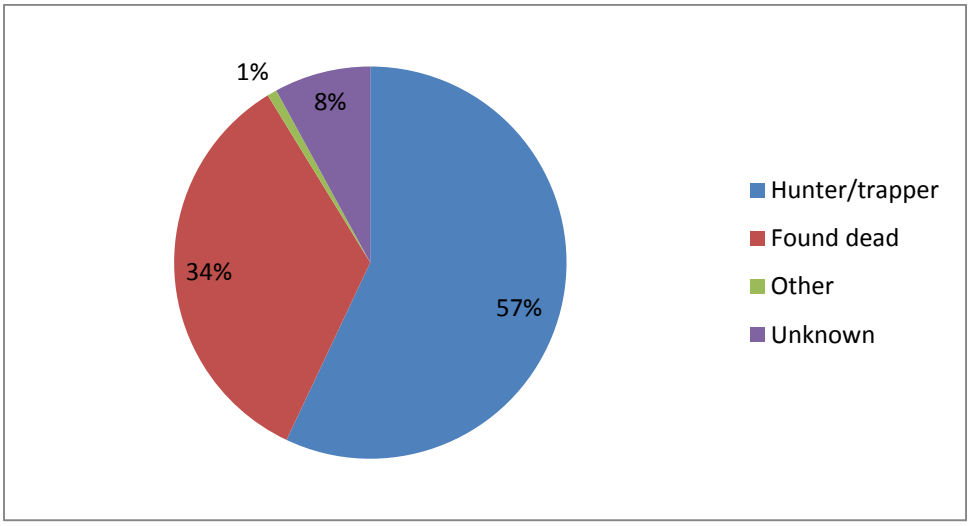
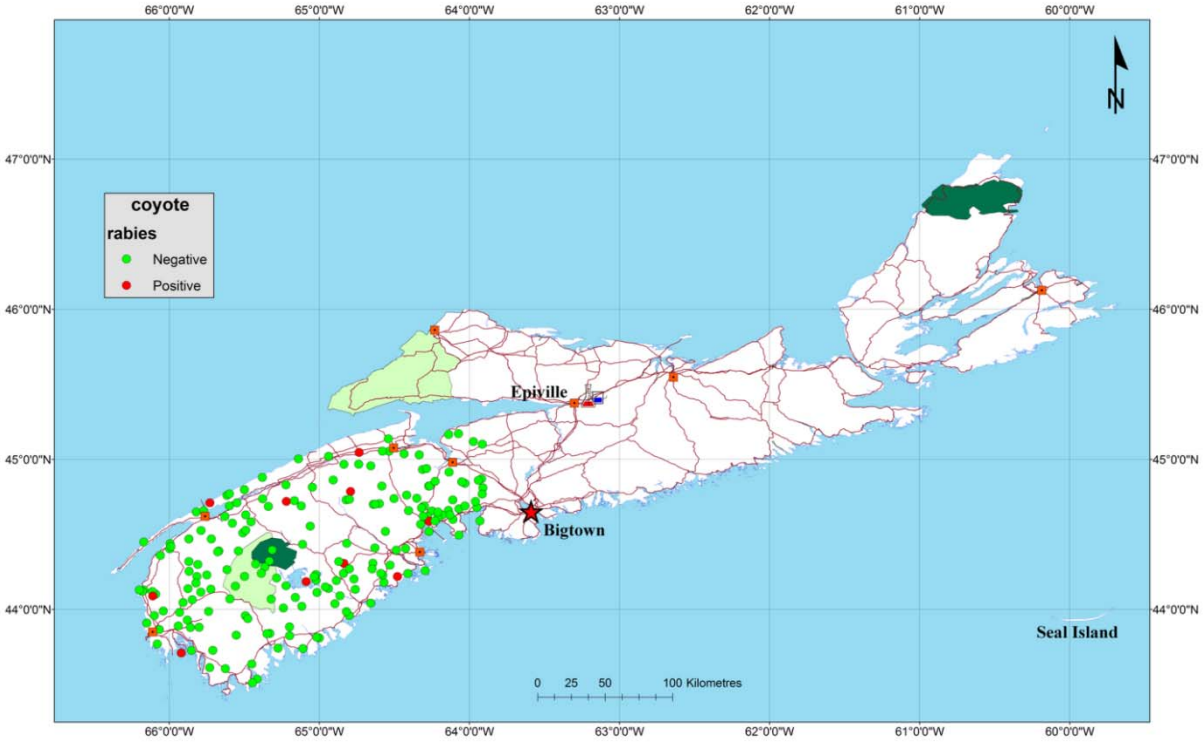


Chart 5: Proportion of animals submitted for rabies testing by how they were found, 2015-2016

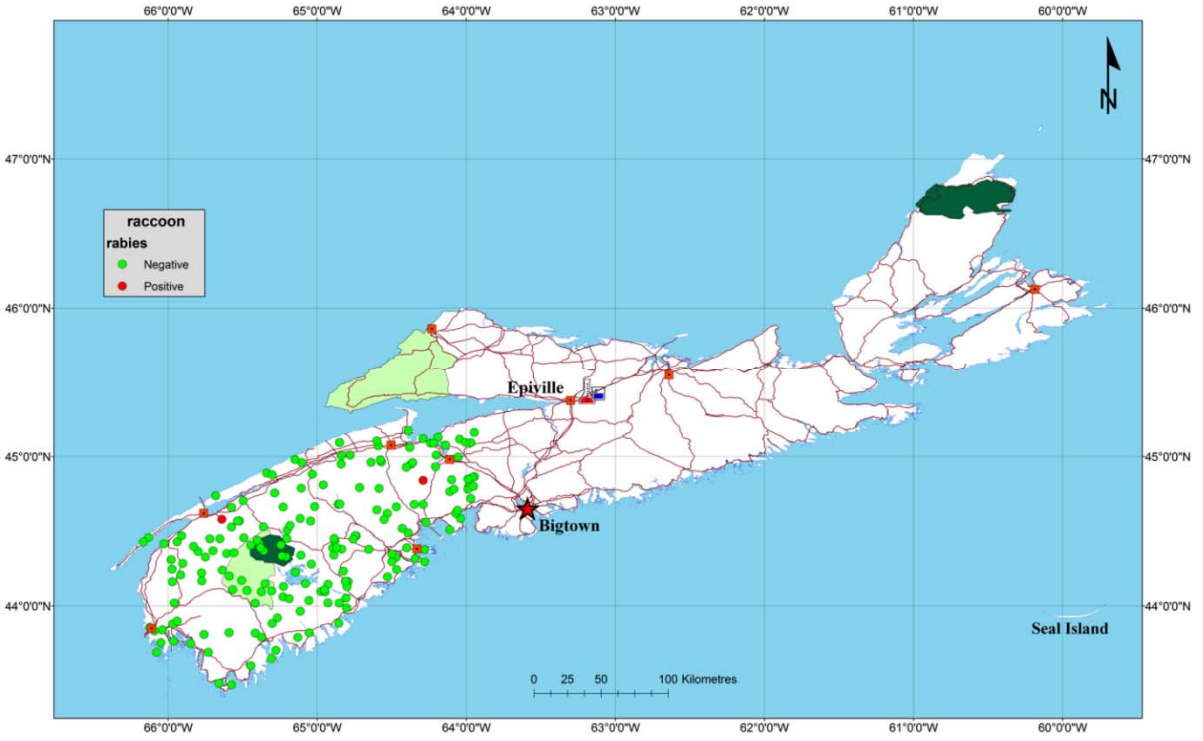


Scenario TS1 Maps:

Map 1: Atlantis rabies surveillance results – raccoons 2015-16



Map 1: Atlantis rabies surveillance results – coyotes 2015-16



Appendix D.2: Targeted Surveillance Scenario TS2 - Foot and Mouth Disease in Atlantis

Background and Design:

Over the past 10 years, reports have been released from other nations, some of which are not far from Atlantis. Of particular concern to the wildlife managers and agricultural health experts was a In 2012, the country of Borduria, located about 230 km from Atlantis across the Hibernian Ocean, reported to the OIE that Foot-and-Mouth Disease (FMD) had been detected and confirmed in wild deer. There is a busy shipping route between Borduria and Atlantis, and in the winter the ocean is frequently ice covered, providing a potential corridor for wildlife movement between the two countries. Because of the important livestock health and trade issues posed by FMD, Atlantis began a targeted wildlife disease surveillance program for FMD in 2013.

