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FOREWORD

The Canadian Cooperative Wildlife Health Centre (CCWHC) is a partnership among Canada's colleges of veterinary medicine and a wide range of government agencies and non-government groups to apply the biomedical sciences to wildlife health and disease (see www.ccwhc.ca) An important purpose of the CCWHC is to promote and support the investigation and diagnosis of disease occurrences in free-living wild animals across Canada. To fulfill this mandate, CCWHC works cooperatively with scientists in many other organizations and draws on expertise throughout Canada. CCWHC maintains a national archive of information on the occurrence of diseases in Canadian wildlife. CCWHC has six regional centres located in Charlottetown, Prince Edward Island; Saint-Hyacinthe, Quebec; Guelph, Ontario; Saskatoon, Saskatchewan; Calgary, Alberta and Nanaimo, British Columbia. The Headquarters office is located at the University of Saskatchewan in Saskatoon. Contact information for each of these Centres is provided on pages 48 and 49 of this Manual. If you would like additional information regarding CCWHC, please call the National Information Line (1-800-567-2033) or visit our website at www.ccwhc.ca

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1. INTRODUCTION

1.1 Purpose of the Manual

This manual is intended as a reference for people investigating wildlife mortality events or illness. **Its primary objective is to ensure that appropriate samples are collected in the field, and that these reach a diagnostic laboratory in suitable condition for detailed examination.** This will ensure an optimal chance to determine the cause and nature of the disease and to assess significance.

It is impossible to provide detailed guidelines that will be appropriate for all circumstances; our intent is to provide general directions that can be modified to fit the circumstances. **Whenever possible, the laboratory that will be examining the specimens or the regional contact (section 3.0), should be contacted before the investigation begins.**

This will establish what type of samples and information are most appropriate, how these should be collected, stored and transported, and how many specimens can be examined. It will also alert the laboratory, so that the personnel can make any special preparations necessary, before the specimens arrive.

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What is Disease?

Since this manual deals with the term ***disease*** repeatedly, it is appropriate to define what we mean. In wild animals, disease is sometimes thought of only in terms of dead animals. However, **death is not a suitable endpoint for distinguishing between health and disease** because many sub-lethal conditions that impair behaviour, growth, maturation, migration, reproduction, and other functions may have important population consequences without directly causing death.

Disease may be caused by infection with some living parasite or pathogen, but non-infectious agents, such as poisons, lightning, heat, cold, nutrition, and environmental conditions also are causes of disease. Diseases often have multiple causes, for example environmental stress, poor nutrition and infection. Thus, a disease investigation must try to gather information on many different components that may contribute to the "cause" of the disease.

Thus, "disease" includes **the effects of any factor that interferes with the normal functions of animals.** These factors may include the effects of infectious agents, poisons, nutrition, weather, aging, genetics, and combinations of these factors. Because disease is often complex, specialists in many disciplines may be required for its understanding; however, the process usually begins with examination of specimens and the circumstances under which the specimens (dead animals most often) were found.

1.2 IDENTIFYING A DISEASE

How does one identify a disease? A "disease" is a complicated mixture of cause and effect; some disease-causing agent has produced an effect on an animal that we recognize as illness or death. The causal agent might be an infectious pathogen or parasite, a physical injury, a problem with nutrition, a natural or man-made poison, a spontaneous cancer, or an electric shock. The effect on the animal may be death, general debility, dysfunction

of a particular body organ or system like the digestive system, kidney or brain, or something very subtle, such as reduced reproduction in the affected population.

Quite often, the ultimate cause of a disease is an environmental change. For example, a lake that normally is safe to drink suddenly kills all animals that drink from it because of a bloom of toxic algae, or a virus that normally does not cause disease in fish because it only grows well at high temperature suddenly causes a fish-kill when water becomes unusually warm.

Thus, correctly identifying a disease - its cause and its effect on the animal - requires a disciplined and step-wise approach.

•Step I: FSI - Field Scene Investigation

The key to a successful disease investigation often is the information gathered at the place the diseased animal is found by the people who find it. This

information, together with specimens collected at the scene, guide all further examinations, laboratory tests, conclusions and interpretations. **Notes should be taken of the general environment and habitat, numbers and species of animals affected and unaffected by disease, signs of disease seen in live affected animals, and other circumstances associated with the disease event.** This information should be passed along, together with specimens of dead of sick animals, to a laboratory participating in Canada's wildlife disease surveillance program (see next section for contact information). Section 6 of this manual provides guidelines for gathering field scene information.

- **Step II: Collection and Shipment of Samples to a Laboratory (see section 5 – pages 26-50)**

- **Step III: General Examination of the Specimens**

  The next step is examination of the

specimens in a general manner. If live animals are available, this might include physical examination and collection of specimens, such as blood, for laboratory examination. Necropsy (autopsy) or post-mortem examination is the most common form of general examination used, and involves a detailed examination of all body organs for abnormalities.

•Step IV: Specific Laboratory Tests

The fourth step is to apply specific laboratory tests to identify causative agents or to confirm the nature of abnormalities seen at necropsy. The choice of which tests to use is based on the circumstances of the mortality event and the necropsy findings, and might include microscopic examination of tissues, culturing for bacteria, viruses or fungi, examination for parasites, toxicological and/or other analyses. In general, the most likely causes are searched for first, low cost tests are used first, and common causative factors are ruled in or out before searching for unusual causes. Because more than

one disease or cause may be present, several tests may be necessary in a single case. Throughout the examination and testing procedure, some specimens are retained in a form suitable for other tests (usually frozen) until the need for such specimens has been eliminated.

- **Step V: Drawing Conclusions**

The last phase of the diagnostic process is to relate findings from the necropsy and subsequent tests to each other to identify **what** disease was present, and then to relate this diagnosis back to the field situation to explain **why** disease occurred under the circumstance prevailing at the time. The information and specimens submitted to the laboratory determine the direction that a laboratory investigation will take and are critical to its eventual outcome. Understanding why a disease event occurred usually requires input from both the laboratory scientist and field personnel.

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2. WHO TO CONTACT FOR ASSISTANCE

The Canadian Cooperative Wildlife Health Centre works with government agencies and others to determine the causes of disease in wild animals in Canada and to apply this information to support public health, animal health and wildlife conservation and management programs.

The CCWHC will assist government agencies and others with the field investigation of wildlife disease occurrences. Arrangements for assistance can be made by contacting either the nearest CCWHC Regional Centre (see listing below) or the National Information Line (1-800-567-2033).

An extensive listing of persons with expertise in wildlife health in Canada has been compiled by CCWHC Headquarters Office (1-800-567-2033). CCWHC will refer inquiries to others when appropriate and consults a wide range of specialists in carrying out its own investigations.

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A list of contacts for assistance in responding to diseased wildlife in each province and territory is provided below.

In British Columbia

Primary Contact: Dr. Helen Schwantje, Provincial Wildlife Veterinarian, Ecosystems Branch, BC Ministry of Environment
Ph: (250) 953-4285 Fax: (250) 356-9145
Email: Helen.Schwantje@gov.bc.ca

In Alberta

Primary Contact: Dr. Margo Pybus, Provincial Wildlife Disease Specialist, Non-game and Wildlife Disease Division, Department of Sustainable Resource Development, 6909 116th St., Edmonton AB T6H 4P2
Ph: (780) 427-3462 Fax: (780) 422-9685
Email: margo.pybus@gov.ab.ca.
For information related to forensic cases, contact B. McClymont at the same address and fax number. Ph: (780) 422-3196 Email: bob.mclymont@gov.ab.ca

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In Saskatchewan

Primary Contact: CCWHC Western/ Northern Regional Centre, Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon SK S7N 5B4
Ph: (306) 966-5815 Fax: (306) 966-7439
Email: ccwhcwesternnorthern@usask.ca

In Manitoba

Primary Contact: Dr. Vince Crichton, Manager, Game, Fur and Problem Wildlife, Manitoba Conservation, Wildlife and Ecosystem Protection Branch, P.O. Box 24, 200 Saulteaux Crescent, Winnipeg MB R3J 3W3
Ph: (204) 945-6815 Fax: (204) 945-3077
Email: Vince.Crichton@gov.mb.ca

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In the Yukon:

Primary Contact: Philip Merchant,
Fish and Wildlife Branch Laboratory,
Department of Environment, Government of Yukon, P.O. Box 2703, Whitehorse YT Y1A 2C6
Ph: (867) 667-5285 Fax: (867) 393-6263
Email: Philip.Merchant@gov.yk.ca

In the Northwest Territories:

