A NECROPSY PROCEDURE FOR SAMPLING DISEASE IN WILD BIRD POPULATIONS*

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INTRODUCTION

When the demography of wild birds is analyzed, disease is an important but often overlooked factor. Although disease can be a primary factor of population regulation, its overall importance is probably more closely related to increasing the susceptibility of the host to other mortality factors (Kennedy 1975; van Riper, in prep.). It is therefore important that researchers be able to determine levels of parasites and diseases, if they are to draw meaningful conclusions concerning demographic parameters of a host population. Our purpose is to outline a procedure which would enable an ornithologist, who does not have sophisticated laboratory facilities, to examine birds correctly and to find answers concerning diseases present within an avian population.

For accurate disease diagnosis it is first necessary to establish a definite postmortem sequence, so that each animal is examined in a similar manner and data are organized and easily retrievable. Ornithologists often feel limited in their ability to understand and diagnose diseases, and in many instances pathologists are not readily available for consultation. Furthermore, budgetary constraints frequently limit the number of specimens that can be sent out for diagnosis; of those that are, the time lag before obtaining results is often considerable. It is therefore important that workers be able to perform their own diagnosis, and to do this the development of a necropsy form applicable to wild bird populations is essential.

The majority of avian necropsy techniques available today have been developed for poultry (e.g., Hungerford 1969; Zander 1975). Those designed specifically for other species usually place emphasis upon caged birds (Keymer 1961; Arnall & Keymer 1975), in particular canaries (Serinus sp.) and the Budgerigar (Melopsittacus undulatus) (Stone 1969). Many necropsy procedures are geared for veterinarian use and consist of pages with only general headings after which findings are placed (Ensley et al. 1976; Carpenter, pers. comm.).

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The necropsy technique discussed herein was developed for small passerine birds, but with slight modifications can be applied to most avian groups. It is based on described disorders present in poultry (Hofstad et al. 1972), pet and caged birds (Petrak 1969; Arnall & Keymer 1975), and wild birds (Davis et al. 1971), and should account for most diseases commonly encountered in birds from the field. Included is a checklist of potential symptoms interspersed with dissection directions (Part I), supplemented with detailed instructions on which parts of a bird to save when a symptom is encoutered (Part II). Short sections are presented which (1) outline the materials and facilities necessary to carry out a postmortem analysis; (2) give general handling techniques which should be used during postmortem analysis; and (3) give detailed instructions on how to prepare different materials which are to be sent to laboratories for diagnosis. Diseases and the avian orders in which they have been reported are summarized in tables. Following the procedures outlined in this paper, most ornithologists should now be able to perform their own postmortem analyses.

MATERIALS AND METHODS

Required Materials

The basic equipment required for this postmortem technique is minimal. It is, however, very important that the working area have limited access so as to reduce bio-hazards. Safety is an important consideration in doing postmortem analysis of any avian species, because many organisms that cause disease in birds are also pathogenic to man. Use standard procedures in handling diseased tissue and liberal amounts of a strong detergent (e.g., Tincture Green Soap) and disinfectant (e.g., Phenol or Pine Oil).

Both a dissecting and compound microscope are necessary. Necropsy of small birds is tedious, and unless fine instruments are used much information can be lost. Opthalmic tools are ideal, and we have found iris microdissecting scissors, watchmakers and microdissection forceps, as well as microprobes invaluable. Other essential equipment should include a small piece of glass for examining the gastrointestinal tract, clean microscope slides and cover slips, sterile swabs and syringes, sterile petri dishes and vials for collecting tissue samples, sterile plastic bags for freezing tissue, and an alcohol lamp.

Required chemicals and solutions for processing necropsy material include: 10% buffered formalin (add a pinch of $CaCO_3$ per gallon); 70% alcohol glycerine-alcohol (90 parts 70% ethyl alcohol, 10% parts glycerine); F.A.A. (50 parts 95% ethyl alcohol, 10 parts commercial formalin, 2 parts glacial acetic acid, 40 parts distilled water); absolute methyl alcohol; sterile transport medium for fungi (e.g., Sabouraud's agar available from Difco Laboratories, Detroit, Michigan 48201; or Mycotic media available from Baltimore Biological Laboratory, Inc., BioQuest

Division, P. O. Bos 243, Cockeysville, Maryland 20030); sterile transport medium for bacteria (e.g., Stuart's medium, a modified form packaged with a sterile swab available from Culturette, American Hospital Supply Corp., McGraw Park, Illinois 60085); and dry ice. Optional, but often extremely useful supplies include: filters, Lugol's solution (5 g iodine, 10 g potassium iodide, 100 ml distilled water; dilute with 5 times the distilled water before use); Hoyer's mounting medium (30 g gum arabic, 50 ml distilled water, 20 ml glycerol, 200 gm chloral hydrate; mix in order listed and filter through fine gauze); 10% solution of potassium hydroxide or 20% solution of sodium hydroxide; sterile transport medium for viruses (available from Colab Laboratories, Chicago Heights, Illinois 60412).