Purpose of the Surveillance program: The purpose of this targeted wildlife disease surveillance program was to detect Foot-and-Mouth disease virus if it emerges in wildlife in Atlantis and, if no virus is detected, to demonstrate that Atlantis remains free of Foot-and-Mouth disease.

Which wildlife species were included in the Targeted wildlife disease surveillance program?

In Atlantis there are 2 wild deer species: white-tailed deer (*Odocoileus virginianus*) and moose (*Alces alces*). The deer population is large but the moose population is much smaller (Table 1). Both species are hunted. The other susceptible wild species in Atlantis is wild boar (*Sus scrofa*). Boar were excluded from the targeted surveillance program because of their small population and because of difficulty obtaining samples.

Table 1: Number of free-ranging white-tailed deer and moose by Wildlife Management Area* and species in Atlantis (data from Atlantis Ministry of Natural Resources, 2013)

Year	Deer				Moose			
	Total	Borderry	CL[1]	ML[2]	Total	Border	CL[1]	ML[2]
Minimum	146130	84420	61710	39200	6500	3230	1125	2145
Maximum	349120	177550	103320	68250	9100	4785	1684	2631

*Wildlife Management Areas are shown in Map 1 below.
[1]CL=Cristolen
[2]ML=Mellen

Where and when was Foot-and-Mouth Disease virus surveillance carried out?

While planning the targeted surveillance program in early 2013, it was decided that the easiest way to access serum samples from deer and moose was by engaging hunters in the surveillance program. Consequently, the majority of samples were collected between September and December in each year (these are the hunting season months in Atlantis). Additional deer and moose found dead or that were hit by cars throughout the year were also tested for Foot-and-Mouth disease virus.

Which wildlife sub-groups should to targeted?

All deer and moose, regardless of age or sex, were included in the targeted surveillance program.

How were the animals caught and samples collected?

Deer and moose hunters were recruited to assist in sample collection. With each hunting license, a sample collection kit, including a data sheet, was distributed. Each hunter was asked to collect a blood sample in the vial provided. The vial and the data sheet were then submitted to surveillance program staff at hunting check points throughout the country; serum was separated from the whole blood and was frozen until tested. The check points were active from September to December of each year. When possible, all deer and moose found dead and reported through the general surveillance system were also tested (using the same specimen collection method). Conservation Officers working for the Ministry of Natural Resources collected these additional specimens.

Test characteristics:

Serological testing (for antibody) was used for the FMD targeted surveillance program in Atlantis. An ELISA for non-structural proteins (NSP) was applied to determine whether or not the animal had been exposed to Foot-and-Mouth disease virus. In cattle, this test has an average sensitivity of 88% and average specificity of 91%. Unfortunately, the test has not been validated for deer or moose.

Scenario TS2 Charts:

Chart 1: Number of cervids sampled and tested for Foot-and-Mouth disease virus, Atlantis 2013-2016

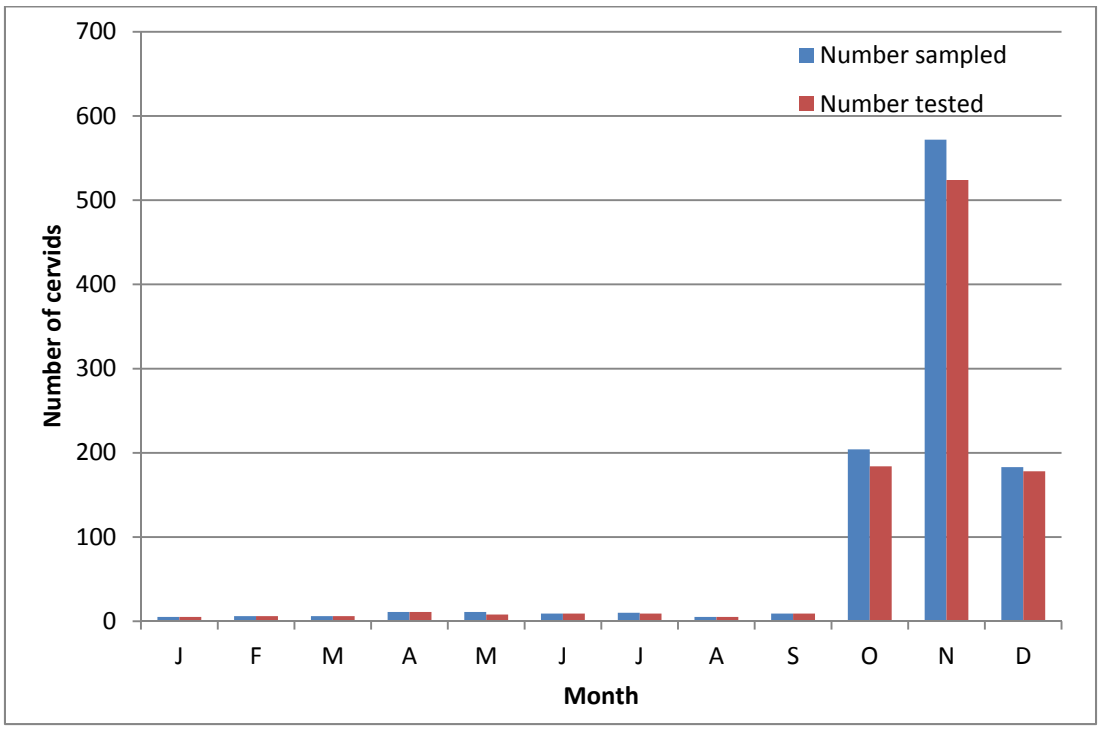


Chart 2: Number of animals submitted from each of the Atlantis Wildlife Management Areas by year

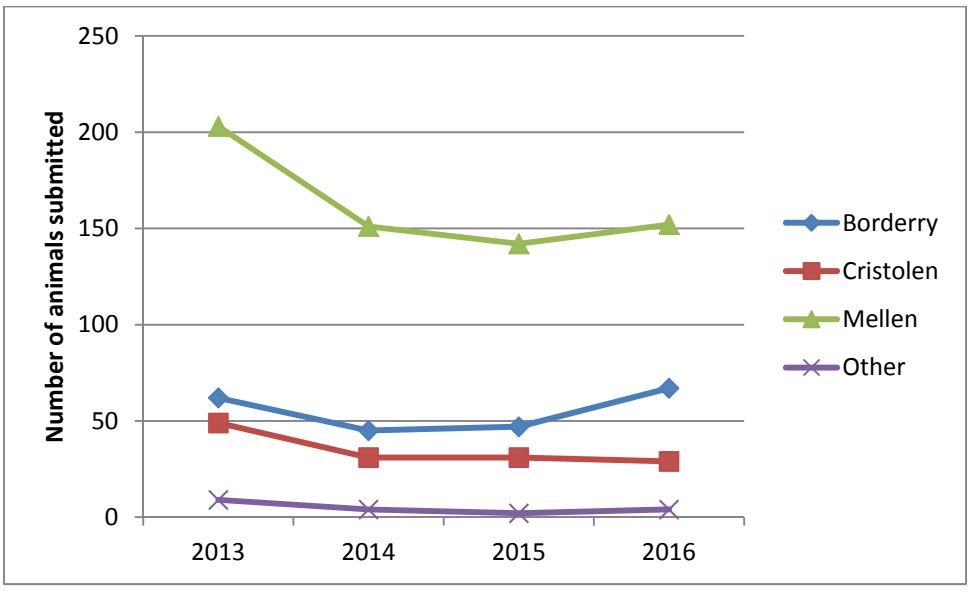


Chart 3: Proportion of cervids submitted for FMD testing by how they were found, 2013-2016