Primary Contact: Dr. Brett Elkin,
Wildlife Veterinarian, Wildlife Division,
Government of Northwest Territories
Environment and Natural Resources,
600, 5102 - 50th Avenue, Yellowknife NT X1A 3S8
Ph: (867) 873-7761 Fax: (867) 873-0293
Email: Brett_Elkin@gov.nt.ca

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In Nunavut:

Primary Contact: CCWHC Ontario/
Nunavut Regional Centre, Ontario
Veterinary College, University of Guelph,
Guelph ON N1G 2W1
Ph: (519) 824-4120 ext. 54662
Fax: (519) 821-7520
Email: ccwhc@ovc.uoguelph.ca

In Ontario:

Primary Contact: CCWHC Ontario/
Nunavut Regional Centre, Ontario
Veterinary College, University of Guelph,
Guelph ON N1G 2W1
Ph: (519) 824-4120 ext. 54662
Fax: (519) 821-7520
Email: ccwhc@ovc.uoguelph.ca

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In Québec:

Primary Contact: CCWHC Québec Regional Centre, Faculté de Médecine vétérinaire, Université de Montréal, 3200 rue Sicotte, Saint-Hyacinthe QC J2S 2M2 Ph: (450) 773-8521 ext. 8346
Fax: (450) 778-8116.
Email: kathleen.brown@umontreal.ca

Atlantic Provinces – CCWHC

Disease conditions in any of the Atlantic provinces can be reported to the CCWHC Atlantic Regional Centre, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown PE C1A 4P3
Ph: (902) 628-4314 Fax: (902) 566-0871
Email: atlantic@ccwhc.ca

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In New Brunswick:

Primary Contact: Dr. James Goltz,
Department of Agriculture & Aquaculture,
Agricultural Research Station
(Experimental Farm), P.O. Box 6000, 850
Lincoln Rd., Fredericton NB E3B 5H1
Ph: (506) 453-5488 Fax: (506) 453-7918
Email: jim.goltz@gnb.ca

In Nova Scotia

Primary Contact: Department of
Agriculture & Fisheries, Veterinary
Pathology Laboratory Services, Hancock
Building, P.O. Box 550, 65 River Rd.,
Truro NS B2N 5E3
Ph: (902) 893-6526 Fax: (902) 895-6684

In Prince Edward Island

Primary Contact: CCWHC Atlantic
Regional Centre, Atlantic Veterinary
College, University of Prince Edward
Island, 550 University Avenue,
Charlottetown PE C1A 4P3
Ph: (902) 628-4314 Fax: (902) 566-0871
Email: atlantic@ccwhc.ca

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In Newfoundland and Labrador

Primary Contact: Animal Health Division, Department of Forestry and Agriculture, PO Box 7400, St. John's NL A1E 3Y5
Ph: (709) 729-6879 Fax: (709) 729-6046

Environment Canada

Environment Canada has special responsibilities and expertise with respect to diseases affecting birds included in the Migratory Birds Convention Act and for all species included in the Species at Risk Act (endangered species).

Environment Canada's wildlife disease specialist is: Dr. Catherine Soos, Wildlife Disease Specialist, Environment Canada, 115 Perimeter Road or 115 Veterinary Road, Saskatoon SK S7N 0X4
Ph: (306) 975-5357 Fax: (306) 975-4089
Email: Catherine.soos@ec.gc.ca

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Parks Canada

Parks Canada can and should be consulted when disease events occur within or near national parks. Parks Canada's wildlife health specialist is:

Dr. Todd Shury, Parks Canada c/o Veterinary Pathology, WCVM, 52 Campus Drive, University of Saskatchewan, Saskatoon SK S7N 5B4
Ph: (306) 966-2930 Fax: (306) 966-7439
Email: Todd.Shury@pc.gc.ca

3. RESPONDING TO DISEASED WILDLIFE

3.1 Respond Immediately

It is important to collect information and specimens as soon as possible after a disease occurrence has been recognized. Sick and dead animals are quickly removed by predators and scavengers, or by decay. If investigation is delayed, it may become impossible to determine the extent and the cause of the disease event.

3.2 Questions to Ask the Caller

The initial report of diseased animals often comes as a telephone call from a member of the public. It is important to collect certain basic information from the caller that can be used to decide if an investigation is necessary, and, if so, what type of investigation is appropriate. This basic information should include:

- **Who is reporting the occurrence? Name, address, telephone number, agency affiliation if any, etc.**

- **What animals are involved?**

Species, number sick or dead, ages, sexes, behavior or other signs of illness, species and numbers that do not seem affected.

- **Where is the incident located?**

General area, precise location of affected animals (latitude/longitude, legal land location, etc.), land owner's name and telephone number, access to the site,

general description of the site in terms of land forms, habitat, and surrounding area.

- When did the event occur?

When were sick or dead animals first observed? Is the event newly occurred, or on-going from some earlier start date? What is known about the timing of the event? How fresh (versus decomposed) are the carcasses?

- What are the circumstances of the disease event?

It is difficult to predict what other information may be important, but questions should be asked about things that happened in the area prior to and at the time sick or dead animals were discovered. Information on weather, habitat conditions, and special features of the animals involved, e.g., that they are migrating, molting, calving, etc. at the time, should be explored. In agricultural areas, it is important to determine if activities such as spraying occurred and what chemicals were used.

3.3 To Investigate or Not?

Whenever possible, disease events should be investigated. Disease in free-living animals is very much like an ice-berg, with the great mass of what is occurring hidden from view. Sick or dead animals rarely become available for examination and even a few specimens may be valuable for understanding health problems in wild populations. Death of a few songbirds in one location may seem inconsequential, but the occurrence may assume much greater importance if a number of such events are investigated and linked to a particular cause, such as a poison. Similarly, assessment of the significance of diseases, such as botulism and avian cholera to waterfowl, must be based on investigation of many separate occurrences.

If in doubt about whether or not to investigate a disease event, discuss the information you have gathered in section 2.2, above, with your agency's wildlife health specialist or CCWHC Regional Centre. CCWHC personnel are available to

assist with field investigations when requested to do so.

Note that the CCWHC and its collaborating provincial veterinary diagnostic laboratories do not charge participating agencies for the examination of specimens (these costs are prepaid). Field personnel are encouraged to submit specimens for examination to these laboratories.

3.4 Go Prepared

If a disease occurrence is to be investigated, you must go prepared to collect specimens and other information. Sick and dead animals disappear rapidly; if they are not collected immediately, there may be no opportunity to return later and collect specimens. The rate at which carcasses disappear is size-related; song bird and small mammal carcasses may disappear within 1 day, while duck-sized carcasses may persist for 2-4 days, on average. Even when carcasses persist longer, decomposition reduces their value

for diagnostic purposes. Some poisons break down quickly in carcasses and in the environment. **It is essential to collect and preserve specimens as soon as possible for future testing. It is much better to be able to discard surplus specimens at some time in the future than to wish you had specimens that you did not collect.**

4. ONE SICK OR DEAD ANIMAL

Each individual sick or dead animal should be regarded as a rare observation that deserves careful examination. Diseased animals rarely are available for examination. For this reason, most health problems in wildlife go unrecognized. It is estimated that important diseases such as Rabies or Foot and Mouth Disease can be present in wild animal populations for months and may spread over extensive areas before being recognized. It is not possible to know in advance whether or not a single dead animal represents an important disease event or a trivial finding. Thus, whenever

possible, individual dead animals should be collected, preserved and delivered to a diagnostic laboratory that participates in Canada's wildlife disease surveillance program.

5. INVESTIGATING AN OUT-BREAK OR DIE-OFF

5.1 Do It Right Now!

Most outbreaks of disease in wildlife are short-lived events in which specimens and information are only available over a short period of time – a few hours to a few days. Because of this, **investigate the occurrence as soon as is possible and go prepared to collect information and samples on the first visit.** Even if carcasses and other specimens can be found a few days later, they may be decomposed and unsuitable for laboratory tests.

5.2 What to do BEFORE Going into the Field

Review all the information you have obtained about the disease event, and consider the species and numbers of animals you may encounter. Consider how you will estimate the number of dead animals, whether or not you will require the assistance of other people on site, whether or not you may have to humanely kill diseased animals, and similar practical matters.

It is advisable to call the laboratory to which you will submit specimens, and the federal and/or provincial/territorial contact person(s) (Section 2) at this stage, to alert them to your suspicions, to seek their advice and to inquire about special methods for collecting specimens or information.

