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A NECROPSY PROCEDURE FOR SAMPLING DISEASE IN WILD BIRDS

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ABSTRACT.—This paper presents a necropsy procedure for examining small wild birds, designed to be used by investigators without experience in avian pathology. It gives instructions on how to conduct a postmortem examination, lists the needed equipment, tells what parts of a bird to save when a disorder in encountered, and lists possible diseases which each may signify. This procedure would enable ornithologists to include parasites and diseases in their investigations of avian populations.

Parasites and diseases should be considered in studying the demographic parameters of a population. Ornithologists often neglect these factors, however, because they lack training or facilities, and pathologists are not readily available for consultation. Our purpose here is to outline a procedure that will enable an ornithologist, without sophisticated laboratory facilities, to conduct postmortem examinations of birds and to diagnose diseases.

For accurate disease diagnosis, it is first necessary to standardize the postmortem technique, so that each animal is examined in a similar manner and data are organized and easily retrievable. Most present-day avian necropsy techniques were developed for poultry (e.g., Hungerford 1969, Zander 1972). Those designed specifically for other species usually emphasize caged birds (Keymer 1961, Arnall and Keymer 1975), particularly the Canary (*Serinus canaria*), and the Budgerigar (*Melopsittacus undulatus*; Stone 1969). Most of the other necropsy procedures are intended for veterinarians and are very general (Ensley et al. 1976, J. Carpenter, pers. comm.). The technique to be presented here was developed for small passerine birds, but with slight modifications, it can be applied to most avian groups. It is based on described disorders present in poultry, caged birds, and wild birds, and should identify most diseases commonly encountered in wild birds.

We present a standardized necropsy procedure, interspersed with dissection directions and supplemented with instructions on which tissues to save and how to preserve them. Other sections (1) outline materials and facilities necessary for a postmortem analysis; (2) give general hints on necropsy procedures, especially those related to the problems of dealing with smaller birds; (3) indicate what possible diseases each disorder listed on the necropsy protocol might indicate; and (4) summarize diseases presently known to occur in birds, from what avian groups they have been reported, and indicate which tissue to collect.

MATERIALS

Certain basic equipment is required for this postmortem technique. Both a dissecting and a compound microscope are necessary. Necropsy of small birds is tedious, and unless fine instruments are used, much information can be lost. Ophthalmic tools are ideal, and we have found iris microdissecting scissors, watchmaker's and microdissection forceps, and microprobes invaluable. Other essential equipment includes a small pane 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 tissues, and an alcohol lamp. A dissecting tray or neoprene cutting board is useful for larger specimens.

Recommended chemicals and solutions for processing necropsy material include: 10% buffered formalin (add a pinch $CaCO_3$ per gallon); 70% ethyl alcohol; glycerine-alcohol (90 parts 70% ethyl alcohol, 10 parts glycerine); formalin-acetic-alcohol (F.A.A.; 50 parts 95% ethyl alcohol, 10 parts commercial formalin, 2 parts glacial acetic acid, 40 parts distilled water); absolute methyl alcohol; 2% potassium dichromate solution; sterile transport medium for fungi (e.g., Sabouraud's or Mycobiotic agar available from Difco Laboratories, Detroit, Michigan 48201); sterile transport medium for bacteria (e.g., Stuart's medium, a modified form

packaged with a sterile swab called a Culturette, American Hospital Supply Corp., McGraw Park, Illinois 60085); and dry ice. Optional but useful supplies include Lugol's solution (5 g iodine, 10 g potassium iodide, 100 ml distilled water; filter; dilute with 5 times the amount of distilled water before use), Hoyer's mounting medium (30 g gum arabic, 50 ml distilled water, 20 ml glycerol, 200 g chloral hydrate; mix in order listed and filter through fine gauze), 10% solution of potassium hydroxide, sterile transport medium for viruses (available from Colab Laboratories, Chicago Heights, Illinois 60412).

Safety is an important consideration in doing any postmortem analysis because some avian disease organisms are pathogenic to man. The working area should have limited access so as to reduce bio-hazard. Use standard procedures in handling diseased tissue and liberal amounts of a strong detergent and disinfectant (e.g., Tincture Green Soap, Phenol, or Pine Oil).

PROCEDURE

GENERAL HANDLING OF A BIRD

Using the necropsy form (Fig. 1) as a guide, perform the necropsy as soon as the bird is received because internal organs decompose rapidly and postmortem migration of parasites and bacterial invasion might occur. Immediately take measurements because weight, in particular, will change. Measurements are important for aging purposes and may later prove useful as indicators of specific diseases within the host population. Feather wear, cloacal protuberance, and brood patch will help define the breeding condition of the specimen. Tag the bird, and label each sample taken from it with the same necropsy number. Indicate if samples are "sterile" or "non-sterile" and the type of medium in which they are preserved. Obtain a detailed history of the specimen as this often gives clues as to what might be wrong. When the necropsy is completed, fill in the preliminary findings under "necropsy summary," but complete the "diagnosis" section only after the laboratory results are returned.