Postmortem Methods

General handling of the bird. A necropsy should be performed as soon as the bird is received because decomposition of internal organs is rapid and postmortem migration of parasites might occur. Take measurements immediately because weight, in particular, will change. Size measurements are important for aging purposes and may later prove useful as indicators of specific diseases within the population. Feather wear, cloacal protuberance, and brood patch will better define the breeding condition of the specimen. Tag and label the bird, and every sample taken from this animal should have the same necropsy number (recorded on the necropsy form); indicate if samples are "sterile" or "non-sterile" and the type of medium in which it is preserved. Obtain a detailed history of the specimen.

Preparation and examination of smears. Several types of smears are useful in the diagnosis of disease. Direct microscopic examination (such as fecal material) is important because some organisms are much more readily detected when alive. By using Lugol's solution, fungal hyphae and protozoa become more visible. Impression smears of organs or exudate prepared for gram or Ziehl-Neelsen stain (fix by drying over heat) are important for laboratory analysis of bacteria. Blood smears and impression smears of organs stained with Giemsa are essential when searching for blood haematozoa; fix for 30 seconds in absolute methyl alcohol.

Collection of blood serum. Serological tests will require blood serum. Collect blood aseptically from the heart and let clot overnight. Centrifuge for 10 minutes and then transfer serum to sterile vials. Refrigerate or freeze for shipment.

Preparation of tissue samples. There are a variety of fixatives used in preparing tissue (e.g., Zenker's is useful for all except nervous system tissue), but a good general fixative for most histopathological work is 10% buffered formalin. Cut tissue samples in pieces no larger than 1 x 2 x 0.5 cm and place in

10 volume equivalents of formalin. After 24 hours the tissue can be packed with less formalin or left as is. When freezing tissue, use dry ice to rapidly lower the temperature below 60°C. Glass may shatter so plastic bags are best for samples. Ship in a styrofoam container with dry ice.

Preparation of cultures. In general, collect samples to be cultured before the intestine is open. If an organ has been collected under nonsterile conditions, sear the surface with a hot spatula, incise tissue, and sample the cut surface. Sample moist membranes and soft organs with sterile swabs. Place the entire swab directly in medium for shipment. Collect joint or nasal exudate with a sterile hypodermic needle or swab. If solid agar is used, place the tissue firmly against agar or embed several small pieces in agar.

Fixation of helminths. Nematodes can be fixed directly in glycerine-alcohol solution and shipped. Cestodes, trematodes, and acanthocephalans should be placed in F.A.A. for 24 hours and then transfered to 70% alcohol before shipment.

Fixation of arthropods. Mites, lice, and small insects may be placed directly in 70% alcohol; larger organisms (such as fleas) may be killed first by placing in steaming water and then transfering to 70% alcohol. For permanent mounts of ectoparasites, drop the specimen directly into Hoyer's mounting medium, pass over a flame to relax specimen, and then cover with a cover slip. Fungal hyphae should be mounted in 10% potassium hydroxide or 20% sodium hydroxide and heated gently to clear the specimen.

Sporulation of Coccidian Oocysts. A fecal suspension should be placed, with a thin layer of 1% formalin, in a petri dish for one to four days so that sporulation will occur and species can be identified. The fecal material may be shipped in this medium.

Laboratory facilities. A problem often occurs in trying to find a laboratory which will process the tissue and identify pathogens. There are several agencies that have well-established laboratories which specialize in avian diseases (e.g., Fish & Wildlife Service), but they are usually hesitant to accept material from independent researchers due to lack of time or personnel to process material. Better places to try include the Department of Agriculture, Department of Health, Veterinary Pathology laboratories, and university laboratories in Medical or Veterinary Sciences. Check with the particular laboratory for their preferences of tissue preservation.

RESULTS

Instructions

The following postmortem analysis (Part I) is organized to include initial cataloging of specimen, the history of the specimen, and necropsy and laboratory analysis. The actual examination is outlined with dissection directions. The examiner need only follow the instructions in parentheses until a particular symptom occurs, check the space, circle the disorder (symptom), and then using the number at the right of the line as a guide, turn to Part II. In Part II are instructions for collection of relevant material necessary for laboratory analysis. The numbers on the postmortem form also are listed in Tables I and II under specific diseases; by referring to the tables the examiner can determine possible disorders to suggest to the laboratory for consideration. Underlined numbers in the tables refer to characteristic symptoms. However, disease symptoms were determined from poultry diseases to a large extent and they may differ in other species of birds. Furthermore, many diseases may share common symptoms, especially those that undergo a septicemic phase. Therefore, in most cases it is only by a thorough necropsy analysis supplemented with laboratory tests that a particular disease can be positively identified.