5.3 What to Take with You

It is impossible to predict what will be found in the field, so it is necessary to take

equipment that is versatile and adaptable. The following equipment is satisfactory under a wide variety of circumstances, and most of it can be packed in a chest-type cooler that can be used later for bringing perishable specimens back to the laboratory. (If you need help in obtaining any of the equipment, contact your agency wildlife disease specialist, veterinary diagnostic laboratory, or the CCWHC).

5.3.1 Specimen containers:

Good quality, leak-proof plastic bags are the most versatile containers available, and are suitable for most types of specimens. However, they are easily perforated and tend to leak fluids. Whenever this is likely, specimens should be double-bagged. A variety of types of plastic bags should be taken into the field:

- Plastic garbage bags for whole specimens, vegetation, etc.
- 8 lb. plastic bags (about 20 x 30 cm)
- Sterile tightly-closable bags in a variety of sizes, available from

scientific suppliers, such as VWR/Canlab. These can be used to contain organs and tissues free of contamination for laboratory tests. If closed properly, they hold fluid well and are suitable for specimens preserved in formalin or other preservatives.

Rigid containers

- Sterile evacuated (Vacutainer®, Venoject®) tubes for blood and other body fluids. These are sterile until opened, and can be used for any liquids in small quantities (1, 3, 5 and 12 ml sizes are available).
- Screw cap plastic vials and bottles of various sizes (lightweight, unbreakable).

Plastic cooler

This can be used to transport equipment into the field and, with addition of ice, is suitable to transport perishable specimens back from the field. Plastic jugs (4L) partially filled with water and frozen, are a

good way of taking ice into the field, as there is no free water to wet specimens as the ice melts.

Clean cloth bags

New burlap or canvas sacks are very useful for intact, dry specimens.

Aluminum foil

Useful for wrapping specimens for toxicology if this is requested by the laboratory.

5.3.2 Preserving Specimens

Cold but Not Frozen: The **most useful all-around method for short term preservation is to place specimens in a cooler containing ice** to keep them cool (3C to 5C) until they can be brought to a laboratory.

A cooler can also be used to keep specimens from freezing in cold weather by placing containers of warm water or

warmed "cold packs" inside the cooler at regular intervals.

Formalin: 10% neutral buffered formalin is preferred for preserving tissue. This is a solution of formaldehyde, water and buffers that keeps the pH at 7.0. Correct pH is crucial to successful preservation. You can obtain a supply 10% neutral buffered formalin from scientific supply companies and also, often, from the laboratory to which you will deliver specimens for examination and testing. If you wish to prepare your own, use the following formula: 100 ml commercial formalin (38 - 40% formaldehyde solution), 900 ml distilled water (tap water generally also is acceptable), 4 g Sodium Phosphate Monobasic, 6.5 g Sodium Phosphate Anhydrous. Alcohol is a poor preservative and should not be used unless specifically requested by the laboratory. (70% ethanol is suitable for preserving parasites).

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5.3.3 Other Equipment:

Protective clothing

Rubber gloves, coveralls, and safety glasses/goggles and rubber boots. Rain suits, waders, and plastic or rubber aprons also are very useful because they keep underlying clothes from being contaminated and can readily be disinfected (see Section 5.6) before leaving the area. If working in an area where pesticide poisoning is suspected, special protective clothing may be advisable (see Section 8.0).

GPS unit and maps of the area, to record precise locations.

Record-keeping system (e.g., field note book and specimen submission forms). A camera and/or video recorder (photographs of the area and site often are very useful later for analyzing why a disease event occurred.

Dissection equipment (see Section 10.).

Materials for labeling specimens

Linen or waterproof tags, soft lead pencils, waterproof markers, adhesive tape (see Section 5.5.3)

Soaps and Disinfectant

Before leaving the site, it is important to disinfect boots, aprons, gloves, tools and equipment used on site, and to wash hands thoroughly (see Section 5.6). Hand soap of some kind, a wash basin, paper towel, chlorine bleach as disinfectant, some source of water, a plastic bucket and a long-handled brush to disinfect boots and equipment is all that is required in most cases. Surfaces should be washed clean before they are disinfected.

5.4 Information to Collect at the Time of Investigation:

Always try to collect as much information about the situation as possible, so that appropriate tests can be chosen when you submit specimens to the

laboratory. Submission forms that request specific information can be obtained from your laboratory, and forms also are available on the CCWHC website at http://www.ccwhc.ca/wildlife_submission_forms_regions.php. Verify or record all of the information you may have asked the person who first reported the disease event to you (see Section 3.2, pages 21 to 23) and include all this information with the specimens when you bring specimens to the laboratory. **Include your own evaluation of the disease event and your suspicions about what might have happened or what might be the cause. Also indicate clearly what you want to know from the laboratory**, e.g., if you need to know the nutritional condition or reproductive state of the animal you have submitted, in addition to why it died, indicate this in the information you submit with the specimens.

5.5 Collection of Specimens for Examination in a Laboratory:

5.5.1 Animals:

Whenever possible, intact, whole animals should be submitted to the laboratory. However, in some situations this may not be possible. In these instances, dissection of the animal(s) and collection of organs and tissues in the field may be the only option available. Directions for dissection of specimens are included in Section 10.

What to Collect

Live animals with well developed signs of disease are excellent specimens for disease diagnosis. If live animals are available but cannot be transported to the laboratory for reasons such as humane treatment of animals, safety or logistics, they should be killed humanely, blood samples collected, and then the blood samples and the carcass kept cool until delivered to the laboratory. If this is not possible, the carcass should be frozen

immediately and delivered to the laboratory in a frozen state.

Dead Animals are the most commonly submitted specimens in wildlife disease investigations.

- Freshly dead animals that have not decomposed and can be kept cool and delivered immediately to the laboratory are the specimens of highest quality and are most likely to result in a conclusive determination of the cause of disease.

- Frozen dead animals also can be excellent specimens, provided the carcass has not decomposed before it is frozen, and provided it is frozen only once and remains frozen until delivered to the laboratory.

Each cycle of freezing and thawing greatly reduces the value of a specimen for disease investigation.

- Rotten dead animals, dried-out carcasses and skeletons offer little hope of

determining the cause of disease. Occasionally examinations of such specimens are successful, and, as such, they are better than nothing.

How many specimens to submit

If only one or a few animals are available, submit them all. In larger die-offs, more dead animals may be available than can be readily transported or examined by the laboratory. In this type of situation, the sample of dead animals collected should include:

- both sick and recently dead individuals, if available.

- representatives of the species affected.

- representatives of the sex and age groups affected.

- as many individuals as (a) can be readily collected and transported to the laboratory and, (b), as the laboratory is able to examine.

When possible, at least 5 individuals of each type (species, sex, age) should be submitted. Some additional specimens also can be frozen, if possible, for later examination should they be needed.

5.5.2 Blood Samples:

Blood is a valuable specimen that can be collected from live animals before they are killed or released alive at the field location. Blood can be used to detect disease agents, antibodies to disease agents, and to monitor the function of body organs. Blood samples should be taken as cleanly as possible, preferably from the vein of a live animal. Satisfactory samples also can be taken by severing the jugular vein of an animal that that has been killed or from the neck of decapitated animals (usually birds). Blood can be collected from the chest cavity or heart of an animal dissected after death, but these samples are less useful.

Blood samples should be collected into sterile tubes. Evacuated glass tubes

(Vacutainer®, Venoject®) containing premeasured anticoagulants are available. Whenever possible, two types of samples should be collected. (a) One should be collected into a tube that contains no anticoagulant (red-stoppered tube or plain glass jar) from which to extract serum. This sample should be kept warm, e.g., in a shirt pocket or other warm (25 °C to 35 °C) location, until the blood has clotted. This will occur within a few hours. The clear straw-colored serum can then be poured or pipetted into a second sterile tube. Serum can be frozen, but if the serum cannot be removed, the tube containing the blood clot and serum should be kept cool but not frozen. (b) The other blood sample should be collected in a tube containing the anticoagulant EDTA (violet-stoppered tube). This sample will remain fluid and should be kept cool until delivered to the laboratory where it can be used to prepare blood smears and to examine for blood parasites and other features.

Blood samples must be protected from freezing. Freezing results in destruction of some blood elements,

making the sample unusable for most purposes. In winter, tubes should be pre-warmed before blood is collected, so that the first drops do not freeze on the cold container wall, and samples can be kept from freezing by carrying them in an inside pocket or in an insulated container, such as a cooler, containing a bottle of warm water.