The actual examination is accomplished by following the directions given in parentheses until a particular disorder is seen. Check the appropriate space, circle the term which best describes the disorder, and then, using the numbers at the end of the line as a guide, consult the Appendix for instructions on how to handle the diseased tissue. Each disorder can also be cross-referenced to a summary of avian diseases (Table 1) so that the investigator has some idea of possible diseases with which he may be dealing. Since the presence of an organism does not necessarily imply a pathogenic condition, careful notes concerning disorders are important.

Example. A bird is collected with extensive lesions on one leg. On the necropsy form (Fig. 1), body measurements, condition of plumage, and a complete history of the specimen are recorded. Section "I. External Analysis" is followed until the subsection on "Legs and Feet" is reached. Line "j" is checked, the word "lesions" is circled, and the numbers at the right of the line (6, 3, 1, 7, 8) are referred to in the Appendix for further directions on handling of tissue. First a smear of any exudate is prepared for gram stain or a bacterial culture (6), then a portion of the lesion is cultured on mycotic medium or examined for fungal hyphae (3). Next, a wet smear of the infected tissue is examined under a compound microscope for the presence of ectoparasites (1), and finally the remainder of the infected tissue is divided in half and placed in 10% buffered formalin and frozen (7, 8). Possible causes of this disorder are cross-referenced to all Ij diseases in Table 1, and include Bumblefoot, Favus, Hematozoa, arthropods (mites), or Avian Pox; Bumblefoot, mites, and Pox are most probable. After the section is completed in the above manner, the investigator continues the necropsy until each disorder is accounted for.

NECROPSY HINTS APPLICABLE TO SMALL BIRDS

Because of their size, smaller birds pose special problems during a necropsy. We have found the following techniques (prefaced by their corresponding sections in Fig. 1) useful in working with passerines.

I. *Body wash for external parasites.* Following the external analysis of a bird, the beak should be taped shut and the specimen washed in a jar with detergent water. This is not only a good way to collect ectoparasites (Watson and Amerson 1967), but also it minimizes flying feathers during the remainder of the necropsy.

II. *Celomic cavity and air sacs.* Careful examination of these structures is critical, especially for the identification of bacteria. To remove the keel, lift the abdominal wall with a forceps and cut transversely; grasp the tip of the keel, lift care-

NECROPSY #:_____

SPECIES:_____ _____ FIELD #:_____ MUSEUM #:_____

Area collected:_____
Collector:_____
Date collected:_____
Date examined:_____
Examiner:_____
Age:_____ Sex:_____
Fat:_____ Skull:_____
Weight:_____g P.M. state:_____
Preserved in:_____
Other:_____
_____

Body measurements—length of:
Body:_____mm Wing:_____mm
Tail:_____mm Beak:_____mm
Tarsus:_____mm Other:_____mm
Condition of plumage:
Body molt:_____ Wing molt:_____
Tail molt:_____ Head molt:_____
Worn plumage:_____ Area:_____
Breeding condition:
Brood patch:_____ Clo. P.:_____
Gonad meas.:_____mm

History of bird:_____

MATERIAL TO LABORATORY

Smears: _____peripheral blood _____brain _____heart _____liver _____spleen
_____fecal _____lung _____kidney _____bone marrow
other:_____

Tissue: _____entire bird _____leg _____feet _____eye _____esophagus
_____crop _____trachea _____brain _____muscle _____blood
_____serum _____heart _____liver _____gall bladder
_____spleen _____pancreas _____bursa _____intestine
_____proventriculus _____gizzard _____lung _____gonad
_____kidney _____nerves _____endocrine glands
other:_____

Body wash:_____ Crop contents:_____

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

Cultures:_____
_____
_____

Other:_____

NECROPSY SUMMARY:

LABORATORY RESULTS:

DIAGNOSIS:

FIGURE 1. A detailed avian necropsy protocol, keyed for tissue preservation and a preliminary disease diagnosis.

I. External Analysis

(Examine bird externally for disorders.)

HEAD AND BEAK

___ a. Face: lesions; crusty or scaly scabs (6,1,7,8)
___ b. Face or sinsuses swollen (6,7)
___ c. Ear disorder (1)

(Cut eyelids back and expose eyeball.)

___ d. Eye: inflamed; swollen; cloudy; exudate; helminths (6,3,2,7,8,9)

(Cut off beak at nostrils; examine with dissecting microscope.)

___ e. Nasal chamber: lesions; nodules; exudate; parasites (6,3,1,7,8,9)
___ Other:_____

BODY AND WINGS

___ f. Keel prominent; loss of weight (9)
___ g. Vent soiled; diarrhea (9)
___ h. Feathers: dry, easily broken, or absent; follicles infected (1)
___ i. Skin: dermatitis, ulceration, swelling; uropygial infected (6,3,1,7,8)
___ Other:_____

LEGS AND FEET

___ j. Legs or feet: lesions; crusty or scaly scabs (6,3,1,7,8)
___ k. Legs or feet: swollen; enlarged bones; inflamed joints (6,7,8)
___ l. Missing appendanges (9)
___ Other:_____

BODY WASH FOR EXTERNAL PARASITES

(Tape bill, shake in soapy water, remove bird, let settle, decant to alcohol.)