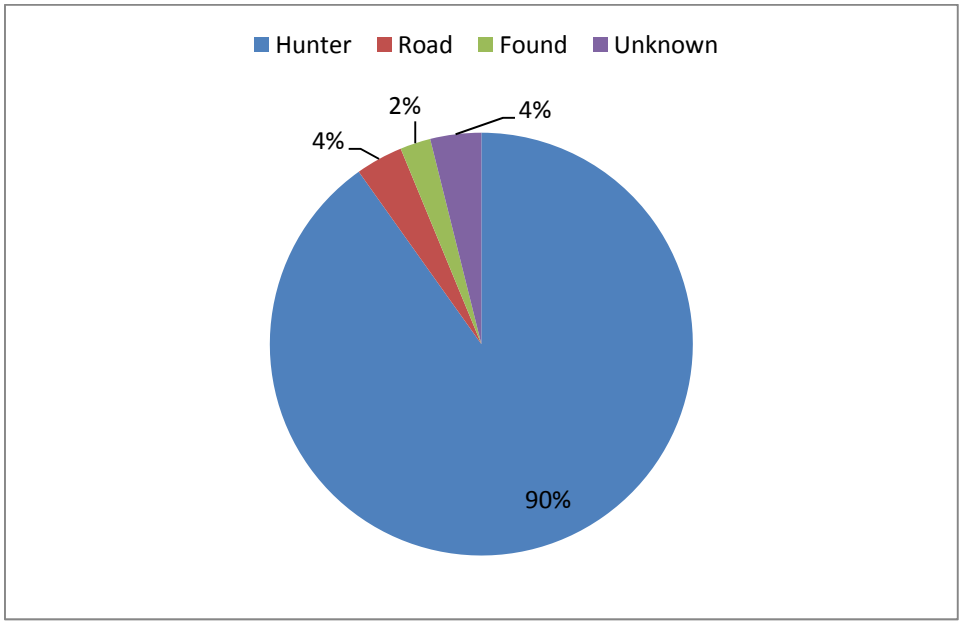
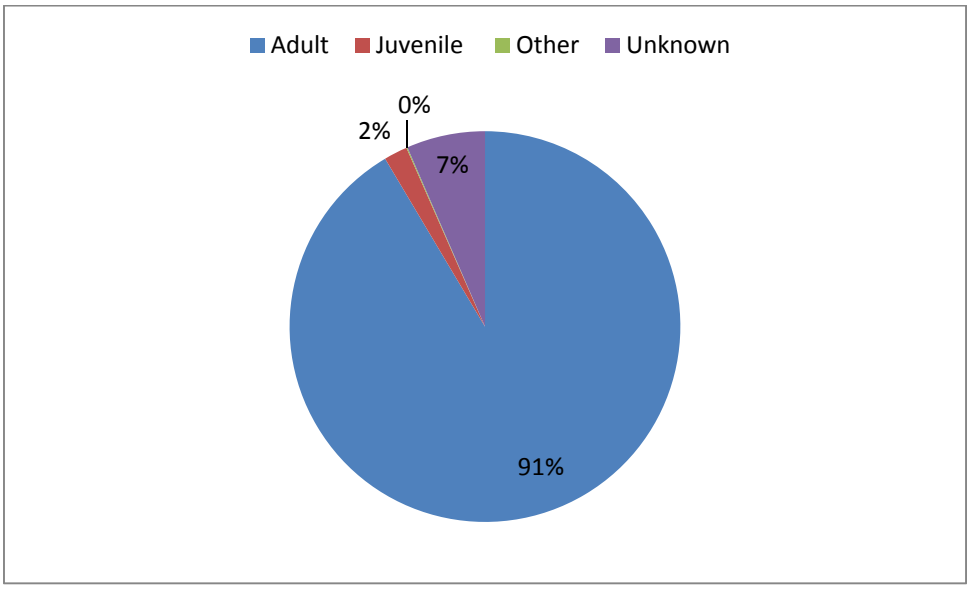
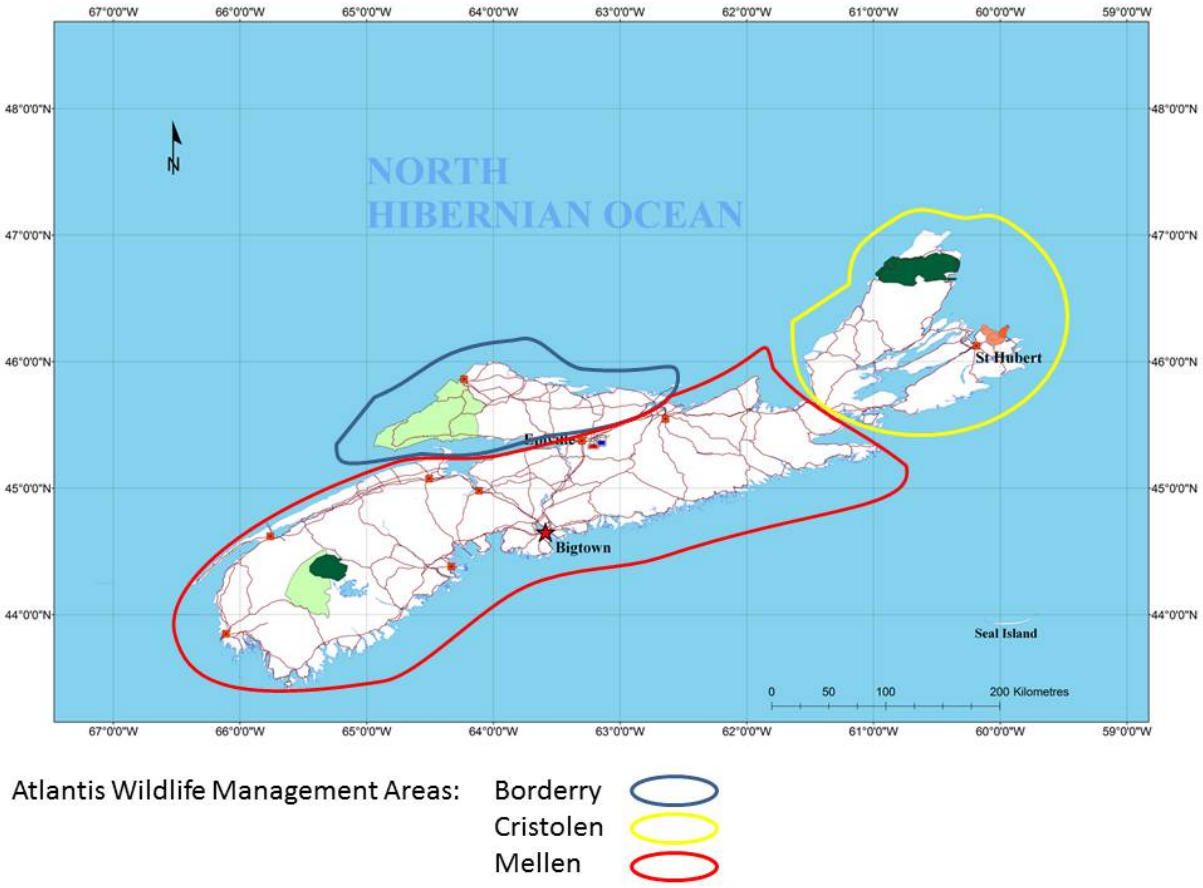


Chart 4: Proportion of cervids submitted for FMD testing by age category, 2013-2016

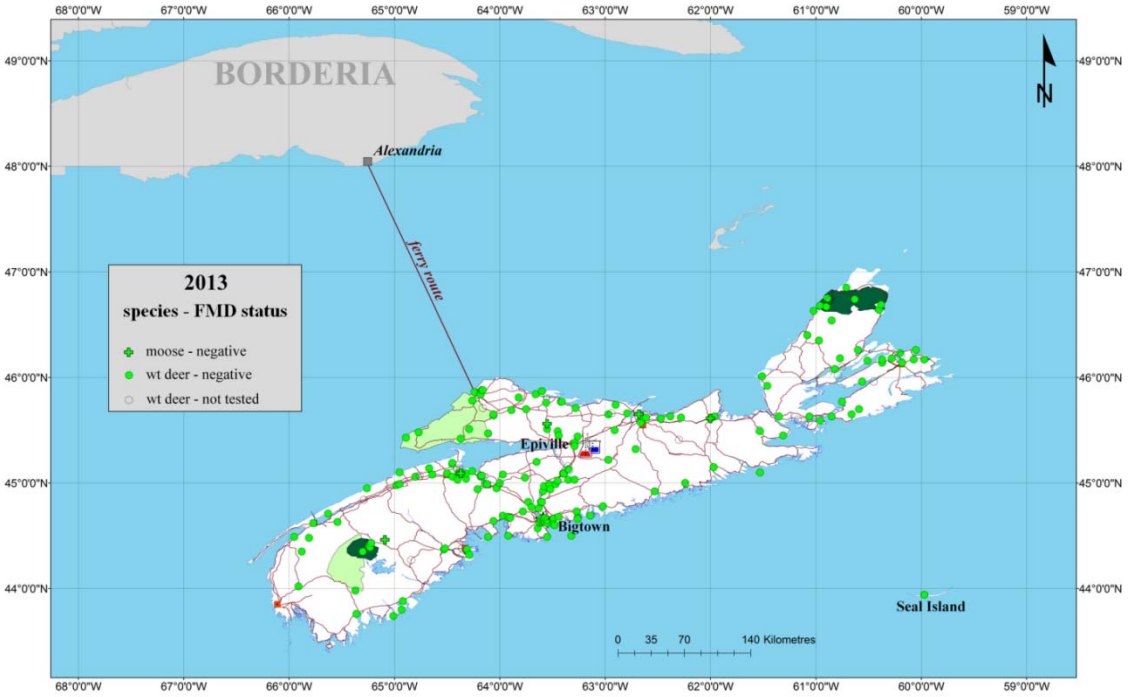


Scenario TS2 Maps:

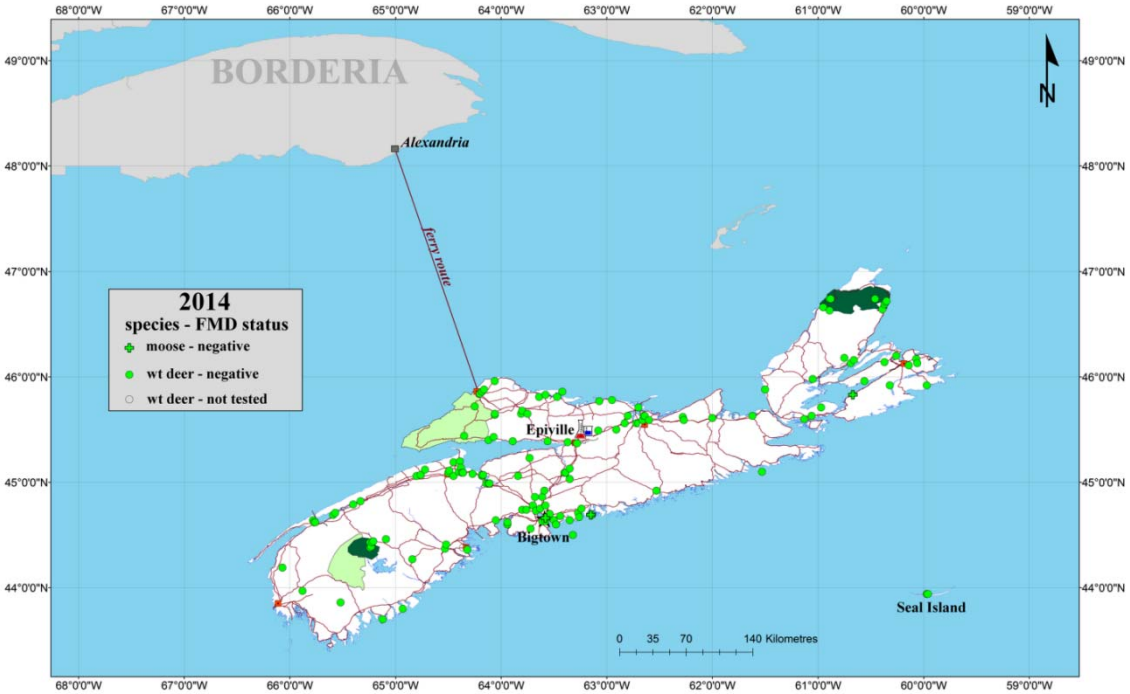
Map 1: The Wildlife Management Areas of Atlantis (2013)



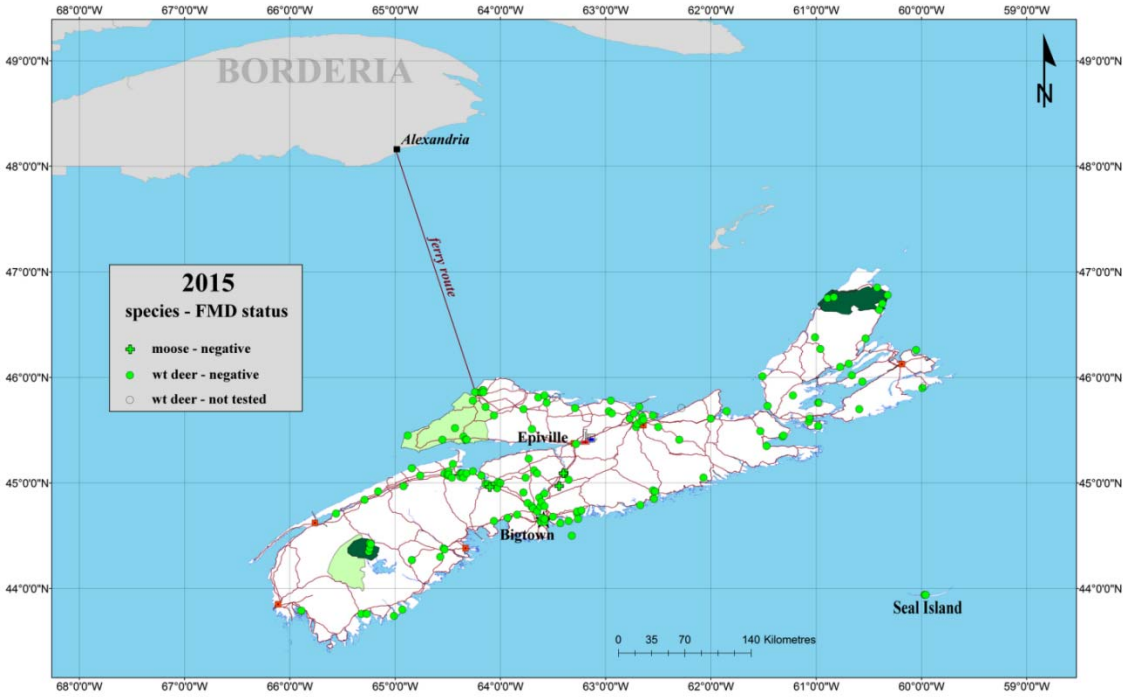
Map 2: Atlantis FMD surveillance results, 2013



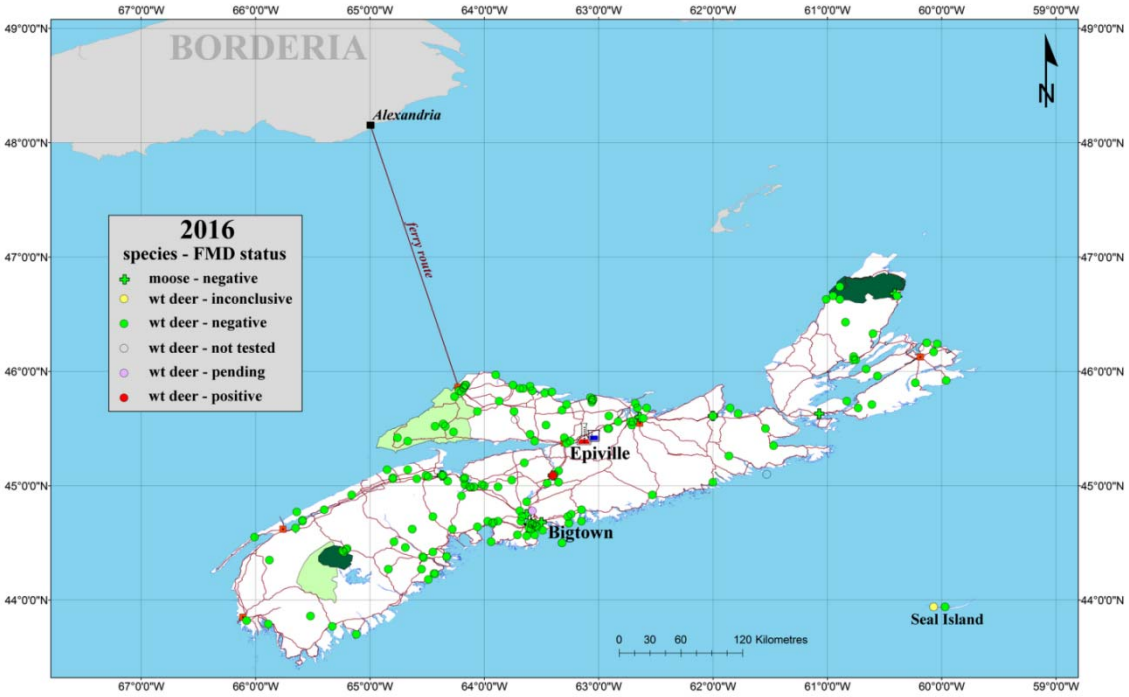
Map 3: Atlantis FMD surveillance results, 2014



Map 4: Atlantis FMD surveillance results, 2015



Map 5: Atlantis FMD surveillance results, 2016



Appendix E

Responses to General and Targeted wildlife surveillance activity questions

In this Appendix, you will find some responses to the questions posed in the exercises in the workbook. These responses are not intended to be comprehensive or the only acceptable responses. Rather, they are intended to permit readers who work through the exercises to assess their own evaluations against a set of responses that are broadly correct and reasonable.