Specimens collected from Dissection in the Field:

General guidelines:

- specimens must always be placed in individual, labeled, leak-proof containers (to prevent cross-contamination)

- **TWO samples of each organ or tissue of interest should be collected routinely**: (a) one fresh and kept cool (+4 °C) or, if necessary, frozen, and (b) one preserved in 10% neutral buffered formalin (10 volumes of formalin to one volume of tissue) (see below for more details)

- it is better to collect and save extra

specimens (that may be discarded later) than to regret not having collected them.

- it is difficult to remove the brain satisfactorily in the field; in most situations, the head should be removed and submitted intact (kept cool or frozen) to the laboratory.

5.5.3 Labeling specimens

Unlabelled specimens are of no value. Specimens must be fully labeled with labels that will remain attached and legible until the specimens reach the laboratory. Where possible, specimens should be double-labeled, with one label inside the container and a duplicate on the outside. Linen or special waterproof tags, written on with soft lead pencil or permanent marker, and tied to specimens with string are preferable. Durable waterproof tags can be made from Tyvek® building paper available from building suppliers. Adhesive tape (type used for bandages) can also be used with pencil or permanent marker. **If you have any doubt**

about the durability of a label, place it within a separate plastic bag that is tied to the specimen. Labels written in marker directly on plastic bags should be avoided, unless you are sure the marker used produces a permanent mark. (Test the marker by wetting the bag and trying to scrub off the label). Paper tags (other than the waterproof type) and water-based inks should not be used for labels).

Samples of Fresh Tissue (not preserved):

These samples most often will be used to detect living microbial disease-causing agents (bacteria, viruses, fungi), so it is essential to: (a) prevent decomposition and (b) keep the microbes alive. Fumes from formalin will kill these microbes. Therefore, fresh tissue must be kept completely separated from tissue preserved in formalin or other chemicals, and must be transported in separate boxes or other containers. Fresh tissue specimens should be held at refrigerator temperature (+4 °C) or on wet ice. Specimens from individual organs must be

placed in individual containers. Sterile plastic bags are very suitable for this type of specimen. Small organs, such as lymph nodes, should be collected intact. For larger organs, such as lung or liver, portions about 5 x 5 x 5 cm should be collected. Lengths of intestine (5-10 cm) must be tied at both ends with string to retain contents. Specimen containers should be placed within a large leak-proof plastic bag that is packed, together with frozen refrigerant packs or wet ice, in an insulated cooler for shipment to the laboratory (See Section 7.0). **Do not use dry ice to keep unfrozen specimens cool; - the specimens are likely to freeze and CO_2 given off from the dry ice will kill some infectious agents.**

Samples Preserved in Formalin

Formalin is used to preserve tissues for microscopic examination. It is essential to use 10% formalin in a solution at pH7.0 (neutral-buffered) (see Section 5.3.2). Tissues must be sliced into thin, flat portions to allow penetration of the fixative.

Decomposition will continue within large blocks of tissue immersed in formalin. In general, a **tissue should be no thicker than 1 cm in one dimension.** Portions of major organs (liver, spleen, kidney, lung, heart, stomach, intestines, muscle) should be preserved, together with portions of any tissue that appears abnormal (be sure to include the abnormal part!).

Specimens should be fully immersed in formalin. The ratio of formalin to tissue should be **10:1 by volume**. Specimens can be left in formalin for an extended period and should always be left immersed for at least 2 days before being prepared for shipment. **Specimens in fixative must not be frozen.**

Formalin is a hazardous substance and should be handled, stored and disposed of in compliance with federal and provincial regulations.

Specimens for parasitological examination:

Ectoparasites (lice, fleas, ticks) can be

preserved for identification in 70% ethanol. If the parasites must reach the laboratory alive, e.g. for isolation of viruses or bacteria, they should be placed in containers that allow air to enter, such as a plastic vial, the opening of which is covered with cloth. This container should contain a bit of moist cotton or sponge to prevent desiccation.

There is great variation of opinion about the best method for preserving worm (helminth) parasites for identification. **Call the laboratory for advice.** If no specific advice is available, worms can be fixed in 70% ethanol or 10% neutral-buffered formalin.

Feces can be valuable in assessing some parasite infections. Feces can be collected from the very end of the lower intestine and kept cool or, if necessary, frozen.

Specimens for toxicological examination:

There is no general test for

poisons; different specimens and tests are required for each individual poison. You must give the laboratory as much information as possible about what poisons might be involved.

As a general guideline, blood, liver, kidney, brain, fat, and content of stomach and intestine should be collected whenever poisoning is suspected. Each specimen should be placed in an individual container and frozen.

The type of container used is of particular importance because some containers may contaminate the samples for some tests, or absorb a significant amount of certain poisons. In the field, specimens should either be placed in clean glass containers or wrapped in clean aluminum foil before being placed within plastic bags and frozen. Large portions of tissue (50-80g) should be collected where possible.

Metal tags or tags with metal grommets should not contact the tissues which are to be analyzed for metal content.

Other types of specimen:

Other samples that might be collected during a disease investigation, such as water, vegetation, soil and suspected poisons, should be held in clean containers at refrigerator temperature (+4°C) or frozen until they can be delivered to the laboratory.

5.6 Preventing Spread of Disease to New Areas: Basic Hygiene

When dealing with diseased wild animals, care must be taken to ensure that you do not inadvertently transfer infectious agents to wild or domestic animals in other areas, or to yourself or others who may handle the specimens and equipment. It is important that resource agencies not be, and not appear to be, careless in this regard.

Some infectious agents can survive for days or weeks in the environment, particularly if within feces, blood, mucus or

tissue.

The basic precautions that need to be taken are quite simple:

- **Carcasses or specimens removed from the outbreak area must be contained in leak-proof containers.** Plastic bags should be doubled and materials should be packaged so that any leakage will be contained within a rigid leak-proof outer container.

- **Protective clothing worn at a disease site, such as coveralls, must not be used in other areas until cleaned. It should be packed in plastic bags prior to leaving the area, and washed thoroughly in hot water before being reused.**

- **Equipment (rubber boots, waders, rain suits, nets, boats, vehicles) that has been in contact with diseased animals, or with a contaminated environment, should be disinfected thoroughly prior to leaving the immediate area.** Equipment must be clean of dirt, tissue and

debris before it can be disinfected. Many disinfectants are available but ordinary household chlorine bleach is suitable, inexpensive and readily available. It should be diluted 1:10 in water for general disinfection and 1:5 for use on heavily contaminated areas. Boots, waders, rain suits, rubber aprons and small equipment can be disinfected using a rubber basin and scrub brush. A pail and long-handled brush are suitable for washing vehicles and boats but a small pressure sprayer may be more convenient. (Prolonged contact with chlorine bleach may cause deterioration of equipment).

- Persons who have been working with diseased animals should not work with healthy animals in other areas on the same day and until they have had the opportunity to shower and have a complete change of clothing. (Use common sense; this guideline is most important in situations, such as with colonial birds and in banding operations, where large numbers of animals will be handled directly).

National Information Line: 1-800-567-2033

6.0 HOW TO TRANSPORT SPECIMENS TO THE LABORATORY

6.1 General Guidelines:

Transport specimens directly to the laboratory rather than shipping them, whenever possible.

Check with the laboratory before shipping specimens.

Ship specimens on a Monday or Tuesday and not on Thursday or Friday. Specimens that spend a weekend in transit are likely to thaw and decompose before they are delivered.

Specimens shipped via common carriers (courier, bus, train, airline) must comply with regulations governing the Transport of Dangerous Goods (TDG). These regulations change with time. At present (October 2010), whole animals or specimens being sent to a veterinary laboratory for general examination are classified as biological specimens and not as specific disease-causing agents to

which various levels of hazard, and thus packaging requirements, are assigned. Advice on TDG issues is available from agency offices and from the nearest Regional Centre of the CCWHC.

Notify the laboratory of the method of shipment and the waybill number. (If a specimen does not arrive, it can be traced).

Perishable specimens must be packaged to prevent decomposition. Insulated cooler chests and frozen refrigerant packs or dry ice are suitable for frozen specimens. There are strict regulations (Transport of Dangerous Goods) regarding the use of dry ice; always check with the carrier before packaging).

Liquids must be packaged in secure containers, then placed within another secure waterproof container that contains absorbent material, so there is no possibility of leakage. (See section 6.3 for a way to reduce the amount of liquid when shipping tissues preserved in

formalin or other chemical preservatives).

Ensure that specimens are labeled with labels that will not become detached or illegible during shipment.

Enclose with the specimens a completed submission form (section 5.4) or other written explanation about the source of the specimens and the reason they are being sent (see Section 3.3). The written information should be in a separate waterproof container (sealed plastic bag) or in an envelope taped to the outside of the package.