___ m. Comments:_____

II. Internal Analysis

BODY SURFACE

(Skin body, neck, head; record fat and skull condition.)

___ a. Muscles: lesions; discolored; hemorrhage (6,1,2,8,9)
___ b. Feather, follicle, or skin parasites (1)
___ Other:_____

UPPER DIGESTIVE AND RESPIRATORY SYSTEMS, SKULL, AND BRAIN

(Cut from mouth down neck exposing trachea and esophagus.)

___ c. Mouth, pharynx, esophagus: lesions; nodules; cheesy masses (6,3,5,7,8)

(Cut esophagus to proventriculus; preserve crop contents in alcohol.)

___ d. Crop: lining thickened; contents sour (5,2,8)

(Cut up length of trachea; examine with dissecting microscope.)

___ e. Trachea and bronchi: lesions; nodules; exudate; parasites (6,1,2,7,8)

(Remove skull; examine brain.)

___ f. Brain and meninges: lesions; nodules; discolored; hemorrhage (6,7,4,8)
___ Other:_____

CELOMIC CAVITY AND AIR SACS

(Cut out breast and slowly lift sternum. Examine air sacs.)

___ g. Air sacs: lesions; nodules; exudate (6,1,7,8)

(Examine internal organs and if any suggest infection, process immediately to avoid contamination.)

___ h. Abdomen: lesions; nodules; exudate (6,1,8)
___ Other:_____

HEART AND PERICARDIUM
(Examine pericardium and remove.)
___ i. Pericardium: exudate; inflamed; discolored; hemorrhage (6,7,8)
(Examine and weigh heart; prepare smear; fix for Giemsa; wait to preserve.)
___ j. Heart: enlarged; lesions; nodules; hemorrhage (6,7,8)
___ Other:_____

LIVER, SPLEEN, GALL BLADDER
(Examine liver, gall bladder; measure spleen:____x____mm.)
___ k. Liver disorder (6,4,7,8)_____
___ l. Spleen disorder (6,4,7,8)_____
___ m. Gall bladder disorder (6,2,7,8)_____
(Remove liver and spleen.)

INTESTINAL TRACT AND PANCREAS
(Examine external appearance of intestinal tract and membranes; do not open.)
___ n. Peritoneum: nodules; discolored; inflamed (6,8)
(Remove gastrointestinal tract and straighten on glass plate.)
(Intestine length:____mm. Separate proventriculus and gizzard.)
(Cut down length of intestine and lay open.)
(Take intestinal and cecal smears; check for protozoan parasites.) (5)
___ o. Intestine: ballooning; lesions; nodules; hemorrhage; helminths (6,2,5,7,8)
(Record location of parasites:_____)
___ p. Ceca: lesions; nodules; exudate; hemorrhage; thickened; helminths (6,2,5,7,8)
___ q. Bursa of Fabricius abnormal (7,8):_____
___ Other:_____
(Cut open proventriculus and gizzard. Remove lining; preserve contents.)
___ r. Proventriculus: lesions; nodules; hemorrhage; erosion; helminths (6,3,2,5,8)
___ s. Gizzard: lesions; nodules; hemorrhage; erosion; helminths (6,3,2,5,8)
___ Other:_____
(Examine pancreas.)
___ t. Pancreas: lesions; chalky; hemorrhage (7,8)

LUNGS
(Examine and remove lungs.)
___ u. Lungs: lesions; nodules; exudate; inflamed (6,3,7,8)
___ Other:_____

UROGENITAL SYSTEM AND ADRENAL GLANDS
(Measure and remove gonads; record sex; examine adrenals.)
___ v. Gonads or associated structures abnormal (6,7,8)
___ w. Adrenal glands abnormal (8,9)
(Examine and remove kidneys.)
___ x. Kidneys: lesions; nodules; discolored; enlarged (6,5,2,7,8)
___ Other:_____

NERVOUS SYSTEM
(Examine nervous plexus.)
___ y. Nerves: lesions; discolored; swollen (8)
___ Other:_____

SKELETAL SYSTEM
(Examine vertebrae; break leg bone and examine bone marrow.)
___ z. Bone marrow, vertebrae, bones, tendons, or joints infected (6,4,7,8)
___ Other:_____