PART I

SPECIES:_____ FIELD #:_____ NECROPSY #_____

Area collected:_____
Collector:_____
Date collected:_____
Date examined:_____
Examiner:_____
Age:_____ Weight:_____g
Sex:_____ Gonad meas.:_____mm
Fat:_____ Skull:_____
P.M.State:_____
Preserved in:_____
_____

Body measurements:
Total length:_____mm
Wing length:_____mm
Tail length:_____mm
Beak length:_____mm
Tarsus length:_____mm

Condition of plumage:
Head molt:_____ Tail molt:_____
Body molt:_____ Wing molt:_____
Worn plumage:_____ Area:_____
Brood patch:_____ Clo.P.:_____

History of bird:_____
_____
_____

MATERIAL TO LABORATORY

Smears: peripheral blood_____, heart_____, liver_____, spleen_____, bone marrow_____, lungs_____, kidney_____, brain_____, fecal_____, other_____

Tissue: entire bird_____, heart_____, liver_____, spleen_____, lungs_____, intestine_____, proventriculus_____, gizzard_____, esophagus_____, crop_____, gall bladder_____, pancreas_____, kidney_____, eye_____, brain_____, gonads_____, nerves_____, trachea_____, bursa of fabricius_____, muscle_____, endocrine glands_____, legs_____, feet_____, other_____

Body Wash:_____ Crop Contents:_____

Parasites: helminths:_____
_____
_____
arthropods:_____
other:_____

Cultures: _____
_____
_____

Other: _____

NECROPSY SUMMARY:

LABORATORY RESULTS:

DIAGNOSIS:

I. External Analysis

(Examine bird externally for disorders.)

a. HEAD AND BEAK

_____Face: lesions; crusty or scaly scabs (1)
_____Face or sinuses swollen (2)
_____Ear disorder (3)
(Cut eyelids back and expose eyeball.)
_____Eye: inflamed; swollen; cloudy; exudate; helminths (4)
(Cut off beak at nostrils; examine with dissecting microscope.)
_____Nasal chamber: lesions; nodules; exudate (5)
_____Nasal parasites (6)
_____Other_____

b. BODY AND WINGS

_____Keel prominent (7)
_____Vent soiled; diarrhea (8)
_____Feathers: dry, easily broken, or absent; follicles infected (9)
_____Skin: dermatitis, ulceration, swelling; uropygial infected (10)
_____Other:_____

c. LEGS AND FEET

_____Legs or feet: lesions; crusty or scaly scabs (1)
_____Legs or feet: swollen; enlarged bones; inflamed joints (11)
_____Missing appendages (12)
_____Other_____

d. BODY WASH FOR EXTERNAL PARASITES

(Tape bill shut, shake in soapy water, let settle, decant to alcohol.)
Comments:_____

II. Internal Analysis

a. BODY SURFACE

(Skin body, neck, head; record fat and skull condition.)
_____Muscles: lesions; discolored; hemorrhage (13)
_____Feather, follicle, or skin parasites (14)
_____Other:_____

b. UPPER DIGESTIVE AND RESPIRATORY SYSTEMS, SKULL, AND BRAIN

(Cut from mouth down neck exposing trachea and esophagus.)
_____Mouth, pharynx, esophagus: lesions; nodules; cheesy masses (15)
(Cut esophagus to proventriculus; preserve crop contents in alcohol.)
_____Crop: lining thickened; contents sour (15)
(Cut up length of trachea; examine with dissecting microscope.)
_____Trachea: lesions; nodules; exudate (16)
_____Tracheal parasites (16)
(Remove skull; examine brain.)
_____Brain: lesions; nodules; discolored; hemorrhage (17)
_____Other:_____

c. CELOMIC CAVITY AND AIR SACS

(Cut out breast and slowly lift sternum. Examine air sacs.)
_____Air sacs: lesions; nodules; exudate (18)
(Examine internal organs and if any suggest infection, process immediately to avoid contamination.)
_____Abdomen: lesions; nodules; exudate (19)
_____Other:_____

d. HEART AND PERICARDIUM
(Examine pericardium and remove.)
_____Pericardium: exudate; inflamed; discolored; hemorrhage (20)
(Examine heart; prepare smear from heart blood and fix for Giemsa stain.)
_____Heart: enlarged; lesions; nodules; hemorrhage (21)
_____Other:_____

e. LIVER, SPLEEN, GALL BLADDER
(Examine liver, gall bladder; measure spleen:_____x_____mm.)
_____Liver disorder (22):_____
_____Spleen disorder (23):_____
_____Gall bladder disorder (24):_____
(Remove liver and spleen.)