ACTIVITY 1

1. Detection of Diseased or Dead Wild Animals: (Who can do this? How could it be organized?)

Government Agency Personnel:	Non-Government Groups
• *Natural Resources* • *Environment* • *Ocean Resources* • *Agriculture (would include veterinary service and various regional offices that might receive specimens, freeze them, ship them, etc.)* • *Aboriginal Government* • *Anguille Original People's Council*	• *Natural History Club* • *Fish and Game Association* • *Calliope International* • *National Fisherman's Union* • *National Farmer's Association* • *Universities – research scientists (biologists, ecologists, etc.)*

2. Identification of Pathogens and Diseases (Who can do this? How could it be organized?)

- *Ministry of Agriculture: National veterinary diagnostic laboratory*
- *Ministry of Health: National medical laboratory*
- *Ministry of Ocean Resources (often have specialized labs in food safety to detect marine toxins such as from harmful algal blooms*
- *University – Atlantis Veterinary College, likely to have a diagnostic laboratory and also research labs with special diagnostic capacities.*

3. Information Management (Who can do this? How could it be organized?)

- *Ministry of Agriculture, Veterinary Services*
  - *may already have a suitable data management system*
- *Ministry of Health*
  - *may already have a suitable data management system*
- *Central government Information Technology service*
  - *may already have a suitable data management system*
- *Universities*
  - *Veterinary college – may have capacity or expertise in diagnostic laboratory, faculty members in epidemiology.*
  - *Computer science departments*

4. Analysis and Communication (Who can do this? How could it be organized?)

- *The OIE Focal Point for Wildlife*
  - *Should play an important role here, either doing the analysis and communication directly or ensuring that it is done.*
- *Ministries of Agriculture (veterinary services), Health, Natural Resources, Environment and Ocean Resources all could participate and contribute expertise.*
- *Veterinary College faculty and staff also could participate*

ACTIVITY 2

*****For each question, the responses for Scenario 1 (Dataset G1) are provided first, followed by responses for Scenario 2 (Dataset G2).*****

What kinds of Errors can you find in the data?
Make a list of the kinds of errors or omissions you have found. Why do you think these different kinds of errors have occurred in the survey data?

Some examples of errors in the datasets for General wildlife disease surveillance are provided below:

Dataset G1 has 317 records - rows

- *Data missing:*
  - *Primary Diagnosis: 6 missing - 1.8%*
  - *Date Found – 48 missing – 15%*
  - *Latin name – 8 missing = 2.5%*
  - *Incorrect Locations – racoons in the ocean*
- *Incorrect Location information:*
  - *Negative Latitude – e.g. A2015-207.1 – longitude and latitude reversed*
  - *Positive Longitude – e.g. 11 records have positive longitude – data entry error*
- *Duplicate entries – same case number for each of three specimens shows the number dead to be 3, when each should have been entered as a single dead animal and three animals entered.*
  - *Bird A2015-100.1 is entered 4 times but is only 1 bird*
  - *A2015-30029.1 is entered 6 times (Primary Diagnosis is easy to spot – proventricular foreign body, sort by primary diagnosis)*
  - *Birds A2015-115.1 to 115.4 – each indicate 4 birds found dead but certainly are 4 individual specimens found together, but now are entered as 16 specimens*
  - *A2015-144.1 is used for two different specimens of different species*
  - *A2015-16.1 – used for 3 specimens, 2 different species.*
- *In addition, there are other similar mistakes here and there.*

Data Set G2 has 312 records – rows

- *Data missing:*
  - *Primary Diagnosis: none*
  - *Date Found – 50 missing – 16%*
  - *Latin name – 9 missing = 2.8%*
  - *Location (Lat/Long missing: 17 entries 5.5%*
  - *Incorrect Locations – racoon in the ocean*
- *Incorrect Location information:*
  - *Negative Latitude – 5 entries*
  - *Positive Longitudes – e.g. 34 records have positive longitude – data entry error*
- *Duplicate entries and similar mistakes:*
  - *Bird A2015-100.1 is used for 2 different specimens; so is A2105-144.1, and many others.*
  - *A2015-30029.1 is entered 6 times (Primary Diagnosis is easy to spot – proventricular foreign body, sort by primary diagnosis)*
  - *Birds A2015-115.1 to 115.4 – each indicate 4 birds found dead but certainly are 4 individual specimens found together, but now are entered as 16 specimens*
- *In addition, there are other similar mistakes here and there.*

Do you see any patterns in the Data?
Make a list of the kinds of patterns you have found
Can you explain why some of these patterns may have occurred?

Dataset G1 - Patterns Present:

- *Spatial clusters:*
  - *Around Bigtown - (where most people live)*
  - *Around Epiville - (location of veterinary diagnostic laboratory)*

- *Very non-uniform distribution of dead animals included in surveillance program – why might this be the case? (reflects human population to large extent)*

- *Clusters in Time*
  - *Some peaks of winter mortality: Reovirus, White Nose Syndrome*
  - *Otherwise, quite uniform throughout year*
  - *Same for Trichinellosis (hunting season), West Nile (mosquito season)*

Dataset G2 - Patterns Present:

- *Spatial clusters:*
  - *Minor cluster around Bigtown - (where most people live)*
  - *Notable cluster around Epiville - (location of veterinary diagnostic laboratory)*
  - *Two spatial clusters are associated with actual disease occurrences: Newcastle disease (east) and Morbillivirus (west of Bigtown)*
  - *Strong tendency to follow roads – (human activity)*
  - *More uniform distribution of dead animals included in surveillance program than in the other dataset (G1)*
  - *May reflect good use of regional government offices – wildlife officers distributed at multiple locations and good publicity so that there is general participation in disease detection by a network of people.*
- *Clusters in Time*
  - *Some peaks of winter mortality: Salmonella in song birds in spring*
  - *Outbreaks of Newcastle Disease and Morbillivirus are clustered in time. Note that Morbillivirus outbreak appears to be increasing at the end of the year, when the surveillance data end.*
  - *Same for Trichinellosis (hunting season), West Nile (mosquito season)*

Do you see any diseases or pathogens that strike you as particularly important for the *Dominion of Atlantis*?
List the pathogens and diseases you think may be important to Atlantis and note your reasons for thinking so.

Dataset G1 - Pathogens:

- *Some pathogens of potential Interest to participants/Atlantis may include:*
  - *Aleutian Disease – mink industry important in country*
  - *HP Avian Influenza – Poultry exports important to country*
  - *Baylisascaris – zoonosis*
  - *Lead and Mercury toxicity – possible sentinel for environmental contamination, food safety*
  - *Morbillivirus in seals, porpoises – conservation and tourism concern*
  - *Plague – zoonosis, tourism*
  - *Reovirus – virus new to science killing large numbers of wild birds, one outbreak is near St Hubert*
  - *Salmonella – seems very common in Redpolls*
  - *West Nile virus – zoonosis*
  - *White Nose Syndrome – conservation and control of agricultural insect pests.*
- *Mortality events in spatial clusters*
  - *The most obvious is Highly Pathogenic Avian Influenza*
  - ***Map G1B** shows the location of HPAI occurrences at the heart of the poultry industry near St. Hubert. Discussion could turn to the source of this HP AI virus, which almost certainly has come to wild birds FROM the poultry industry. There would surely have been en epidemic in the Atlantis poultry industry, probably well known to the Atlantis wildlife focal point, but that does not show up in the wildlife surveillance data.*
  - *This map also shows occurrence of a Low Pathogenicity AI virus (H7N3) at a different location.*
- *Mortality events clustered in time/season*
  - *Salmonella – nearly all cases occur in song birds in March*
  - *HP Avian Influenza – all cases found from 1 November to 15 December*
  - *Various others – less evident*
  - *Reovirus – 20 January to 6 February*

Dataset G2 - Pathogens:

- *Some pathogens of potential Interest to participants/Atlantis may include:*
  - *Aleutian Disease – mink industry important in country*
  - *H7 low pathogenicity Avian Influenza – must be reported, poultry exports important to country*