COMMENTS:_____
_____
_____

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

Disease	Pathogen(s)	Postmortem findings[2]	Tissue to collect[3]	Avian hosts[4]
PARASITIC INFECTIONS				
Arthropods	Biting insects, lice, mites	I a, c, d, e, f, **h**, **i**, **j** II a, **b**, e, g, h, u	par	Probably all orders susceptible
Helminths	Acanthocephala; Cestoda, Nematoda, Trematoda	I b, d, e, f, g II a, c, d, e, h, k, m, **o**, **p**, **r**, **s**, t, u, v, x	par	Probably all orders susceptible
FUNGAL DISEASES				
Aspergillosis	*Aspergillus fumigatus*	I b, d, e, h, i II **c**, e, f, **g**, **u**	lu, as	Sp, Str, Rh, Ti, Ga, Pro, Pel, Ci, A, F, G, Gr, Ch, C, Ps, St, **T**, Cor, P
Candidiasis	*Candida albicans*	I h II c, **d**, k, r, s	ms, c	Sp, Rh, Ci, A, G, Gr, Ch, C, Ps, Cu, Ap, Pic, P
Favus	*Trichophyton* sp.	I a, e, f, **h**, **i**, j II e, g, o, u	sk, l	**G**, P
Moniliasis (see Candidiasis)				
Other Mycosis	*Absidia* sp., *Cryptococcus* sp., *Histoplasma* sp., others	**I** e II f, g, k, l, o, u, x	l	Sp, Ci, A, G, C, Ps, P
Sour Crop (see Candidiasis)				
Thrush (see Candidiasis)				
White Comb (see Favus)				
PROTOZOAN DISEASES				
Blackhead (see Histomoniasis)				
Coccidiosis	*Isospora* sp., *Eimeria* sp., others	I **f**, **g** II **o**, **p**, x	ic, cm, i	Str, Ti, Pro, Pel, Ci, A, F, G, Gr, Ch, C, Ps, Cu, St, Cor, Pic, P
Frounce (see Trichomoniasis)				
Hematozoa	*Haemoproteus* sp., *Leucocytozoon* sp., *Plasmodium* sp., *Trypanosoma* sp., *Aegyptianella* sp., *Lankesterella* sp., others	II a, f, g, i, j, **k**, **l**, o, p, u, x, z	bs; ois:li, s, bm, br	Sp, Str, Pod, Pel, Ci, A, F, G, Gr, Ch, C, Ps, Mu, Cu, St, Cap, Ap, T, Cor, Pic, P. (Probably all orders susceptible to some type of blood protozoa if vectors are present.)

TABLE 1. Continued.

Disease	Pathogen(s)	Postmortem findings[2]	Tissue to collect[3]	Avian hosts[4]
Histomoniasis	*Histomonas meleagridis*	I **g** II i, **k**, n, o, **p**	li, ce	G
Intestinal Amebiasis	*Entamoeba* sp., *Endolimax* sp., others	II k, **o**, **p**	ic, cm, i	A, F, G
Intestinal Flagellates	*Hexamita* sp., *Trichomonas* sp., others	I f, **g** II a, **o**, **p**	ic, cm, i	Ca, Pel, A, F, G, Gr, Ch, C, Cu, St, Cap, P
Sarcosporidiosis	*Sarcocystis* sp.	II **a**, j, k, l, o, x	mu	Ci, A, F, G, Ch, Ps, St, P
Toxoplasmosis	*Toxoplasma gondii*	I d, **f**, g II f, **i**, **j**, **k**, **l**, o, u	ois:br, lu, li, s, h, bm, e; br	Sp, A, G, C, Pic, P
Trichomoniasis	*Trichomonas* sp.	I d, f II **c**, **d**, k, r	ms, c, es, li	Ca, A, F, G, **C**, P
BACTERIAL DISEASES				
	Actinobacillus sp. (gram neg; rod)	I d, k II e, g, r	rt	A, Ps
Air Sac Disease (see Colibacillosis)				
Arizonosis	*Arizona* sp. (gram neg; rod)	I **d**, g II **f**, h, j, k, n, v	li, b, s, go, lu, k, i	A, **G**, Gr, Ps, P
Anthrax	*Bacillus anthracis* (gram pos; rod)	II l, u	b, s, bs	Str, Ci, A, F, G, C, P
Avian Hemorrhagic Septicemia (see Cholera)				
Bacillary White Diarrhea (see Pullorum Disease)				
Arthritis (see Staphylococcosis)				
Bacterial Endocarditis (see Streptococcosis)				
Botulism	*Clostridium botulinum* (gram pos; rod)	I h II y	ser, ic	Ga, Pod, Pel, Ci, A, F, G, Ch, C, St, P
Bumblefoot	*Staphylococcus* sp., *Streptococcus* sp., *Escherichia coli*, *Pasteurella* sp., *Mycobacterium* sp.	I **j**, k	f	Ci, A, F, G, Gr, C, St, P
Chlamydiosis	*Chlamydia psittaci* (gram neg; coccoid)	I b, d, **e**, f, g II **g**, h, **i**, j, **k**, **l**, m, o, **u**	li, s, k, re, ic	Ga, Pro, Pel, Ci, A, F, G, Gr, Ch, C, **Ps**, St, Ap, Cor, Pic, P
Cholera	*Pasteurella multocida* (gram neg; bipolar rod)	I b, d, e, g, k II e, f, g, h, **i**, **k**, l, n, **o**, u, v, **z**	ois:li; bm, b, li, mn, re	Sp, Pod, Pro, Pel, Ci, **A**, F, G, Gr, Ch, C, Ps, St, P

TABLE 1. Continued.

