

ELEPHANT TAG/SSP RESEARCH AND NECROPSY PROTOCOL

(Elephas maximus and Loxodonta africana)



*The American Zoo and Aquarium Association
Elephant Species Survival Plan*

April, 2019

TABLE OF CONTENTS

| | |
|---|-------|
| Abstract/summary | 3 |
| Introduction | 4 |
| Elephant Herpesvirus Alert | 5 |
| Elephant Tuberculosis Alert..... | 5-6 |
| Internet Sites..... | 6 |
| Equipment Checklist | 7 |
| Logistics / Necropsy Tips..... | 8 |
| Carcass disposal | 9 |
| Gross Examination Worksheet..... | 10-12 |
| Tissue Check List..... | 13 |
| Researchers Interested in Participating in Necropsies | 14 |
| Research requests..... | 15 |
| Form for requesting elephant tissue/blood samples..... | 23 |
| Literature cited..... | 24 |

ABSTRACT / SUMMARY

Due to the length of this protocol, a brief summary is provided here as a reminder for those who have previously performed an elephant necropsy. Those persons or institutions who have not previously performed an elephant necropsy should read the protocol in its entirety to ensure completion of a **safe**, efficient, and accurate necropsy procedure.

This necropsy protocol should be used in conjunction with the optional SSP research and tissue requests (included at the end of this document) to facilitate collection of a complete tissue, sample, and data set. Several pathologists, clinical veterinarians, and scientists are potentially available to assist institutions with elephant necropsies. Assistance may be in the form of telephone consultation or, if given sufficient notice and time to travel, on-site guidance (contact information available at the end of this document). Two of the more important disease processes in elephants include **endotheliotropic herpes virus (EEHV) infection** and **tuberculosis** (caused by the human pathogen, *Mycobacterium tuberculosis*). Specific sample collection protocols are available for these diseases; some procedures are delineated here, and others are covered in supplemental documents (the **Elephant Endotheliotropic Herpesvirus Research and Necropsy Protocol Supplement** is available at eehvinfo.org and www.aazv.org under the SSP/TAG protocols; and Guidelines for the Control of Tuberculosis in Elephants protocol is available through the USDA website – http://www.aphis.usda.gov/animal_welfare/index.shtml) and Recommendations for the Diagnosis, Treatment and Management of Tuberculosis (*Mycobacterium tuberculosis*) in Elephants in Human Care through the IEF website - <https://elephantconservation.org/stay-informed/recommendations-for-tb-in-elephants-in-human-care/>.) and should be followed in detail if either disease is suspected. If the TB test status of the elephant is unknown, suspect, or positive, close attention should be paid to the tuberculosis alert in this protocol to ensure the safety of staff participating in the necropsy and to prevent contamination of the surrounding areas or animals. Most equipment listed in the protocol is similar to that used in smaller animal necropsies with the addition of heavy equipment (tractor), chain saw or reciprocating saw, an axe, numerous large knives, chains, straps, and the very important TB protective equipment. To complete a detailed necropsy, a team of at least 6-8 people should be assembled for 8-10 hours of work. Team members should be organized and assigned specific roles (supervising pathologist or clinician, prosectors, dedicated knife sharpener, and various assistants to collect samples, take notes, and take photos). Heavy equipment or chain hoists should be used to remove and move large body parts (limbs, head, etc.) for safety and efficiency reasons. The gastrointestinal tract of the elephant is massive but relatively simple, and the remaining organs are similar to those in other mammals (with some exceptions listed in the protocol). The thoracic cavity should be examined last and in those cases with unknown, suspect, or positive TB-results, special precautions are required (see TB alert). Removal of the brain is difficult and requires use of a chain or reciprocating saw. The protocol provides some guidance to facilitate this task. Disposal of an elephant carcass is a job in and of itself. Ideally, the necropsy should be performed within or adjacent to a burial site large enough to accommodate the carcass. Special burial permissions may be required depending on city, county, and state regulations, and those agencies should be contacted as soon as possible.

Post-mortem examination of an elephant can be a daunting task, but with proper personnel, planning, and experience, the procedure can be done safely and efficiently. If at all possible, institutions should make preparations or contingency plans for the movement, necropsy, and disposal of an elephant ahead of time to avoid the stress of planning at the time of death. The information gained from an elephant necropsy is hugely valuable to institutions, the AZA, and to elephants in captivity and in the wild.

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April 2019

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INTRODUCTION

This protocol is an effort of the Elephant Species Survival Plan (SSP) Propagation Group of the American Zoo and Aquarium Association (AZA). The purpose is to provide a format for the systematic collection of information and samples that will add to our knowledge of elephants. All North American institutions holding elephants will receive a copy.