  - *Baylisascaris – zoonosis*
  - *Lead and Mercury toxicity – possible sentinel for environmental contamination, food safety*
  - *New-to-science Morbillivirus in White-tailed deer (wild ungulate) – potential emerging disease that might be like rinderpest or PPR*
  - *Plague – zoonosis, tourism*
  - *Major loss of marine wildlife to a harmful algal bloom – possible negative impact on tourism, commercial seafood harvest, human health*
  - *Salmonella – seems very common in Redpolls*
  - *West Nile virus – zoonosis*
  - *White Nose Syndrome – conservation and control of agricultural insect pests.*
- *Mortality events in spatial clusters:*
  - *Newcastle Disease in wild birds, not very far from St Hubert and concentration of poultry industry*
  - *New-to-science Morbillivirus in White-tailed deer (wild ungulate) – potential emerging disease that might be like rinderpest or PPR. This seems to have been detected first in dead dolphins on shore, and then to occur as a developing major epidemic in white-tailed deer (locally abundant wild ungulate)*
  - *Petroleum oil – a problem on sea coasts – suggests marine source of oil (shipping)*
- *Mortality events clustered in time/season (as noted above):*
  - *Newcastle Disease in wild birds, not very far from St Hubert and concentration of poultry industry*
  - *New-to-science Morbillivirus in White-tailed deer (wild ungulate) – potential emerging disease that might be like rinderpest or PPR. This seems to have been detected first in dead dolphins on shore, and then to occur as a developing major epidemic in white-tailed deer (locally abundant wild ungulate)*
  - *Same for Trichinellosis (hunting season), West Nile (mosquito season)*

Do you see any pathogens or disease occurrences that should be reported to the OIE?
List the pathogens and disease occurrences you find that would fall under each of these categories.

Dataset G1:
- *OIE List Pathogens:*
  - *Avian Influenza HP H5N1*
  - *Avian Influenza Low Path H7N3*
  - *Rabies*
  - *Trichinellosis*
  - *West Nile virus*
  - ***NOTE –** Trichomonosis in birds is NOT a listed disease. For the OIE, "Trichomonosis" refers to bovine genital infection only*
  - ***Potential Emerging Diseases**:*
    - *Reovirus*
- *OIE Non-list Wildlife Pathogens:*
  - *Avian Pox*
  - *Baylisascaris*
  - *Infection with Batrachochytrium dendrobatidis*
  - *Canine Distemper*
  - *Lead Poisoning (chemical)*
  - *Mercury Poisoning (chemical)*
  - *Morbillivirus in aquatic mammals*
  - *Organophosphate poisoning (chemical)*
  - *Pasteurellosis (P. multocida)*
  - *Petroleum Oil toxicity (chemical)*
  - *Plague (Y. pestis)*
  - *Sarcoptic Mange*
  - *Trichomonas sp.*
  - *White Nose Syndrome of bats*

Dataset G2:
- *OIE List Pathogens:*
  - *Newcastle Disease*
  - *Avian Influenza Low Path H7N3*

- *Rabies*
- *Trichinellosis*
- ***NOTE –** Trichomonosis in birds is NOT a listed disease. For the OIE, "Trichomonosis" refers to bovine genital infection only*
- ***Potential Emerging Diseases:***
  - *Morbillivirus epidemic in White-tailed deer*

- *OIE Non-list Wildlife Pathogens:*
  - *Avian Pox*
  - *Baylisascaris*
  - *Infection with Batrachochytrium dendrobatidis*
  - *Canine Distemper*
  - *Lead Poisoning (chemical)*
  - *Mercury Poisoning (chemical)*
  - *Morbillivirus in aquatic mammals*
  - *Organophosphate poisoning (chemical)*
  - *Pasteurellosis (P. multocida)*
  - *Petroleum Oil toxicity (chemical)*
  - *Plague (Y. pestis)*
  - *Sarcoptic Mange*
  - *Trichomonas sp.*
  - *White Nose Syndrome of bats*

Based on your review of the data, what IMPROVEMENTS do you think are needed in the surveillance program of the Dominion of Atlantis?
What improvements would you suggest?
How could Atlantis achieve the improvements you think are needed?

Some of the improvements that may be suggested include:

1. *Reducing the amount of missing data*
   - *How could Atlantis achieve this goal?*
     - *Training/education of personnel regarding data recording so everyone understands what data should be recorded in each data field.*
     - *Training on use of GPS or other was to determine latitude and longitude, and correct way to record these (positive and negative numbers, etc)*
     - *Forms to fill out for each specimen collected in the field, to make data collection uniform*
     - *Immediate follow-up with people who detected each incident with missing data to try to find those missing data – someone would have to be assigned to do this, and would have to review data for new entries very soon after the data were received.*

2. *Acquiring samples from places distant from Bigtown to achieve vigilance for disease occurrences and samples more uniformly across the country (Data set G1)*
   - *How could Atlantis achieve this goal?*
     - *Wider publicity about the surveillance program*
     - *Expansion of the network of people engaged in detection of sick or dead animals to include the whole country.*
     - *Better use of regional offices of the Ministries of Natural Resources, Agriculture, Ocean Resources, etc.*
     - *Engage participation of NGO, naturalist groups with active members in different regions.*

ACTIVITY 3

A. Low disease or pathogen prevalence – 2%

		True disease/pathogen status		Total	Predictive values
		+	-		
Diagnostic test result	+	*20*	*98*	*118*	*PPV=20/118=17%*
	-	*0*	*882*	*882*	*NPV=882/882=100%*
Total		*20*	*980*	1000	

True prevalence=2% **Apparent prevalence=12%**
Sensitivity=99%; Specificity=90%

B. High disease or pathogen prevalence – 40%

		True disease/pathogen status		Total	Predictive values
		+	-		
Diagnostic test result	+	*396*	*60*	*456*	*PPV=396/456=87%*
	-	*4*	*540*	*544*	*NPV=540/544=99.3%*
Total		*400*	*600*	1000	

True prevalence=40% **Apparent prevalence=46%**
Sensitivity=99%; Specificity=90%

C. Describe the effect of true prevalence on the interpretation of the test results.
What happened to the predictive values of the test when true prevalence was changed?
What happened to the apparent prevalence?

When the true prevalence increased (from 2% to 40%) the PPV increased from 17% to 87% and the NPV decreased very slightly (from 100% to 99.3%).

The apparent prevalence increased from 12% to 46% when the true prevalence increased from 2% to 40%.

*The take home message from this example – **the closer the true prevalence of disease is to 50%, the better diagnostic tests perform** (higher predictive values overall and better agreement between the true and apparent prevalence).*

ACTIVITY 4

A. Low Sensitivity

		True disease/pathogen status		Total	Predictive values
		+	-		
Diagnostic test result	+	*80*	*90*	*170*	*PPV=80/170=47%*
	-	*20*	*810*	*830*	*NPV=810/830=97.6%*
Total		*100*	*900*	1000	

True prevalence=10% ***Apparent prevalence=17%***
Sensitivity=80%; Specificity=90%

B. High Specificity

		True disease/pathogen status		Total	Predictive values
		+	-		
Diagnostic test result	+	*80*	*9*	*89*	*PPV=80/89=90%*
	-	*20*	*891*	*911*	*NPV=891/911=97.8%*
Total		*100*	*900*	1000	

True prevalence=10% ***Apparent prevalence=9%***
Sensitivity=80%; Specificity=99%

The effect of reducing the sensitivity is more marked at higher disease/pathogen prevalence; try redoing the calculations with prevalence at 40% and see what happens.

C. What happened to the predictive values of the test when the specificity increased?

When the specificity of the test was increased from 90% to 99%, the PPV increased from 47% to 90% and there was very little change in the NPV. If the sensitivity was increased instead, the NPV (already high) would be even higher.

*The higher the sensitivity the more likely it is that the animal really is **NOT** infected if it tests negative (high sensitivity rules out infection – high NPV);*

*The higher the specificity, the more likely it is that the animal really **IS** infected if it tests positive (high specificity rules in infection – high PPV).*

D. There are times when tests with higher sensitivity are preferred and other times when higher specificity is needed. Propose some examples of surveillance objectives when tests with higher sensitivity, or with higher specificity, would be advantageous.

High sensitivity: When you don't want to miss any infected animals; tests with high sensitivity provide very few false negative results.
Examples:
- *detecting important notifiable diseases*
- *demonstrating freedom or detect infection*

High specificity: these tests provide very few false positive results
Examples:
- *determining disease prevalence*
- *identifying disease trends.*

ACTIVITY 5

For each question, the responses for Scenario TS1 (Rabies) are provided first followed by responses for Scenario TS2 (FMD).