Disease	Pathogen(s)	Postmortem findings[2]	Tissue to collect[3]	Avian hosts[4]
Chronic Respiratory Disease (see Mycoplasmosis)				
Colibacillosis	*Escherichia coli* (gram neg; rod)	Respiratory: I b, d, e II e, **g**, i, k, u, v Septicemic: I d, g, k II **a**, g, h, i, j, **k**, l, n, o, p, v, x, z	p, li, rt, ov, ic	Str, A, F, G, Gr, Ch, C, Ps, St, P
Duck Septicemia (see Infectious Serositis)				
Erysipelas	*Erysipelothrix* sp. (gram pos; rod)	I a, e, **g**, i II a, g, **i**, **j**, **k**, **l**, o, p, r, s, t, u, v, x, z	li, s, bm, h, b, ce	Sp, Pod, Pel, Ci, A, F, G, Gr, Ch, C, Ps, Cu, St, P
Fowl Typhoid	*Salmonella gallinarum* (gram neg; rod)	I e, g II i, j, **k**, **l**, n, o, p, r, s, u, v, x	li, s, ser	Str, A, G
Gangrenous Dermatitis (see Wound infection)				
Infectious Coryza	*Haemophilus gallinarum* (gram neg; rod)	I **b**, **d**, **e**, g II e, g, u	re	G
Infectious Enteritis (see Vibrio infections)				
Infectious Serositis	*Pasteurella anatipestifer* (gram neg; rod)	I b, **d**, e, f, **g**, i, k II e, f, g, **i**, **k**, l, n, u, v, y, z	bm, b, li, s, lu, br, re	**A**, G
Infectious Synovitis (see Mycoplasmosis)				
Limberneck (see Botulism)				
Listeriosis	*Listeria monocytogenes* (gram pos; rod)	II **i**, **j**, k, l, r	b, li, s, h, br	Pel, A, F, G, Gr, Ch, C, Ps, St, Pic, P
Mycoplasmosis	*Mycoplasma* sp. (gram neg; coccoid)	Respiratory: I b, d, **e** II **e**, **g**, u Skeletal: I f, g, **k** II a, g, k, l, o, x, **z**	ser, re, lu, br, li, s, k, j	F, G, C, Ps, P
Necrotic Dermatitis (see Wound infection)				
Necrotic Enteritis (see Ulcerative Enteritis)				
New Duck Disease (see Infectious Serositis)				
Ornithosis (see Chlamydiosis)				

TABLE 1. Continued.

Disease	Pathogen(s)	Postmortem findings[2]	Tissue to collect[3]	Avian hosts[4]
Paratyphoid	*Salmonella* sp. (gram neg; rod)	I d, f, **g**, k II c, g, **i**, j, **k**, **l**, n, **o**, p, u, v, **x**, z	cm, ic, li	Probably all orders susceptible
Pasteurellosis (see Cholera)				
Pseudotuberculosis	*Pasteurella pseudotuberculosis* (gram neg; rod)	I g II a, g, **k**, **l**, n, **o**, p, u	li, s	Sp, Pel, A, F, G, Gr, Ch, C, Ps, Cu, St, T, Cor, Pic, P
Psittacosis (see Chlamydiosis)				
Pullorum Disease	*Salmonella pullorum* (gram neg; rod)	I d, **g**, k II a, i, **j**, **k**, l, n, o, **p**, **s**, **u**, v, x	li, s, i, h, go	Ci, A, G, C, P
Quail Disease (see Ulcerative Enteritis)				
Salmonellosis (see Fowl Typhoid, Paratyphoid, and Pullorum Disease)				
Spirochetosis	*Borrelia anserina* (spiral)	I f, **g** II k, **l**, o, x	bs, li, k	A, F, G, C, P
Spondylitis (see Staphylococcosis)				
Staphylococcosis	*Staphylococcus aureus* (gram pos; coccoid)	I g, i, **k**, l II **k**, l, o, x, **z**	b, j, li, s, h, ver	Probably all orders susceptible
Streptococcosis	*Streptococcus* sp. (gram pos; coccoid)	I a, f, g, i, k II i, **j**, **k**, **l**, n, v, z	b, li, s, h	Str, Ci, A, G, Gr, Ch, C, Ps, St, Ap, P
Tuberculosis	*Mycobacterium avium* (gram neg; acid-fast; rod)	I a, **f**, g, k II g, h, i, **k**, **l**, **o**, s, t, u, v, x, **z**	li, s, l, pt, bm	Sp, Str, Rh, Ca, Ti, Ga, Pel, Ci, A, F, G, Gr, Ch, C, Ps, Cu, St, Ap, Cor, Pic, P (Probably all orders susceptible.)
Ulcerative Enteritis	*Clostridium colinum* (gram pos; rod)	I **g** II k, l, **o**, **p**	i, li, s	Str, Ca, Ci, F, G, C, Ps, P
Vibrio Infections	*Vibrio* sp. (gram neg; spiral)	Hepatic: I **g** II i, j, **k**, l, o, v, x, z Enteric: I **g** II **l**, **o**, p Other: I b, e II a, k, n	bi, li, s, k, lu, i	Sp, A, G, Gr, C, P
Vibrionic Hepatitis (see Vibrio infections)				

TABLE 1. Continued.