f. INTESTINAL TRACT AND PANCREAS
(Examine external appearance of intestinal tract and membranes.)
_____Peritoneum: nodules; discolored; inflamed (25)
_____Intestine: external ballooning or hemorrhage (26)
(Remove gastrointestinal tract and straighten on glass plate.)
(Intestine length:_____mm. Separate proventriculus and gizzard.)
(Cut down length of intestine and lay open.)
_____Intestine: lesions; nodules; hemorrhage: helminths (27)
(Record location of parasites:_____
_____Ceca: lesions; nodules; exudate; hemorrhage; thickened; helminths (27)
_____Bursa of Fabricius abnormal (28):_____
_____Other:_____
(Take intestinal and cecal smears; check for protozoa parasites.)
_____Coccidiosis, Trichomoniasis, Histomoniasis, Other (27):_____
(Cut open proventriculus and gizzard.)
_____Proventriculus: lesions; nodules; hemorrhage; erosion; helminths (30)
_____Gizzard: lesions; nodules; hemorrhage; erosion; helminths (30)
_____Other:_____
(Examine pancreas.)
_____Pancreas: lesions; chalky; hemorrhage (31)

h. LUNGS
(Examine and remove lungs.)
_____Lungs: lesions; nodules; exudate; inflamed (32)
_____Other:_____

i. UROGENITAL SYSTEM AND ADRENAL GLANDS
(Measure and remove gonads; record sex; examine adrenals.)
_____Gonads or associated structures abnormal (33):_____
_____Adrenal glands abnormal (34)
(Examine and remove kidneys.)
_____Kidneys: lesions; nodules; discolored; enlarged (35)
_____Other:_____

j. NERVOUS SYSTEM
(Examine nervous plexus.)
_____Nerves: lesions; discolored; swollen (36)
_____Other:_____

k. SKELETAL SYSTEM
(Examine vertebrae; break leg bone and examine bone marrow.)
_____Bone marrow abnormal (37):_____
_____Vertebrae or other bones infected (37):_____
_____Other:_____

l. COMMENTS:_____
_____

PART II

(1) Face, legs, or feet: lesions; crusty or scaley scabs.

Scrape part of a lesion onto a microscope slide (with water or mineral oil) and examine for mites; preserve in 70% alcohol. If exudate is present, prepare a smear for gram stain. Divide the remainder of infected tissue into three parts; preserve one in 10% formalin, freeze part on dry ice, and culture the rest in a mycotic medium.

(2) Face or sinuses swollen.

Smear a portion of the exudate on two clean slides and fix for gram stain. Either freeze an exudate sample or swab the sinus area and place in a virus transport medium.

(3) Ear disorder.

If the ear is crusty, examine a wet smear for mites. Preserve in 70% alcohol.

(4) Eyes: inflammed; swollen; cloudy; exudate; helminths.

Examine the eyes for helminths; fix and preserve. Smear exudate on a clean slide for gram stain. Fix the infected tissue in 10% formalin, cutting a window through the eyeball so that fixatives can reach the internal structures. Be aware that this is often a secondary symptom and primary disease disorders will probably occur elsewhere. However, if there were nervous symptoms before the bird died, preserve the brain and nerve tissue at end of necropsy by freezing on dry ice.

(5) Nasal chamber: lesions; nodules; exudate.

From the exudate prepare a smear for gram stain and collect two swabs. Place one swab in a transport medium for virus and the other in bacteria transport medium. Preserve half of the infected tissue by freezing and the other half in 10% formalin. This may be a secondary symptom; therefore, examine the remaining respiratory system carefully.

(6) Nasal parasites.

Examine the nasal chamber with a dissecting microscope; preserve and fix parasites.

(7) Keel prominent.

A prominent keel is often indicative of a chronic disease; however, since it is also a secondary manifestation of many disorders, look for other symptoms.

(8) Vent soiled; diarrhea.

A soiled vent indicates diarrhea, a symptom of many diseases. Look for other disorders and be especially careful to examine the digestive tract.

(9) Feathers: dry, easily broken, or absent; follicles infected.

Examine feathers under a microscope for ectoparasites; preserve in 70% alcohol. The entire feather may be placed in alcohol rather than removing the parasite. Follicles and inner shaft should be examined for mites. If the skin is dry, or scaley and powdery, preserve a section in 10% formalin.

(10) Skin: dermatitis, ulceration, swelling; uropygial infected.

Proceed as in #(1). If possible collect parasites and preserve in alcohol.

(11) Legs or feet: swollen; enlarged bones; inflammed joints.

Using a sterile syringe, collect fluid from joints (including wing joint), and smear for gram stain. Place a portion (or a swab) into a bacteria medium and either freeze (-60°C) the remaining exudate or place in viral transport medium. Preserve some infected tissue by freezing and the rest in 10% formalin.

(12) Missing appendages.

If appendages are missing consider the bird's history in terms of trauma (e.g., freezing) or past viral (e.g., Pox) infections. Be sure to include this in the history of the bird.

(13) Muscles: lesions; discolored; hemorrhage.

If nodules are obvious on pectorals, open one and examine for nematode larvae or mites; preserve in 70% alcohol. Smear necrotic lesions and prepare for a gram stain. If exudate is present (e.g., blood) prepare a swab and place in a bacteria medium. Fix infected tissue in 10% formalin. This is often a secondary symptom so be careful to look for other indications of disease.

(14) Feather, follicle, or skin parasites.

Examine the internal surface of the skin for mites; if present preserve in alcohol. Proceed as outlined in #(9).