1. What errors or inconsistencies did you find in the datasets?

Scenario 1 (Rabies)

- *Inconsistent entry for test result – Negative and Positive entered for samples received late in 2016; Neg and Pos entered for samples received earlier*
- *Incorrect and missing dates*
- *Missing information about how the animals were found and their age category*

Scenario 2 (FMD)

- *Inconsistent entry for sex – M and F in 2013; Male and Female in other years*
- *Incorrect and missing dates - especially years (e.g. there are entries for 2011, 2012, and 2051)*
- *Incorrect locations – refer to points in the sea on the map*
- *Missing information about how the animals were found*
- *Missing test results*

2. What patterns are present in the data? (Spatial, temporal, other...)

Scenario 1 (Rabies)

- *Spatial (refer to maps): only animals in south-west Atlantis were included in surveillance (within 150km of Kejimkujak)*
- *Temporal (refer to charts): both species caught throughout the year; more coyotes tested than raccoons earlier in the surveillance program (2015), some spikes in animal submissions.*

Scenario 2 (FMD)

- *Spatial (refer to maps and charts): most animals were from Mellen WMA; very few from the other WMAs in Atlantis*
- *Temporal (refer to charts): most animals caught and tested in the fall of each year (Oct-Dec). Consider how this could affect the surveillance program timeliness.*

3. Do you have all the data that you need to interpret the information provided and meet the stated objectives of the targeted surveillance program? If not, make a list of the additional information that is needed.

Scenario 1 (Rabies)

- *There are no good data for either coyote or raccoon populations in Atlantis. Both are considered to have large and increasing populations, likely over 100000 each*
- *Test sensitivity and specificity are provided in the background document.*
- *Currently no rabies control or vaccination program in Atlantis.*

Scenario 2 (FMD)

- *Population of deer and moose in Atlantis and in 3 of WMAs are provided in the background sheet*
- *Test sensitivity and specificity (for ELISA in cattle) are also provided.*
- *FMD vaccination is not practiced in Atlantis.*

4. Are there any important biases that would affect how you interpret the targeted surveillance data results? Describe how the data might be biased and if this is a problem or not.

Scenario 1 (Rabies)

- *A large proportion of the animals included in the rabies surveillance program were found dead. These animals may or may not be representative of the general raccoon or coyote population. Consider how this could impact the accuracy of the prevalence estimates (if these animals are more likely to be*

infected then we may overestimate the prevalence of rabies in the population). Consider if age or sex could affect the detection of rabies (unlikely).

- *It is also important to note that there were a few test positive animals collected quite close to the surveillance area boundary. It may be necessary to extend the surveillance area to ensure that no positive animals were missed and that the new rabies strain has not already spread further into Atlantis.*

Scenario 2 (FMD)

- *Because most of the samples come from hunted animals, a greater proportion of the animals tested are male and most are adults. How could sex and age affect the surveillance results? FMD virus should not preferentially infect and age or sex group so this is unlikely to bias the results.*
- *However, the hunted animals are more likely to be disease-free than the general population because hunters tend to select the larger and "healthier" animals. This selection bias reduces the likelihood that the animal is infected with FMD virus and thus can affect the predictive value of the diagnostic tests used. In this situation, the true prevalence is believed to be very low (close to 0) so this is unlikely to bias the surveillance results to date.*

5. What are the important surveillance findings? How should these findings be reported to the OIE? Who else should be informed about the surveillance findings?

Scenario 1 (Rabies)

- *A new coyote-adapted strain of rabies has been identified in 11 coyotes and 2 raccoons in 2015-2016. In 2015: 7/49 (8.9%) coyotes tested positive; in 2016: 4/115 (3.5%) coyotes and 2/141 (1.4%) of raccoons tested positive.*
- *Rabies is an OIE-listed disease and this is a new strain. According to the terrestrial animal health code, "the first occurrence of a new strain of a pathogen of a listed disease in a country" must be provided to OIE within 24 hours (see article 1.1.3 and the rabies specific chapter for further discussion).*
- *Rabies is an important zoonotic pathogen and as such ministries responsible for public health and agriculture need to be made aware that a new variant of rabies is circulating in Atlantis.*

Scenario 2 (FMD)

- *1 deer tested positive for FMD virus in 2016 and another had an inclusive test result.*
- *Atlantis is an FMD-free country where vaccination is not practiced. In the years prior to 2016, FMD was not detected and the information from the targeted surveillance program describing which susceptible animals were tested, how many and what the test results were needs to be provided to the OIE annually, along with other information in the FMD chapter of the Terrestrial Animal Health Code.*
- *In 2016, 1 animal tested positive and a second one had an inconclusive test result. Prior to formal notification, these test results should be confirmed with additional testing. Should it be determined that the positive test result is truly positive, the OIE must be notified within 24 hours as FMD is a listed disease and this would be the first occurrence in Atlantis.*
- *Although the case of FMD in 2016 was observed in a wild animal, FMD is an important infectious disease of livestock; the Ministry of Agriculture must be informed.*

ACTIVITY 6

For each question, the responses for Scenario 1 (Rabies) are provided first followed by responses for Scenario 2 (FMD)

Are the data gathered through the program adequate to meet the stated objectives?

Scenario 1 (Rabies)

- *Refer to objectives in background document.*
- *Using Ausvet, how many animals needed to be included in the surveillance program to estimate the prevalence of rabies in the population? Refer to the background documents for the estimated prevalence in raccoons and coyotes and the test characteristics. Calculate the sample size and compare this to the number of samples tested:*
    - *Coyote: estimated prevalence is 10%; desired precision is 5% - sample size needed is 169 animals. Raccoons: estimated prevalence is 2%; desired precision is 5% - sample size needed is 58 animals*
    - *** Consider whether these numbers should be sample each year or over the full 2 years presented here.*

- *Based on these results, the data are adequate to meet the stated objectives over the 2 year study period. Additional animals are needed to achieve the desired level of precision for the prevalence in coyotes if the estimates are done by year.*
- *The data do not enable Atlantis to know the distribution over the entire country; only for the area from which animals were collected.*

Scenario 2 (FMD)

- *The purpose of the surveillance program was to detect Foot-and-Mouth disease virus if it occurred in wildlife in Atlantis. Refer to objectives in background document. Based on the dataset, Table 1 (below) can be created.*

Table 1: FMD testing of wild cervids from Atlantis by year

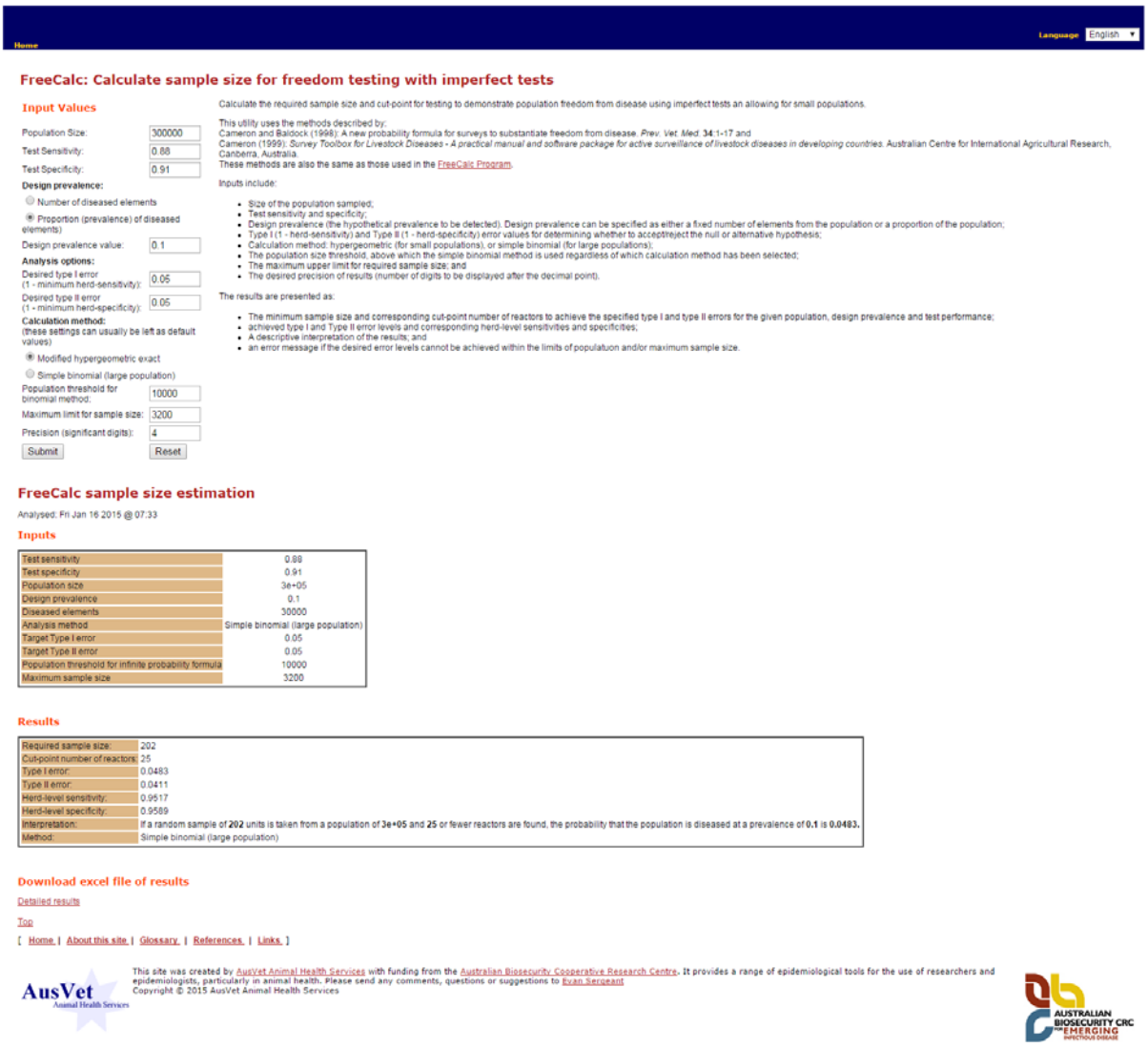
Year	Epiville Lab	
	# Samples submitted	# Samples tested[1]
2013	323	274
2014	231	221
2015	222	212
2016	252	244
Total	1028	951