Disease	Pathogen(s)	Postmortem findings[2]	Tissue to collect[3]	Avian hosts[4]
Western Duck Sickness (see Botulism)				
Wound Infection	*Staphylococcus aureus*, *Clostridium* sp., *Escherichia coli*	I a, **i**, j II **a**, k, l, u, x	l, li, s, lu, k	Probably all orders susceptible
VIRAL DISEASES				
Arbovirus	...	I g II **f**, y	ser, br, h, s, li	A, G, C, P
Avian Encephalomyelitis	...	I d II f, **s**, y	br	A, **G**, C
Avian Infectious Bronchitis	...	I b, d, e II **e**, g, u, v, x	lu, tr, k, ov, ser	Pro, **G**
Avian Influenza	...	I b, d, **e**, g II **e**, f, **g**, i, k, l, n, **u**, v, x	rt, li, s, b, ser	**A**, F, **G**, Ch, C, Ps
Avian Pox	...	I **a**, e, i, **j**, l II **c**, e	sk, l, ser	Str, Pod, Pel, A, F, G, Gr, Ch, C, Ps, St, Ap, Pic, P
Bluecomb	...	I f, g II a, d, k, l, **o**, p, **t**, x	ic, cm, bF	G
Coronaviral Enteritis (see Bluecomb)				
Duck Virus Enteritis	...	I e, f, g II c, g, **h**, i, k, l, **o**, p, q, r, t, u, x	li, s, ser, h, k, es, cl, bF, e	A
Duck Virus Hepatitis	...	II **k**, l, x	li	A
Encephalitis (see Arbovirus)				
Equine Encephalomyelitis (see Arbovirus)				
Duck Plague (see Duck Virus Enteritis)				
Fowl Plague (see Avian Influenza)				
Hemorrhagic Enteritis	...	I g II a, f, j, k, **l**, **o**, r, s, t, u, x	ic, s	G
Inclusion Body Hepatitis	...	II **a**, **k**, l, q	li	F, **G**, St
Infectious Anemia (see Inclusion Body Hepatitis)				
Infectious Bursal Disease	...	I g II a, l, o, **q**, x	bF, s	G
Laryngotracheitis	...	I b, d, **e** II c, **e**, g, u	tr, lu, ser	G
Monocytosis (see Bluecomb)				

TABLE 1. Continued.

Disease	Pathogen(s)	Postmortem findings[2]	Tissue to collect[3]	Avian hosts[4]
Neoplasms: Erythroblastosis (Leukemia); Hemangioma; Leukosis (Big Liver Disease); Marek's Disease; Myeloblastosis; Myelocytomatosis; Nephroblastoma; Osteopetrosis (Marble Bone, Thick Leg Disease)	...	Circulatory: I f, g, i II a, i, **k**, **l**, u, x, **z** Skeletal: I h, **k** II a, k, l, x, **z** Kidney: II **x** Leukosis I f II g, h, j, **k**, **l**, n, **q**, u, v, x, z Marek's I **d**, f, g, h, i II a, f, h, j, k, l, n, o, r, t, u, **v**, w, x, **y**, z	l, bF, n, bm, bo (Marek's Disease: k, l, s, b)	Probably all orders susceptible; Leukosis and Marek's Disease most widespread; **G**, **Ps**, most often reported with neoplasms or diseases of neoplastic origin.
Newcastle Disease	...	I d, **e** II **e**, **f**, **g**, i, k, l, m, **o**, r, s, t, u, v, **y**	lu, br, s, li, ki, bm	Sp, Str, Rh, Ca, Pel, Ci, A, F, G, Gr, Ch, C, Ps, Cu, St, Cor, Pic, P
Puffinosis	...	I d, **j**	f	**Pro**, A, **Ch**, C
Quail Bronchitis	...	I d, e II **e**, **g**, u	re	A, **F**, **G**, P
Turkey Viral Hepatitis	...	II **k**, m, t	ic, li, s	G
Viral Arthritis	...	I **k** II **z**	sf, s	G

[1] Information not our own was taken from the following sources: Arnall and Keymer (1975), Ashford et al. (1976), Bennett and Herman (1976), Daoust (1978), Davis et al. (1971), Dawson et al. (1976), Greiner et al. (1975), Hacking and Sileo (1977), Hitchner et al. (1975), Hofstad et al. (1978), Keymer (1972), Kirmse (1967), Kocan, Potgieter, and Kocan (1977), Kocan, Snelling, and Greiner (1977), McClure et al. (1978), Merck Chemical Division (1975), Pellérdy (1974), Petrak (1969), Stoner and Stoner (1945), Ward and Gallagher (1926), Windingstad et al. (1977), Winterfield and Berkhoff (1977).

[2] Letters refer to categories in parts I and II on the necropsy form (Fig. 1); they indicate a disorder likely to be encountered with a particular disease. Boldface letters indicate characteristic findings. Use caution in interpretation as postmortem findings may differ between acute and chronic cases and between young and adult hosts, or unusual hosts.