(15) Mouth, pharynx, esophagus: lesions; nodules; cheesy masses. Crop: lining thickened; contents sour.

Lesions in the mouth should be smeared for gram stain or preserved in 10% formalin. If cheesy, culture on a mycotic medium for shipment. A wet smear may reveal protozoa (especially if crop lining is thickened); proceed as outlined in #(29). Preserve crop contents and tissue in formalin. Fix and preserve any helminths.

(16) Trachea: lesions; nodules; exudate.

Freeze or swab exudate and place in viral transport medium. Prepare an exudate for gram stain. Preserve lesions and tracheal tissue in 10% formalin and on dry ice. The entire length of the trachea should be examined for nematodes; fix and preserve.

(17) Brain: lesions; nodules; discolored; hemorrhage.

Swab tissue and place in bacteria medium. Make two impression smears (one fixed for gram stain and one for Giemsa). Divide the remaining brain tissue in half; freeze part and place the rest in 10% formalin. Collect a sample of body fat; freeze.

(18) Air sacs: lesions; nodules; exudate.

Make a tissue smear for gram stain and search tissue for mites. Prepare diseased tissue by freezing and in 10% formalin. Also freeze (even though they may appear normal) sinus tissue, trachea, lungs, and cloaca.

(19) Abdomen: lesions; nodules; exudate.

Lesions or exudate in the abdomen should be smeared for gram stain. If the lesions are nodular, examine for mites or nematode larvae; fix and preserve. The remaining diseased tissue should be place in 10% formalin.

(20) Pericardium: exudate; inflammed; discolored; hemorrhage.

Prepare two smears (for gram stain and Giemsa) from exudate. Preserve the remaining tissue in 10% formalin. Since this is often a secondary symptom, samples of tissue from the kidney, bone marrow, bursa of Fabricius, lungs, liver, spleen, and brain should be routinely collected.

(21) Heart: enlarged; lesions; nodules; hemorrhage.

Prepare lesion, organ impression, and heart blood smears for gram stain and Giemsa stain. Swab tissue and place in a bacteria medium. Preserve what remains of the heart in 10% formalin. Tissue from other representative areas of the body (e.g., liver, spleen, kidney, bone marrow, lung, brain--freeze part--and bursa of Fabricius) should also be collected.

(22) Liver disorder.

Prepare two organ impression as well as lesion and exudate smears for gram stain and Giemsa stain. If tubercles are present on the liver smash a small (2 mm) tubercle between two slides and prepare for Ziehl-Neelsen stain. Swab tissue and place in bacteria medium. Divide the remaining tissue, placing part in 10% formalin and freezing the rest on dry ice for shipment. A sample of heart blood should be prepared for Giemsa stain and blood serum collected. Collect spleen, heart tissue, lung, and bone.

(23) Spleen disorder.

Follow the procedure outlined in #(22) and be sure to collect a sample of the liver.

(24) Gall bladder disorder.

Using a sterile syringe collect some bile and place in bacteria medium. Examine the bile ducts for helminths and if present, preserve.

(25) Peritoneum: nodules; discolored; inflammed.

Prepare a gram stain from lesion. If small tubercles are present smash some between two slides and fix for Ziehl-Neelsen stain. The remaining tissue should be placed in 10% formalin.

(26) Intestine: external ballooning; hemorrhage.

Examine carefully without opening the intestine. Prepare smears of any lesions for gram stain.

(27) Intestine: lesions; nodules; hemorrhage; helminths.
Ceca: lesions; nodules; exudate; hemorrhage; thickened; helminths.

Before proceeding, prepare a swab from lesions and place in bacteria medium. Omit if intestinal contents have contaminated the area such that lesions cannot be seared and incised.

Examine a wet smear of intestinal contents at several places for intestinal protozoa (see #[29]). Smears of lesions should be prepared for gram stain. Fix the intestinal and cecal contents in 1% formalin and the tissue in 10% formalin. Freeze sections of the bursa of Fabricius, liver, spleen, bone marrow, and take blood serum. Sections of the heart, lung, kidney, liver, and spleen should be placed in 10% formalin. Examine the length of the intestine for helminths and if present, preserve.

(28) Bursa of Fabricius abnormal.

Divide the organ in half and preserve part in 10% formalin and freeze the rest on dry ice.

(29) Intestinal Protozoa.

To check for protozoa parasites, a wet smear will usually suffice. However, Lugol's solution will often facilitate observation.

(30) Proventriculus: lesions; nodules; hemorrhage; erosion; helminths.
Gizzard: lesions; nodules; hemorrhage; erosion; helminths.

If lesions are present, swab and place in a bacteria medium. Prepare a smear from heart blood for gram stain. Any cheesy exudate should be transfered to a mycotic medium for culture and/or placed in 10% formalin. Be sure to remove the gizzard lining and examine for helminths; if present, preserve.

(31) Pancreas: lesions; chalky; hemorrhage.

Place the entire organ in 10% formalin.

(32) Lungs: lesions; nodules; exudate; inflammed.