6.2 Animal Carcasses

When carcasses are transported to the laboratory, they must be packed so that there is no leakage, and decomposition is reduced as much as possible. The time until specimens can be delivered, size of the animal(s), weather, and whether the specimen is frozen or unfrozen, may influence the techniques used.

6.2.1 If delivery can be made within 24 hours:

Large animals (e.g., deer, bear):

Unfrozen: In summer, do not leave carcasses exposed to sun. Cover with a tarp in a way that air can circulate. In winter, try to prevent freezing, e.g., by covering with tarp or other insulating material.

Frozen: No special precautions are necessary because of the slow rate of thawing of large carcasses.

Medium and Small-sized animals (e.g. rabbit, goose, robin, mouse):

Unfrozen: Place each animal in an individual bag, then within a heavy plastic bag. Pack with frozen refrigerant packs or wet ice in an insulated rigid container (e.g. a cooler chest or 5 gallon plastic pail with sealing lid). Surround specimens with crumpled newspaper (increases insulation and will absorb fluid).

Frozen: The objective is to prevent thawing and to deliver carcasses that are still completely frozen. The packaging recommended above for un-frozen carcasses will also insulate frozen carcasses and help keep them frozen.

6.2.2 If delivery can be made within 48 hours:

Large animals that are not frozen must be kept as cool as possible or they will decompose. Actions such as wetting the carcass or placing it in a cooler overnight, should be used. Frozen carcasses should be protected from direct sunlight and kept as cool as possible if they cannot be kept in a freezer or outdoors in freezing temperatures.

6.2.3 If delivery will be delayed more than 48 hours:

In general, **do not submit unfrozen carcasses under these circumstances.** Contact the laboratory to determine if they

prefer that you (a) freeze the entire carcass or (b) perform a necropsy and preserve tissue specimens. However, if the specimen can be kept refrigerated, or in cool conditions during spring and autumn, when the daily temperature is about +4°C, small and medium-sized specimens can remain adequately preserved for several days, if they are kept cool and out of direct sunlight.

6.3 Other Specimens

Perishable specimens (fresh, not preserved):

Blood: Whole blood should be kept cool (+4°C) but not allowed to freeze. Wrap tubes in protective material, e.g., corrugated paper, to prevent breakage, and pack with frozen refrigerant packs in rigid insulated containers. Special mailing containers for blood tubes and refrigerant packs are available, and can be ordered from the website of a scientific supplier, such as VWR/Canlab.

Serum should be frozen and shipped to the laboratory in insulated containers with frozen refrigerant packs.

Unfrozen tissues: A three-layered container system should be used (see Fig. 1). Each specimen is packed in a separate leak-proof container (plastic bottle or sealed bag), together with a suitable label. These bags are then placed in a second heavy plastic bag, together with frozen refrigerant packs, and sealed. This bag is then placed in a leak-proof rigid container for shipment. The most suitable containers are special foam mailer-shippers, which can be ordered from a scientific supplier, such as VWR/Canlab.

Frozen tissues: The same three-container system used for unfrozen tissue should be used. Dry ice may be used but, if so, the container and labeling must meet Transport of Dangerous Goods labeling and packaging requirements.

Tissues in Fixative: To reduce the risk of spillage of formalin during shipment to the laboratory:

- Ship tissues in non-breakable containers (plastic bottles or bags).

- After tissues have fixed for at least 2 full days, most of the formalin can be removed, leaving only enough to keep the specimens moist. Use a small piece of cotton wool, paper towel or sponge within the container to hold sufficient formalin to ensure the specimens stay moist.

- Place the primary containers in a plastic bag that contains cat litter or other absorbent material. (This will cushion the specimens and absorb leakage).

- Place the bagged specimens in a suitable rigid, leak-proof shipping container.

Fixed and perishable specimens should be shipped in separate containers, because formalin fumes will destroy living pathogens in fresh tissue placed in the same container.

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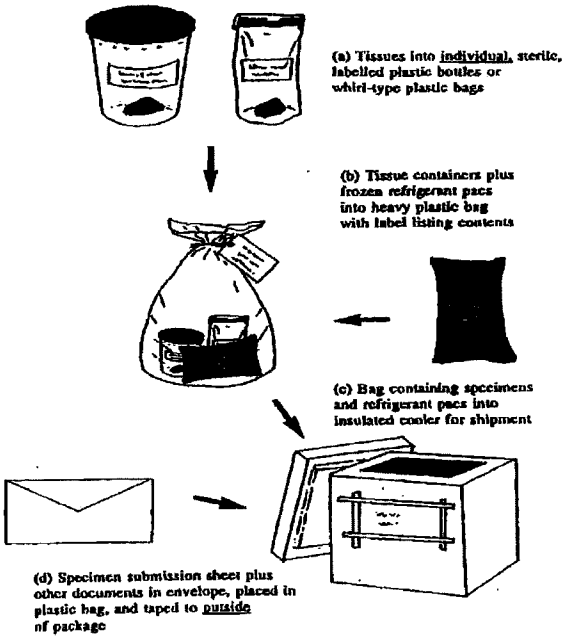

Figure 1. The preferred method for packaging fresh and frozen tissue specimens for shipment to the laboratory. Absorbent material, such as cat litter or paper towel, should be placed in the outer bag to absorb spills.

7.0 SPECIMENS FOR FORENSIC (MEDICO-LEGAL) EXAMINATION

Specific requirements for forensic examinations vary among jurisdictions, and enforcement officers are familiar with those that apply in their area. One general requirement is the maintenance of control over specimens that may be used as evidence, i.e. maintenance of a **chain of evidence**. The following are general guidelines that should be confirmed for your area:

Telephone the laboratory to determine if they can perform the required analysis. (The laboratory will tell you how to package, store and deliver specimens).

Advise the laboratory scientist immediately on submitting specimens that a forensic examination is required.

Whenever possible, deliver the specimen, in person, to the laboratory scientist who will be responsible for the

examination. (It may be permissible to ship specimens to the laboratory in a sealed package, together with an explanatory letter. The acceptability of this method should be confirmed in your area).

Sign the specimen labels or tags to indicate that you have provided the specimen to the scientist, and ensure that he/she also signs to indicate receipt of the specimens. **Record the time and date of this transaction.**

Tell the scientist exactly what you expect from the examination.

Tell the scientist exactly what specimens you will need to have retained and returned to you, e.g., bullet fragments, pelt, entire carcass.

Call the laboratory before returning to pick up specimens to ensure that the examination is complete, and that the scientist will be available to return the specimens to you in person.

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When you pick up specimens from the laboratory, ensure that the scientist that examined them signs them over to you, with time and date recorded.

Advise the scientist if his/her testimony is likely to be required in court.

8.0 PROTECT YOURSELF

There are at least three potential risks associated with investigation of diseased animals in the field:

In some areas, bears may be using and defending the carcasses. (Be Alert!).

Pesticides or other hazardous substances applied recently to fields or carcasses may represent a risk. When investigating suspected poisoning events, determine what chemicals have been used and when they were applied; if in any doubt, wear a chemical filtering mask and full protective clothing. Always wear rubber

gloves when handling poisoned animals to prevent skin exposure to the toxic chemicals.

Some diseases that occur in wild animals are infectious to humans. The risk of infection while handling sick animals can be reduced greatly by common sense and a few simple precautions:

- **Always wear rubber gloves when handling sick or dead animals**. (Ordinary household rubber gloves are satisfactory).

- **Avoid contaminating your skin and clothing with blood, body fluids or droppings from animals.**

- **Wash your hands and any contaminated skin thoroughly with soap and water after handling sick or dead animals.**

- **If you become ill after working with sick animals, see a physician and tell him/her that you may have been exposed to an animal disease.**

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Suggest that the physician call the laboratory (if you have submitted specimens), the provincial contact person (Section 3) or the CCWHC (1-800-567-2033) for information about diseases that might be involved.

The serious disease to which you are most likely to be exposed is **rabies**. This disease occurs in all parts of Canada but is much more common in some areas than in others. To reduce the risk of exposure:

- Treat any mammal showing abnormal behaviour, e.g. lack of fear, "friendliness", staggering, stumbling, circling, or viciousness, as though it is rabid.

- Treat wild carnivores and bats that appear sick as potentially rabid.

- Rabies virus is present in the saliva of rabid animals. Never allow a sick animal the opportunity to bite you; avoid exposure to saliva.

- Do not dissect an animal that has

you suspect may have rabies or a similar disease. Submit the intact animal to the laboratory.