[1] *Where the number of samples tested is less than the number submitted, the tissue samples received by the lab were not adequate for testing purposes.*

- *Using Ausvet, you can enter different prevalence estimates to see if the level of sampling in Atlantis was adequate to meet the surveillance program objectives.*

- *Below is one example from Ausvet assuming that the test has the same characteristics in deer as it does in cattle (test sensitivity of 88% and specificity of 91%). A prevalence to be detected of 10% was entered and the population entered was an estimate of the entire deer population (including moose) in Atlantis. From the results, the required sample size was 202 animals and up to 25 animals could test positive and you would still be >95% confident that Atlantis was free of FMD. The sample size in each of the 4 years is adequate to meet the stated objectives.*

    - *With the imperfect diagnostic test, up to 25 animals could test positive and you would still be 95% confident that, if present, the prevalence of FMD was less than 10%. However, the positive and inconclusive test results should still be further evaluated to help confirm that these are truly false positive results.*



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Language English

FreeCalc: Calculate sample size for freedom testing with imperfect tests

Input Values

Population Size: 300000
Test Sensitivity: 0.88
Test Specificity: 0.91
Design prevalence:
Number of diseased elements
Proportion (prevalence) of diseased elements)
Design prevalence value: 0.1
Analysis options:
Desired type I error (1 - minimum herd-sensitivity): 0.05
Desired type II error (1 - minimum herd-specificity): 0.05
Calculation method: (these settings can usually be left as default values)
Modified hypergeometric exact
Simple binomial (large population)
Population threshold for binomial method: 10000
Maximum limit for sample size: 3200
Precision (significant digits): 4
Submit Reset

Calculate the required sample size and cut-point for testing to demonstrate population freedom from disease using imperfect tests an allowing for small populations.

This utility uses the methods described by:
Cameron and Baldock (1998): A new probability formula for surveys to substantiate freedom from disease. *Prev. Vet. Med.* 34:1-17 and
Cameron (1999): *Survey Toolbox for Livestock Diseases - A practical manual and software package for active surveillance of livestock diseases in developing countries*. Australian Centre for International Agricultural Research, Canberra, Australia.
These methods are also the same as those used in the FreeCalc Program.

Inputs include:

- Size of the population sampled;
- Test sensitivity and specificity;
- Design prevalence (the hypothetical prevalence to be detected). Design prevalence can be specified as either a fixed number of elements from the population or a proportion of the population;
- Type I (1 - herd-sensitivity) and Type II (1 - herd-specificity) error values for determining whether to accept/reject the null or alternative hypothesis;
- Calculation method: hypergeometric (for small populations), or simple binomial (for large populations);
- The population size threshold, above which the simple binomial method is used regardless of which calculation method has been selected;
- The maximum upper limit for required sample size; and
- The desired precision of results (number of digits to be displayed after the decimal point).

The results are presented as:

- The minimum sample size and corresponding cut-point number of reactors to achieve the specified type I and type II errors for the given population, design prevalence and test performance;
- achieved type I and Type II error levels and corresponding herd-level sensitivities and specificities;
- A descriptive interpretation of the results; and
- an error message if the desired error levels cannot be achieved within the limits of population and/or maximum sample size.

FreeCalc sample size estimation

Analysed: Fri Jan 16 2015 @ 07:33

Inputs

Test sensitivity	0.88
Test specificity	0.91
Population size	3e+05
Design prevalence	0.1
Diseased elements	30000
Analysis method	Simple binomial (large population)
Target Type I error	0.05
Target Type II error	0.05
Population threshold for infinite probability formula	10000
Maximum sample size	3200

Results

Required sample size:	202
Cut-point number of reactors:	25
Type I error:	0.0483
Type II error:	0.0411
Herd-level sensitivity:	0.9517
Herd-level specificity:	0.9589
Interpretation:	If a random sample of **202** units is taken from a population of **3e+05** and **25** or fewer reactors are found, the probability that the population is diseased at a prevalence of **0.1** is **0.0483.**
Method:	Simple binomial (large population)

Download excel file of results

Detailed results

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AusVet Animal Health Services

This site was created by AusVet Animal Health Services with funding from the Australian Biosecurity Cooperative Research Centre. It provides a range of epidemiological tools for the use of researchers and epidemiologists, particularly in animal health. Please send any comments, questions or suggestions to Evan Sergeant
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AUSTRALIAN BIOSECURITY CRC for EMERGING INFECTIOUS DISEASES

- *Perhaps you would prefer that the prevalence was lower than 10%. If you entered a prevalence of 5% instead, the required sample size would be 703 animals and up to 76 could test positive (screen shot not shown). In this case, the sample size for each year is not adequate but over the 4 years together, the sample target is achieved.*
- *However, it is very possible that the test works differently in deer than cattle. If you assumed a perfect test, just 59 animals would have to be tested (with no reactors) to be 95% confident that, if present in Atlantis, the prevalence of FMD was less than 5% (screen shot not shown). In this situation, the annual sample number is adequate to meet the stated objective. However, with 1 positive reactor in 2016, Atlantis can no longer be confident that it remains free from FMD. It would be helpful if the test was validated for use in wild deer or if a different type of test with less potential variation between species was available.*
- *Regardless, both the positive reactor and the animal with the inconclusive test result should be further investigated and additional testing is recommended. If a validated test is available it should be applied; if not, another test that may exhibit less variation in performance across species should be used.*

How would you improve the program?

Scenario 1 (Rabies)

- *Carry out surveillance across all of Atlantis*
- *Include additional susceptible wild animal species*
- *Link wildlife rabies surveillance data to surveillance done in domestic animals*

- *Establish a threshold for implementation of the control program ahead of time*

Scenario 2 (FMD)

- *In Atlantis, most of the moose and deer tested have come from the Mellen WMA (63%). If wild moose and deer infected with FMD are able to move freely, they are most likely to arrive in Atlantis from Borduria and therefore arrive first on the west coast of Atlantis (in Borderry). If the west coast is considered highest risk for introduction from Borduria, it might be most appropriate to focus surveillance activities there.*
- *Since 2013, the level of wild deer and moose testing in Atlantis has been adequate to detect FMD virus at a prevalence of 5% (with 95% confidence – assuming that the test sensitivity and specificity estimates are appropriate for wild deer) . But the 1 positive (and 1 inconclusive) test result in 2016 are cause for concern and require further testing, investigation and follow-up. A validated test for use in wild deer would greatly improve the program.*
- *Because wild boar are highly susceptible to FMD, it would be worth developing a plan to incorporate wild boar into the surveillance program.*

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