From the exudate prepare two swabs; place one in a bacteria medium and one in a transport medium for viruses. Fix an exudate smear for gram stain. Any cheesy exudate should be cultured on a mycotic medium. Preserve half the remaining tissue in 10% formalin and freeze the rest. Fix a heart blood smear for Giemsa stain and separate serum into a sterile vial.

(33) Gonads or associated structures abnormal.

Prepare a swab for bacteria medium and make two impression smears for gram stain and Giemsa stain. Freeze some of the remaining tissue and place the remainder in 10% formalin. Samples of the bone marrow, heart blood, liver, spleen should also be collected.

(34) Adrenal Glands abnormal.

Look for other disorders, but note especially if shock might be suspected (e.g., hemorrhage of heart).

(35) Kidney: lesions; nodules; discolored; enlarged.

Prepare swabs from lesions for bacteria medium. Make impression smears for gram and Giemsa stain. Divide the remaining tissue in half and freeze part; examine the other half carefully for trammodes and Protozoa and if present, preserve. Collect blood serum in sterile vials and refrigerate. Also collect tissue from the heart, liver, and spleen.

(36) Nerves: lesions; discolored; swollen.

Preserve nerves that are diseased in 10% formalin. Prepare a heart blood smear for Giemsa stain and see #(17) for possible treatment of the brain. Even if brain appears normal, preserve it in 10% formalin.

(37) Bone marrow abnormal; vertebrae or other bones infected.

Prepare two bone marrow smears, one for gram stain and one for Giemsa stain. Freeze part of the tissue and preserve the rest in 10% formalin. If vertebrae are implicated, check carefully for joint involvement and place exudate in bacteria medium.

SUMMARY

More work needs to be done on disease in wild birds, especially studies which delimit the entire parasitic fauna present within a host population. Recording levels of a single pathogen, as most surveys to date have done, cannot possibly determine the impact disease is playing upon wild populations. A multi-disease approach is necessary, one which reveals the inter-relationship between all parasites and diseases within a host population.

We have presented this postmortem technique in hopes that more ornithologists will be inspired to attempt such multi-disease studies. These surveys will hopefully provide enough information so that disease interactions can be defined, possibly simulated in laboratory situations, and control measures can be found which would be applicable to the natural state.

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TABLE 1. Summary of diseases in avian hosts[1].

Disease	Pathogen	Major Host Symptoms[2]	Hosts Reported Susceptible[3]
Bacterial Diseases			
Arizonosis	Arizona sp.	Septicemic; (4,17,18, 19,21,22,27,32,33,36)	A,G,PS,P
Anthrax	Bacillus anthracis	Nervous	Str,Ci,A,F,C
Botulism	Clostridium botulinum	Nervous; (17,36)	Pod,Ga,Pel,Ci,A,F,G,Ch,C,St, P
Chlamydiosis (Ornithosis)	Chlamydia psittaci	Respiratory; Circulatory; (4,17,18,19,20,21,22, 23,24,31,32,33,36)	Ga,Pro,Pel,Ci,A,F,G,Gr,Ch,C, Ps,St,Ap,Cor,Pic,P
Cholera	Pasteurella multocida	Septicemic; (2,4,5, 15,16,17,18,19,22,25, 27,30,32,33,36)	Sp,Pod,Pro,Pel,Ci,A,F,G,Gr, Ch,C,Ps,St,P
Clostridia	Clostridium sp.	Wound infection; Digestive; (10,13, 22,27,30,35,37)	G (necrotic enteritis); probably all species susceptible to wound infection or gangrene
Colibacillosis	Escherichia coli	Various; (1,2,4,5,10, 11,13,16,18,19,20, 21,22,23,25,27,32, 33,35)	Str,A,F,G,Gr,Ch,Ps,St,P
Erysipelas	Erysipelothrix insidosa	Septicemic; (1,10,11, 13,18,20,21,22,23, 27,30,31,32,33,35)	Sp,Pod,Pel,Ci,A,F,G,Gr,Ch,C, Ps,Cu,St,P
Infectious Coryza	Hemophilus sp.	Respiratory; (2,4,5, 16,18,32)	G,C

TABLE 1—Continued[1].