- If you work in an area where rabies is common, consider being immunized against the disease. Consult local public health authorities about immunization.

9. KILLING SICK ANIMALS SAFELY AND HUMANELY

Three major factors should be considered when it is necessary to kill wild animals in the field:

- The method used should not place you or others at risk.

- The animal must be killed humanely, i.e., it must receive a quick and painless death.

- The method used should interfere as little as possible with subsequent examinations and tests.

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Acceptable methods for euthanasia (induction of a painless death) are available for most domestic species. However, many of these are impractical in field situations because:

- Wild animals are usually not physically confined and cannot be captured and/or restrained for euthanasia without risk to the persons attempting restraint, and stress and risk of injury to the animal.

- Facilities and equipment for administration of anaesthetic gas generally are not available in the field.

- Injectable lethal drugs, such as the barbiturates, are dangerous to handle and their use is controlled under narcotic drug legislation, so that they generally are not available to field workers.

Many wildlife officers now are trained and equipped to carry out chemical immobilization of wild animals. In some circumstances, chemical immobilization prior to euthanasia may be the most appropriate and humane approach.

Any method used to kill an animal should render the animal irretrievably unconscious as rapidly as possible and with the least possible distress. If chemical immobilization equipment is available, animals should be immobilized and then killed with injectable euthanasia agents. The following are methods that are practical under field conditions.

9.1 Birds

If a number of birds are to be killed in a situation that allows pre-planning, use of carbon dioxide (CO_2) is suitable. This requires a chamber into which the birds can be placed and a source of CO_2, either as compressed gas in a cylinder or dry ice. The chamber should be prefilled with 100% CO2 gas before the birds are placed inside. If dry ice is used, it must not contact the birds. Birds should be left in the chamber for a few minutes after all movement has ceased.

Cervical dislocation (separation of the

brain from the spinal cord by applying pressure to the base of the skull and to the spinal column) is suitable for smaller birds. Dislocation is difficult in larger birds, particularly in waterfowl, and decapitation is preferable in these larger species.

None of the above methods interferes significantly with diagnostic tests.

9.2 Mammals

Carbon dioxide can be used for killing small mammals, using the same methods described for birds (above).

Cervical dislocation and decapitation are suitable for small animals (rodents).

Shooting is an effective method of humanely killing animals in the field, if done carefully. Unfortunately, the most effective means, a shot to the brain, destroys the possibility of diagnosing most diseases of the brain. The best compromise is a shot to the neck that severs the vertebral column and spinal cord. This shot should be made

as high as possible on the neck (i.e. close to theskull, so that it has an effect similar to decapitation. Shooting should be done from close range and, where possible, from above rather than from the side. If large animals cannot be approached closely, a shot with a suitable caliber rifle that passes through the heart area will result in rapid death with only moderate interference with subsequent tests.

Stunning by a blow delivered to the central skull bones with sufficient force to produce massive brain hemorrhage results in rapid unconsciousness but damages the brain for subsequent tests. (The degree of damage is less than with gunshot). After stunning, the major blood vessels must be cut to result in exsanguination and preclude recovery.

Discretion is required whenever animals must be killed, because no method is aesthetically pleasing and most are distressing to the public.

10. NECROPSY: Dissection in the Field

10.1 General considerations

Necropsy (dissection and examination of organs and tissues after death) is a basic tool in the investigation of disease. It consists of systematic examination of all body parts and organs. **Whenever possible, necropsy should be done by a trained pathologist** experienced in recognition and interpretations of abnormalities in tissue.

A few factors apply to every necropsy:

1. The objective is to recognize abnormalities; this requires the ability to distinguish between normal and abnormal tissues. Road-killed animals and animals killed by hunters provide an opportunity to become familiar with the appearance of tissues of healthy animals

2. Tissues and organs must be examined in a systematic manner. Important disease processes often are overlooked because some organs and tissues are not examined carefully. The precise method used for a necropsy is less important than

establishing a routine in which each body system is examined fully.

3. A complete and careful record must be kept of what was observed during the necropsy. (A sample necropsy record form is available at http://www.ccwhc.ca/wildlife_submission_forms_regions.php; an advantage of using such a form is that the organs to be examined are listed, with space for recording observations. This fosters a complete examination). The report should be descriptive and self-explanatory. **Abnormalities should be described in terms of location, size, shape, color, consistency and content.** Photographs of abnormalities, taken during necropsy, are very helpful to laboratory personnel working with specimens collected from an animal. Digital photos can be sent electronically to the laboratory at the time the specimens collected at necropsy are shipped or delivered.

4. Samples of abnormal tissues should be collected for further study in the laboratory (see Section 5.5.3).

5. **Protective clothing, (rubber gloves, eye protection, coveralls, rubber apron)**

must be worn during necropsies.

The equipment required for necropsy depends on the size of the animal, but is not extensive.

Small and medium-sized mammals, birds: sharp knife, scissors, forceps, bone shears, string (to tie lengths of intestine to retain contents), containers for specimens, preservatives (see Section 5.3).

Large mammals: the above, plus large bone shears or axe to cut ribs (*"pruning shears"* used for trimming trees are very suitable), stone and steel to keep knife sharp, a butcher's saw for cutting pelvis and other bones, and rope and/or block and tackle to assist in moving the carcass.

Basic techniques for examination of birds and mammals are described below. If an animal is killed for necropsy, blood samples should be collected before or immediately after death (see Section 5.5.2). The animals should be weighed if possible, and the species, weight, sex and age recorded. The external surface of the body should be examined carefully, with particular attention to the body orifices. Any discharge from these should be noted. The

condition of the plumage/pelage is assessed and any abnormalities, such as presence of ectoparasites, hair loss, crusts, or foreign matter (e.g. oil) is noted. Ectoparasites should be preserved in 70% ethanol for identification. The limbs should be manipulated to check for fractures or dislocations.

10.2 Necropsy of a bird

The bird is placed on its back and the skin is opened to expose the body wall and neck (see Fig. 2). The amount of subcutaneous fat and fullness of the muscles are good indicators of nutritional condition and should be recorded. The limbs are skinned so that the muscles and joints can be inspected. (Fix a portion of breast muscle in formalin). It is useful to break a leg bone to assess bone strength (normal bones break cleanly with a distinct snap). The body cavity is opened posterior to the sternum (keel) and the sternum is lifted. At this point, the air sacs can be inspected. In a normal bird, these should be thin transparent membranes in the abdomen and thorax. (Cloudiness or presence of white or yellow material within the membranes indicates disease). The

sternum with the attached breast muscle is removed by cutting the ribs and the two larger bones that support the shoulder joint of the wing on both sides, with bone shears (see Fig. 3). This exposes the viscera, which should be inspected before any organs are removed. It is convenient to remove the heart at this time by cutting through the vessels at its base. A cut should be made across the heart. The cut surface of the muscle and the inner surfaces of the heart chambers should be examined. A portion of heart should be fixed in formalin and another retained fresh.

The other body systems are now examined in place, and then by removing and opening any organs that have a lumen to determine the nature of the content.

Digestive system: Beginning at the bill, open the esophagus to the thorax with scissors. Cut off the esophagus just above the proventriculus (stomach) and remove the proventriculus, gizzard, liver, spleen, pancreas, and intestine from the body cavity as a unit (see Fig. 4). The spleen and liver can be dissected free from the proventriculus and examined. (Fix portions of liver and spleen in formalin and save duplicate portions fresh). The intestine is

straightened by gentle pulling and limited cutting of the membranes so that it can be laid out (see Fig. 5) prior to opening. The proventriculus, gizzard, and intestine are opened with scissors to examine their content and the condition of the inner surfaces. Parasites should be collected. The gizzard content should be examined carefully for lead shot (particularly in waterfowl and raptors). Sections of intestine from the sites indicated in Fig. 5 should be placed in formalin.

Respiratory: The trachea (windpipe) is opened longitudinally with scissors. The lungs remain in the carcass after the digestive tract has been removed; they should be observed in place, and then one lung should be removed by a combination of pulling gently with forceps and cutting with scissors, inspected, fixed in formalin. The other lung should be removed and retained fresh.

Reproductive tract: The gonads are examined in place, and their size and the sex of the bird noted.

Kidneys: The kidneys are examined in place; at least one kidney should be removed for examination and portions fixed

in formalin and retained fresh.

Brain: in general, the brain should not be examined in the field; the entire head should be kept cool or frozen, and submitted to the laboratory.