[3] Abbreviations refer to tissue that should be cultured or collected if a particular disease is suspected. In general, always use diseased tissue, even if not listed. Key: as = air sacs, b = blood, bF = bursa of Fabricius, bi = bile, bm = bone marrow, bo = bone, br = brain, bs = blood smear, c = crop, ce = ceca, cl = cloaca, cm = cecal material, e = eye, es = esophagus, f = foot or leg, g = gizzard, go = gonad, h = heart, i = intestine, ic = intestinal contents or fecal material, j = joints, k = kidneys, l = lesions or tumor or tubercles, li = liver, lu = lung, mn = meniges, ms = mucosal scrapings, mu = muscle, n = nerve, ois = organ impression smear, ov = oviducts, p = pericardium, par = parasites, pt = peritoneum, re = respiratory exudate, rt = respiratory tract, s = spleen, ser = serum, sf = synovial fluid, sk = skin, tr = trachea, ver = vertebrae.

[4] Abbreviations refer to the orders of birds in which the diseases have been commonly reported. Wild, domestic, cage, and laboratory birds that have shown susceptibility are included. However, care should be exercised in interpretation as many groups have not been well studied. Boldface indicates an order in which that disease is especially critical or more 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 = Charadriiformes, C = Columbiformes, Ps = Psittaciformes, Mu = Musophagiformes, Cu = Cuculiformes, St = Strigiformes, Cap = Caprimulgiformes, Ap = Apodiformes, Col = Coliiformes, T = Trogoniformes, Cor = Coraciiformes, Pic = Piciformes, P = Passeriformes.

fully, and cut anteriorly across the ribs with a scissors (for larger birds bone shears will be necessary). Do not touch the surface of any internal organs lest they become contaminated. Cut through the coracoids and clavicles to complete removal of the sternum. If any of the internal organs indicate bacterial infection, make cultures now, before their surfaces become contaminated.

II.l. *Spleen disorder.* The spleen is an important indicator organ, but is sometimes difficult to find in small birds. It is usually reddish-brown, either spherical or slightly oblongate, and lies on the cranio-dorso side of the gizzard. It can be seen by rotating the gizzard 180° to the left (counter-clockwise) with the forceps.

II. *Intestinal tract and pancreas.* In order to avoid contaminating the remaining organs, remove the gastrointestinal tract as an entire unit. Cut the abdominal wall around the cloaca, and remove the tract whole, cutting as far anterior of the proventriculus as possible. Stretch the intestine on a dry glass plate; separate the proventriculus-gizzard and set them aside. Measure the length of the intestine so that the location of all parasites can be pinpointed. A 45° angle iridectomy scissor is a useful tool for opening the small intestine. Some intestinal helminths of small birds are minute and need to be manipulated with instruments as small as microprobes. These probes can be cheaply made by inserting or melting an insect pin into a swizzle-stick.

II.r. and s. *Proventriculus-Gizzard.* Examine the proventriculus and gizzard after the intestinal tract as they dry out less rapidly. Be sure to carefully tease the gizzard lining apart from the muscle, because many parasitic helminths occur there.

II. *Nervous system.* Remove all internal organs so that the nerve plexes can be seen crossing over the various bones.

DISCUSSION

Research is needed on disease in wild birds, especially to delimit the parasite fauna present within a host. Recording the levels of a single pathogen cannot possibly determine the impact of diseases upon wild bird populations. A comprehensive approach is necessary in order to reveal how diseases are interrelated. We hope that our postmortem technique will assist ornithologists to attempt such multi-disease studies.

Investigators should remember, however, that disease categories have been largely determined from poultry disorders, and they may differ in other avian groups. Moreover, diseases may share common signs, especially those that undergo a septicemic phase. Therefore it is usually only by a thorough necropsy analysis, supplemented with laboratory tests, that a particular disease can be positively identified.

Problems may arise in trying to find a laboratory that will process tissues and identify pathogens. Several agencies have well-established laboratories specializing in avian disease (e.g., U.S. Fish and Wildlife Service), but they are usually reluctant to accept material from outside researchers due to lack of time or personnel. Instead, try state departments of agriculture or health, veterinary pathology laboratories, university medical laboratories, or veterinary science departments. The U.S. Department of Agriculture Veterinary Services Diagnostic Laboratory at Ames, Iowa, might also be consulted.

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APPENDIX

The following section is an outline of how to handle diseased tissue for diagnosis or shipment to a diagnostic laboratory. Be sure to check with the laboratory for its preferred procedure before sending specimens. Numbers preceding each paragraph correspond to the numbers at the ends of the lines in the necropsy form (Fig. 1).

If you suspect a PARASITIC infection:

(1) *Arthropod Parasites.* Examine suspect area (e.g., nasal chamber, inner surface of skin, feather vane or shaft, inner feather shaft, follicles) with dissecting microscope for parasites. Scrape lesions and suspect tissue (e.g., irritated areas, crusty scales or scabs, dry skin, nodules) onto a slide with mineral oil or water and make a wet mount; examine with a compound microscope. Preserve mites, lice, and small insects in 70% alcohol. For permanent mounts of ectoparasites drop the specimen directly into Hoyer's mounting medium, pass over a flame to relax the specimen, then place on a cover slip; store in a horizontal position until dry.