Disease	Pathogen	Major Host Symptoms[2]	Hosts Reported Susceptible[3]
Bacterial Diseases (Con't.)			
Infectious Serositis (Duck Septicemia)	Pasteurella anatipestifer P. septicaemiae	Septicemic; (4,5,16, 17,18,20,22,23,25, 32,33,36)	A,G
Listeriosis	Listeria monocytogenes	Septicemic; (4,17,18, 19,20,21,22,23,27, 32,35,36)	A,F,G,Gr,Ch,C,Ps,St,P
Mycoplasmosis	Mycoplasma sp.	Respiratory; Skeletal; (2,5,10, 11,16,18,19,20,22, 32,35)	F,G,C,Ps,P
Pseudotuberculosis	Pasteurella pseudotuberculosis	Septicemic; (13,18, 19,22,23,27,32)	A,F,G,Gr,Ch,C,Ps,Cu,St,T, Cor,Pic,P
Salmonellosis	Salmonella sp.(over 1000 pathogenic sp.)	Digestive; Septicemic; (4,11, 15,17,18,19,20,21, 22,23,25,27,30,32, 33,35,36)	Sp,Str,Ga,Pel,Ci,A,F,G,Gr, Ch,C,Ps,Cu,St,Ap,Cor,Pic,P
Spirochaetosis	Borrelia anserina	Blood; (22,23,27,35)	A,G,C,P
Staphylococcus	Staphylococcus aureus	Skeletal; (1,2,3,10, 11,22,37)	Str,Ci,A,F,G,Gr,Ch,C,Ps,Ap, C,P (common on skin and mucous membrane)
Streptococcus	Streptococcus sp.	Septicemic; (1,2,9,10, 11,17,19,20,21,22,23, 25,33)	Ci,A,F,G,Gr,Ch,C,Ps,P

TABLE 1—Continued[1].

Disease	Pathogen	Major Host Symptoms[2]	Hosts Reported Susceptible[3]
Bacterial Diseases (Con't.)			
Tuberculosis	Mycobacterium avium	Viscera; (1,11,18,19, 22,23,27,32,33,37)	Sp,Str,Rh,Ca,Ti,Ga,Pel,Ci,A, F,G,Gr,Ch,C,Ps,Cu,St,Ap,Cor, Pic,P
Ulcerative Enteritis	Corynebacterium sp.	Digestive; (22,23,27)	F,G,C
Vibrio Infections	Vibrio sp.	Liver; Digestive; (20, 21,22,23,27,33,35)	Sp,Pod,Pro,Pel,Ci,A,F,G,Gr, Ch,C,Ps,St,P
Fungal Diseases			
Aspergillosis	Aspergillus fumigatus	Respiratory; (1,2,4, 5,9,10,15,17,18,22,32)	Sp,Str,Rh,Ti,Ga,Pro,Pel,Ci, A,F,G,Gr,Ch,C,Ps,St,T,Cor, P
Candidiasis	Candida albicans	Digestive; (15,27,30)	Sp,Rh,Ci,A,G,Gr,Ch,C,Ps,Cu, Ap,Pic,P
Cryptococcus	Cryptococcus neoformans	Meningitis; (17)	G,C,Ps,P
Favus	Microsporum sp. Trichophyton sp.	Skin; (1,5,9,10, 16,18,32)	G,P
Protozoan Diseases			
Coccidiosis	Isospora; Eimeria; and others.	Digestive; (26,27, 29,35)	Pro,A,G,C,Ps,P others?
Haemoproteus	Haemoproteus sp. Parahaemoproteus sp.	Blood; (17,18,22, 23,32)	Pod,Ci,A,F,G,Gr,Ch,C,Ps,Cu, St,Cap,Ap,T,Cor,Pic,P

TABLE 1--Continued[1].

Disease	Pathogen	Major Host Symptoms[2]	Hosts Reported Susceptible[3]
Protozoan Diseases (Con't.)			
Histomoniasis	Histomonas meleagridis	Digestive; (22,27,29)	G
Leucocytozoonosis	Leucocytozoon sp.	Blood; (17,21,22,23)	Ci,A,F,G,Gr,Ch,C,Ps,Cu,St, T,Cor,Pic,P
Malaria	Plasmodium sp.	Blood; (17,22,23)	Sp,Ci,A,F,G,Gr,Ch,C,Cu,St, Cor,Pic,P
Other blood Protozoa	Aegyptianella, Lankestrella, Toxoplasma	Blood; (11,17,18, 20,21,22,23,27,32, 35)	Ci,A,F,G,Gr,Ch,C,Ps,Cu,St, Cap,Ap,Cor,Pic,P
Sarcosporidiosis	Sarcocystis rileyi	Muscle; (13,21)	Ci,A,F,G,St,P
Trichomoniasis	Trichomonas sp.	Digestive; (5,15,16, 18,19,20,22,27,29, 30,31,32)	Ca,Pel,F,G,C,Ps,P
Trypanosomiasis	Trypanosoma sp.	Blood; (37)	Ci,A,F,G,Gr,C,Cu,St,Ap, Pic,P
Viral Diseases			
Arbovirus		Nervous; (17,21,36)	A,G,C,P
Bluecomb		Digestive; (26,27,31,35)	G
Duck Virus Enteritis (Duck Plague)		Septicemic; Digestive; (5,15,16,17,18,19,20,22, 23,27,28,31,32,35,36)	A
Duck Virus Hepatitis		Liver; (17,22,23,35)	A,G

TABLE 1--Continued[1].