10.3 Necropsy of a mammal

Small animals are usually dissected on their backs. Larger animals are done lying on their side. Ruminants are most conveniently dissected while lying with the left side down, with the abdomen facing you. (This keeps the large forestomachs out of the way). After external examination, the skin is opened along the ventral midline from the mouth to near the anus. (The cut should pass to one side of the mammary glands or the penis/scrotum). The skin is reflected and the legs are reflected by cutting through the muscle between the foreleg and torso; the hip joint is disarticulated to allow the rear leg to lie flat (see Fig. 6). It is convenient to examine the mammary glands of adult females at this time.

The abdominal cavity is opened by carefully cutting through the abdominal wall just posterior to and parallel to the last rib,

and then dorsally, caudally and ventrally in a half-circle to reach the ventral midline near the pelvis, exposing the viscera. The surface of the viscera is inspected for abnormalities and the amount and nature of fluid in the abdominal cavity are noted.

The thorax is opened by cutting through the ribs near the sternum and along the vertebral column with bone shears (see Fig. 7). The thoracic wall can then be removed (see Fig. 8). The thoracic viscera are examined in the same manner as for the abdomen. Incisions are made along the inside of both mandibles so that the tongue can be retracted (see Fig. 9). The hyoid bones at the base of the tongue are cut and then the tongue, trachea and esophagus are dissected free all the way to the opening of the chest cavity. The thoracic viscera are removed as a unit by pulling on the trachea and esophagus, while cutting along the attachment of the lungs to the vertebral column, and cutting the esophagus and large blood vessels as they enter the diaphragm.

After removal, the trachea is opened with scissors down into the major airways of the lung. The air passages are examined for the presence of fluid, froth, mucus, pus

or parasites, all of which are abnormal. The lungs should be carefully examined and palpated for the presence of firm areas. (Normal lung has a uniform soft spongy texture and is light pink in color). Several small portions from different areas of the lung should be fixed in formalin and portions of any firm areas should be both fixed in formalin and retained fresh. The heart should be examined while attached to the lungs. The pericardial sac that surrounds the heart is opened. The surface of the heart should be moist and glistening. A cut should be made across the heart about 1/3 of the length from the tip to the base (see Fig. 10).

The ventricles should be opened so that the inner surfaces of the heart chambers and the heart valves can be examined. (Fix a portion of heart in formalin and save one fresh).

The stomach(s), liver, spleen, pancreas and intestines often can be removed from the body as a unit. After examining the surfaces of the liver it can be dissected free from the stomach and several cuts made to inspect the internal surface. (Fix a portion of liver in formalin and retain a portion fresh). The gall bladder

(not present in cervids) should be identified and opened. The size and consistency of the spleen should be noted (fix a portion of spleen and save a portion fresh). The stomach and intestines are separated by gentle traction and limited cutting, so that each portion can be opened with scissors to inspect the content and internal surface. Fix portions of intestine in formalin. If the content or internal surface appears abnormal when opened, tie off 2-3 cm of the immediately adjacent section that has not yet been opened, and save this tied-off segment fresh. While separating the intestines, the lymph nodes in the mesentery can be observed.

The kidneys and adrenal glands remain in the carcass after removal of the stomach and intestine. They should be removed and cut with a knife so the interior can be inspected; a portion of kidney is fixed in formalin and another retained fresh. The bladder should be opened and inspected. The condition of the uterus and ovaries should be examined and the uterus opened to determine if embryos are present. The scrotum of males is opened and the testicles should be cut to inspect the internal surface.

The limbs are skinned so that major muscles can be inspected. A portion of muscle should be fixed in formalin. Large joints of the limbs should be opened for inspection. If the animal is judged to be in poor nutritional condition with little or no subcutaneous or internal fat, the femur should be dissected free and opened to examine the marrow. In most species, the femur can be cracked open by striking it sharply with a heavy object. In adult ruminants, the marrow cavity should be filled with hard white fat; red or gelatinous material is abnormal and indicates emaciation.

The brain should not be removed in the field. The head should be submitted intact to the laboratory. If you suspect rabies, handle the head as little as possible. Otherwise, to reduce the bulk and weight for shipping, the head can be skinned, the lower jaw removed and the nose removed by cutting across the skull with a saw just anterior to the eye sockets. (The lower jaw may be needed for aging, so include it with specimens sent to the laboratory).

If an animal has evidence of spinal cord injury, (such as paralysis), the

vertebral column should be dissected free, by cutting off the ribs and pelvic bones, and freeing it of most of the muscle, and submitting it intact to the laboratory. **Be very careful when cutting bones or working around cut bones that you do not puncture your gloves and skin on a sharp fragment.**

10.4 Specimens to collect at necropsy

(a) in 10% neutral buffered formalin: portions of liver, kidney, lung, heart, stomach, muscle from leg, spleen, and intestine (from several sites along its length), together with any other tissues you think may be abnormal. (Include the abnormal areas).

(b) in individual sterile plastic containers for microbiology: liver, kidney, lung, and portion of intestine near the cecum (tied off with string at both ends).

(c) Other specimens, depending on disease suspected. In most cases, the head should be submitted intact, fresh or frozen.

(See Section 5.5 for detailed instructions)

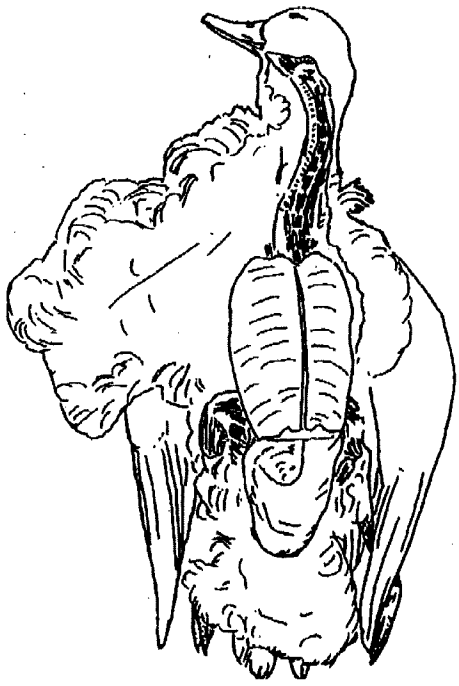

Figure 2. After inspecting the body surface, the skin is opened from beak to vent and reflected. The amount of subcutaneous fat and muscle mass should be noted.

Figure 3. The body cavity is opened by cutting through the ribs and collar bones on both sides and by opening the abdominal wall. The condition of the air sacs is assessed as this is done.

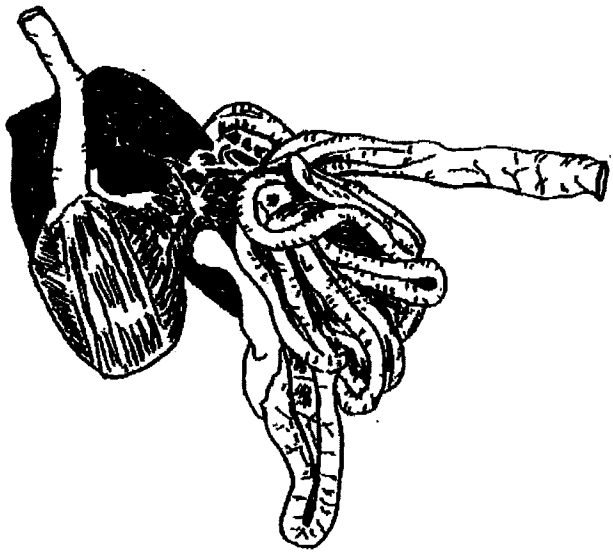

Figure 4. The proventriculus, gizzard, liver, spleen, pancreas and intestines are removed from the body as a unit.

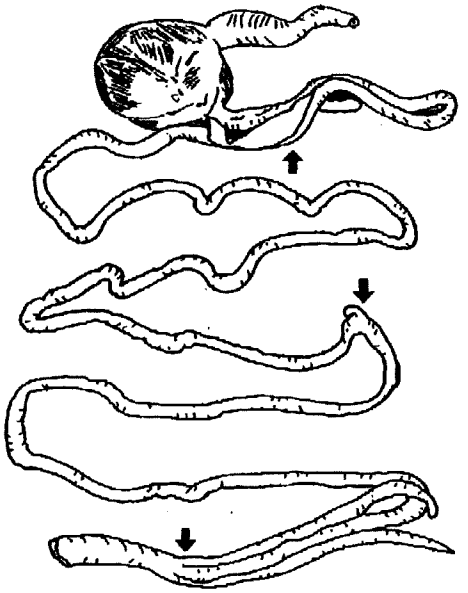

Figure 5. After removing and inspecting the liver and spleen, the intestine is separated by gentle traction. Scissors are used to open the full length of the digestive tract. Portions in formalin should be taken at the points marked with arrows.