(2) *Helminth Parasites.* Examine appropriate area (e.g., under gizzard lining, under nictitating membrane of eye, inside trachea, in bile ducts, along intestine and ceca) with compound microscope. Nematodes may be fixed directly in glycerine-alcohol solution and shipped. Cestodes, trematodes, and acanthocephalans should be placed in F.A.A. for 24 h and then transferred to 70% alcohol for shipment. To examine fecal material for helminth ova, follow procedure outlined under digestive tract protozoa.

If you suspect a FUNGAL infection:

(3) Culture suspect tissue on mycotic medium. Sear the surface with a hot spatula if tissue has been contaminated, incise tissue, and sample cut surface. If no medium is available, place sample in 10% formalin. Fungal hyphae may be observed using a wet mount (Lugol's solution will make them more visible but is not necessary); they may be permanently mounted in 10% potassium hydroxide, relaxed and cleared with gentle heat.

If you suspect a PROTOZOAN infection:

(4) *Blood Protozoa.* Prepare a smear for Giemsa stain using heart blood, bone marrow, organ impressions (touch cut surface of organ to clean slide) of liver, spleen, and brain. Fix in absolute methyl alcohol for 30 s, and stain according to standard technique. Examine using oil immersion lens.

(5) *Digestive Tract Protozoa.* Prepare a wet smear from appropriate material (e.g., mouth lesions, intestinal and cecal contents) and examine under compound microscope. Lugol's solution will be helpful but is not necessary. If coccidia are present and sporulation is desired, place sample in 2% potassium dichromate and examine again in four days.

If you suspect a BACTERIAL infection:

(6) Most bacterial infections cannot be positively diagnosed without identification of the pathogen, and for this reason it is best to collect a sample for bacterial culture. If an organ has been collected under nonsterile conditions, sear the surface with a hot spatula, incise the tissue, and sample the cut surface. Sample moist membranes and soft organs with sterile swabs; place the entire swab directly in medium for shipment and refrigerate. Collect joint or nasal exudate with sterile hypodermic needle or swab. If solid agar is used, place the tissue firmly against agar or embed several small pieces. Use appropriate material from lesions, exudate, or abnormal tissue. If certain diseases are suspected, one tissue will be preferred over others (see Table 1). Otherwise the liver, spleen, heart and any diseased tissue are usually good choices.

Sometimes a presumptive diagnosis can be made on the basis of slides showing the pathogen. To prepare a smear for gram stain, use appropriate material (e.g., exudate, necrotic lesions, organ impression smears, heart blood, bone marrow, joint fluid collected with a sterile syringe). Fix by drying over heat and stain according to standard methods. Use the same procedure for staining with Giemsa, except fix in absolute methyl alcohol for 30 s without heat. Ziehl-Neelsen stain should be used if acid-fast bacteria are suspected or tubercles are present. Squash a small (2 mm) piece of tissue between two slides and fix over heat.

For serological testing, collect blood aseptically from the heart and centrifuge immediately. Transfer serum to sterile vials and freeze for shipment. If little blood is available, sealed capillary tubes are usually sufficient.

If you suspect a VIRAL infection:

(7) Most viral infections cannot be diagnosed without isolating the pathogen. Viral culture medium is acceptable, but freezing is usually preferred. Freeze the sample, using dry ice to rapidly lower the temperature below −60°C (glass often shatters so plastic bags may be used). Use material from infected tissue, exudate, or joint fluid (collect with a sterile syringe). If one section of the respiratory system (e.g., air sacs) is involved, it is good to freeze tissue from other respiratory areas (e.g., sinus, trachea, lungs), and cloaca, even though the tissue may appear normal. Other suggested representative tissue to collect includes liver, spleen, bone marrow, blood or serum, and bursa of Fabricius. See Table 1 if a particular disease is suspected. Collect blood for serological testing as described under section 5 above; do not freeze whole blood.

If tissue is to be for GENERAL HISTOPATHOLOGY:

(8) Preserve infected tissue in 10% buffered formalin (some laboratories prefer other media such as Zenker's). When nerves are diseased, check the brain carefully for abnormalities and preserve in 10% formalin, even if it appears normal. With any disease, take representative sections of the body; sections of heart, lung, respiratory system, gastrointestinal tract, kidney, liver, brain (especially if nerves are diseased), spleen, gonads, muscle, and skeleton are recommended. Cut tissue samples in pieces no larger than 1.0 × 2.0 × 0.5 cm and place in 10 times the amount of formalin. After 24 h the tissue may be packed with less formalin or left as is.

If SECONDARY SYMPTOMS and NERVOUS SIGNS are present before death:

(9) Many signs and symptoms are of a secondary nature, and when they are encountered the investigator should be aware of other abnormalities. Especially note the condition of the respiratory or digestive systems. Earlier trauma (e.g., missing toes due to freezing or pox infections) or shock signs should be noted in history of the bird. If nervous signs were noticed before the bird was collected or died, preserve the brain and nerve tissue at end of necropsy on dry ice and submit for viral and histopathology analysis. If poisoning is suspected, collect and freeze a sample of the body fat.

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