Disease	Pathogen	Major Host Symptoms[2]	Hosts Reported Susceptible[3]
Viral Diseases (Con't.)			
Encephalomyelitis		Nervous; (4,17,30,36)	A,G,C
Hemorrhagic Enteritis		Digestive; (13,21,23,27, 30,35)	G
Infectious Bronchitis		Respiratory; (2,5,16,18, 32,33,35)	G
Infectious Bursal Disease		Bursa of Fabricius; (13, 23,28,30,35)	G
Influenza (over 80 types) (Fowl Plague)		Respiratory; (2,4,5,16, 17,18,19,20,22,23,25,32, 33,35)	A,G,Ch,C,Ps
Laryngotracheitis		Respiratory; (2,4,5,15, 16)	G
Monocytosis		Digestive; Viscera	G
Newcastle Disease		Systemic; Nervous; Respiratory; (4,5,16,17,18,20, 22,23,24,27,30,32,33,36)	Sp,Str,Rh,Ca,Pel,A,Ci,F,G, Gr,Ch,C,Ps,Cu,St,Ap,Cor, Pic,P
Pox		Skin; Mucous membranes; (1,5,10,11,12,15,16)	probably all species of birds
Puffinosis		Skin (feet); Nervous; (1, 17)	A,Ch,C,Pro
Quail Bronchitis		Respiratory; (2,4,5,16, 18,32)	A,G,F,P

TABLE 1--Continued[1].

Disease	Pathogen	Major Host Symptoms[2]	Hosts Reported Susceptible[3]
Viral Diseases (Con't.)			
Turkey Viral Hepatitis		Liver; (22,31)	G
Viral Arthritis		Skeletal; (11)	G
Neoplastic Diseases			
Erythroblastosis		Circulatory; (9,10,13,18, 22,23,32,35,37)	G
Hemangioma		Skin; Viscera; (9,10)	G
Leukosis complex		Viscera; (18,19,21,22,23, 28,30,32,33,35,37)	Ci,A,G,C,Ps,P
Marek's Disease		Nervous; Viscera; (1,4, 13,17,19,21,22,23,25,27, 28,30,31,33,35,36)	A,F,G,C,Ps,St,P
Myeloblastosis		Bone marrow; (9,22,23,35, 37)	G
Myelocytomatosis		Skeletal; (11,13,37)	G
Nephroblastoma		Kidney; (35)	G
Osteopetrosis		Skeletal; (11,37)	G
Other Neoplasms		Viscera; Various sites	Str,G,C,Ps,P

[1] Information not our own is from Garnham (1966), Davis et al. (1971), Hofstad et al. (1972), Arnall and Keymer (1975), Greiner et al. (1975), McClure et al. (1978).

[2] Numbers refer to symptoms listed on postmortem form and outlined in Part II; these numbers indicate the symptoms most likely to be found when that disease is present, and the underlined numbers are very characteristic symptoms.

[3] Letters refer to the orders of birds in which the diseases have been reported. Wild, domestic, cage, and laboratory groups that have shown susceptibility are included. The underlined orders indicate a host group in which that disease is particularly common.

Key to orders: Sp = Sphenisciformes, Str = Struthioniformes, Rh = Rheiformes, Ca = Casuariiformes, Apt = Apterygiformes, Ti = Tinamiformes, Ga = Gaviiformes, Pod = Podicipediformes, Pro = Procellariiformes, Pel = Pelecaniformes, Ci = Ciconiiformes, A = Anseriformes, F = Falconiformes, G = Galliformes, Gr = Gruiformes, Ch = Charadiiformes, C = Columbiformes, Ps = Psittaciformes, Mu = Musophagiformes, Cu = Cuculiformes, St = Strigiformes, Cap = Caprimulgiformes, Ap = Apodiformes, Col = Coliiformes, T = Trogoniformes, Cor = Coraciiformes, Pic = Piciformes, P = Passeriformes.

TABLE 2. Summary of parasites and other disorders of avian hosts[1].

Disease	Major Host Symptoms	Parasite Location
Helminth and Arthropod Parasites		
Helminth		
Acanthocephalans	Digestive; (27)	Intestine
Cestodes	Digestive; (27)	Intestine
Nematodes	Digestive; Respiratory; (4, 5,6,13(larvae),15,16, 19(larvae),22,23,24,27,30)	Intestine, proventriculus, gizzard, trachea, eye, & ceca are most common, but many other sites possible.
Trematodes	Viscera; (4,5,16,22,24,27, 30,31,33,35)	Various sites throughout body.
Arthropods		
Biting insects	Irritation of skin; (10)	Free-living or in nest; some on the body or the feathers.
Lice	Feather destruction; (1,9,10)	Feathers
Mites	Various, from feather damage to respiratory symptoms; (1, 3,5,6,9,10,13,16,18,19,32)	Feathers; Respiratory; Skin; Viscera
Miscellaneous Disorders		
Diet deficiencies--not analyzed as they are not usual in wild populations.		
Poisoning	Various symptoms; Nervous; (17)	----
Trauma	Various symptoms; (9,10,12, 13,17)	----

[1] Specific pathogens and orders of birds susceptible are not included because parasites are often not identified below the taxonomic level given, and probably all species of birds are susceptible.