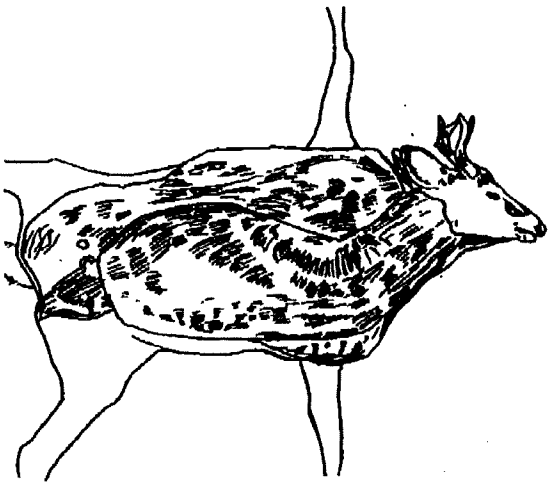

Figure 6. The skin is opened along the midline from between the jaws to the anus. This cut should pass to the side of the mammary gland or penis/testicles. The muscles between the shoulder and the body wall and those around the hip joint are cut so the legs can be laid back.

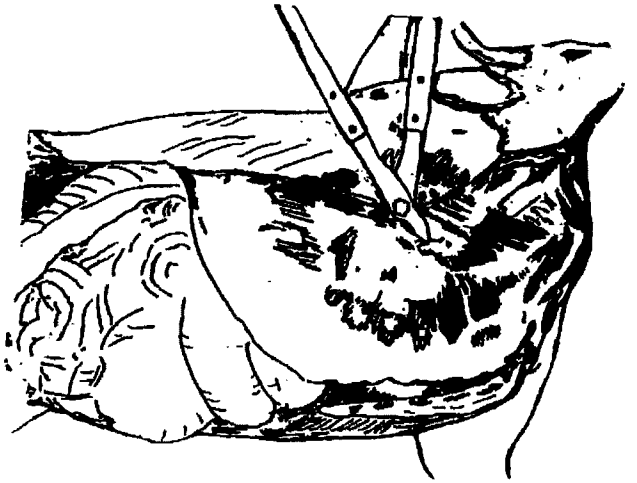

Figure 7. The abdominal wall is opened and reflected and the rib cage is removed by cutting the ribs along the sternum and spinal column with shears.

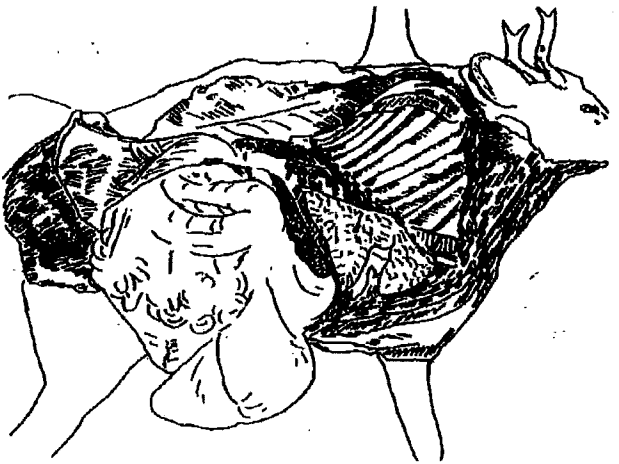

Figure 8. After the rib cage is opened, the abdominal and thoracic viscera should be inspected and the presence of fluid or other material in the body cavities noted.

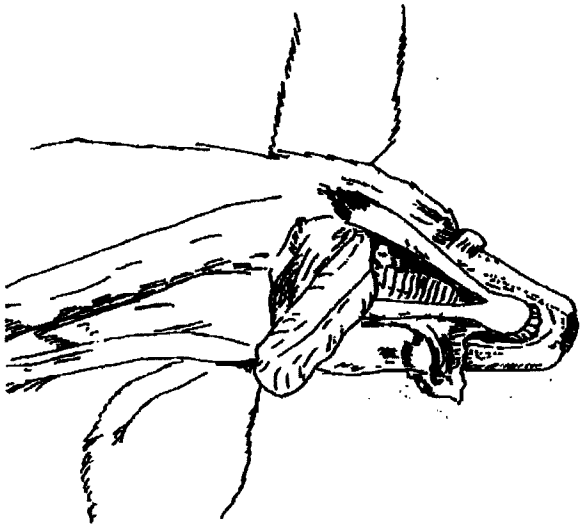

Figure 9. Cuts are made on the inside of both lower jaws so that the tongue can be freed. The hyoid bones at the base of the tongue are cut and then the entire tongue, esophagus and trachea can be dissected free down to the lung.

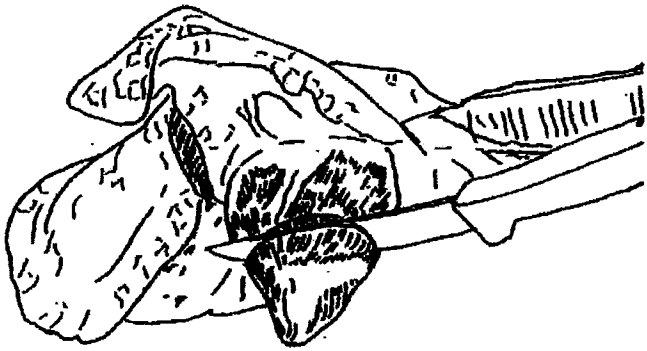

Figure 10. The heart and lungs are removed from the chest as a unit. The trachea is opened with scissors down into the lungs. The heart should be examined while it is attached to the lungs. The first step is to cut across the heart so the muscle and the size of the ventricles can be examined. The chambers are then opened to examine the inner chamber surfaces and valves.

National Information Line: 1-800-567-2033

11. DISPOSING OF ANIMAL CARCASSES

When dead wild animals are present in an area, it may be necessary to dispose of the carcasses and "*sanitize*" the area. In some instances, there is a valid biological reason for carcass disposal to reduce the risk of further disease occurrence. Animals dead of infectious diseases, such as avian cholera in waterfowl or anthrax in bison, are a major source of contamination that may result in infection of other animals. Carcasses may also serve as substrate for growth of *Clostridium botulinum* that causes botulism and birds may become poisoned by consuming a carcass or maggots associated with carcasses. Poisons in carcasses, including lead and some pesticides, may result in secondary poisoning of scavengers. The need for carcass collection and disposal should be judged on the basis of the nature of the disease that is present (which usually requires laboratory diagnosis) and the situation in which the carcasses are found.

When carcass disposal is necessary, it should be done on the site so as to reduce the risk of spread of disease to other areas, and in a manner that (a)

allows counting of the carcasses so that the size of the disease occurrence can be recorded, (b) results in complete destruction of the carcasses, and (c) conforms to standards relating to environmental pollution.

Persons involved in carcass disposal should wear protective clothing (rubber gloves, rainwear, rubber boots) and clothing and equipment must be thoroughly cleaned and disinfected before leaving the site (see Section 6.7).

Burying and burning (the methods used most commonly in the past) are increasingly at variance with environmental regulations regarding ground water and air pollution. Approval must be obtained from appropriate environmental agencies before these methods are used.

If carcasses are to be buried, a site should be chosen that does not result in ground water contamination, and where there is sufficient soil depth so that carcasses will not be excavated by scavengers (allow for 1 m of soil coverage). Carcasses can be covered with unslaked

lime or formalin, prior to burying, to discourage scavenging.

Carcasses are difficult to burn. Portable garbage incinerators of the type used in parks may be available in some locations and are suitable for small numbers of birds or other small animals. In most situations, carcasses will have to be burned in open fires, either on the surface or in a pit, using fuels such as wood, tires or fuel oil. To obtain a complete burn, it is necessary to maintain a hot fire and ensure that air can get under the burning carcasses. This can be
done by placing carcasses on a metal rack over the fire. (A suitable fire is likely to become hot enough to deform light metal racks). Only one layer of carcasses should be burned at a time.

National Information Line: 1-800-567-2033

TO CONTACT THE NEAREST OFFICE OF THE CANADIAN COOPERATIVE WILDLIFE HEALTH CENTRE

The CCWHC has six Regional Centre offices across Canada.

Contact information for each centre is given below. This information is also available on the CCWHC Website: http://www.ccwhc.ca and from the CCWHC National Information Line: 1-800-567-2033

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