An elephant necropsy may be a daunting procedure, however, it should be viewed as an important learning opportunity. Although it may not be feasible to collect all the information and samples requested in each case, collection of as much as possible is encouraged. With the increased availability of digital cameras, photographic documentation of both normal and pathologic structures for future reference is also strongly recommended.

Sample and data collection information for research is presented at the end of this document. The requested data sets are optional. Some of these observations may be applied to live animals. Therefore, this protocol, the **Elephant Endotheliotropic Herpesvirus Research and Necropsy Protocol Supplement, Guidelines for the Control of Tuberculosis in Elephants**, and **Recommendations for the Diagnosis, Treatment and Management of Tuberculosis (*Mycobacterium tuberculosis*) in Elephants in Human Care** should be referred to when planning a procedure that might facilitate data collection.

Familiarity with the protocols in these documents and having the necessary equipment ready will facilitate sample collection. It is suggested that a necropsy team be designated in advance; the ability to mobilize skilled individuals quickly will save valuable time particularly in the event of a sudden death. Veterinarians, anatomists, and pathologists from nearby universities and zoos may be enlisted to assist the institution's staff. In addition, a list of researchers interested in participating in elephant necropsies is included in this protocol.

The Elephant TAG/SSP Research and Necropsy Protocol will be revised as required. Contact Jaime Landolfi with any changes. **A copy of the completed final necropsy and any other lab reports should be forwarded, with digital images if applicable, to Dr. Landolfi for inclusion in the TAG/SSP pathology archive.**

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ELEPHANT HERPESVIRUS DISEASE ALERT

All elephants that die or are euthanized for any reason should have a systematic collection of information and samples that will contribute to our knowledge of Elephant Endotheliotropic Herpesvirus (EEHV). Please refer to the Elephant Endotheliotropic Herpesvirus Research and Necropsy Protocol Supplement (available at www.eehvinfo.org and www.aazv.org) for some disease-specific protocol guidance. Sample and research requests are also included at the end of this document. Contact the Elephant SSP Veterinary Advisors or listed researchers for specific questions.

ELEPHANT TUBERCULOSIS ALERT

An intense search for lesions of tuberculosis (TB) is encouraged in all elephant necropsies. **This should include all elephants that die or are euthanized for other reasons (even if TB is not suspected).** Be advised that elephant TB is likely to be caused by *Mycobacterium tuberculosis* which is contagious to humans. Therefore, participants must be prepared with proper protective apparel, and any suspicious organs or lesions should be contained as soon as possible.

Ideally, elephants should be bled for currently available TB diagnostic test development and validation, and trunk wash(es) collected just prior to euthanasia. Elephants that die naturally should have a post mortem trunk wash performed, and serum should be harvested from post mortem blood for serological assays. Available online documents (**2017 Recommendations for the Diagnosis, Treatment and Management of Tuberculosis (*Mycobacterium tuberculosis*) in Elephants in Human Care** [http://www.nasphv.org/Documents/ElephantTB_NASPHV.pdf] and **Guidelines for the Control of Tuberculosis in Elephants** [https://www.aphis.usda.gov/animal_welfare/downloads/elephant/TB-Guidelines-Final-8-November-2010.pdf] and) may be consulted for further information.

Protective equipment for tuberculosis cases

Respiratory protective equipment should be available for all elephant necropsy procedures regardless of the historical TB testing status of the animal. In animals with an unknown, suspect, or positive TB test history, respiratory protection should be considered **mandatory**. OSHA standards (29CFR1910.134) require that “workers present during the performance of high hazard procedures on individuals (humans) with suspicious or confirmed TB” be given access to protective respirators (at least N-95 level masks). Similar precautions should be taken during an elephant necropsy. According to the draft CDC guidelines for the prevention of transmission of tuberculosis in health care settings, respiratory protective devices used for protection against *M. tuberculosis* should meet the following criteria:

1. Particulate filter respirators approved include (N-,R-, or P-95,99, or 100) disposable respirators or positive air pressure respirators (PAPRs) with high efficiency filters
2. Ability to adequately fit wearers who are included in a formal respiratory protection program with well-fitting respirators such as those with a fit factor of greater than or equal to 100 for disposable or other half-mask respirators
3. Ability to fit the different face sizes and characteristics of wearers. This can usually be met by supplying respirators in at least 3 sizes. PAPRs may work better than half-masks for those persons with facial hair.

See website links below for OSHA and CDC guidelines.

Necropsy procedures

All elephants require careful examination of the tonsillar regions and submandibular lymph nodes for possible tuberculous lesions. These lymph nodes may be more easily visualized following removal of the tongue and laryngeal structures during the dissection. All lymph nodes (including extra-thoracic) should be carefully evaluated for lesions since other sites may also be infected (e. g. reproductive or gastrointestinal tract). Any nodes that appear caseous or granulomatous should be collected for both culture (freeze or ultrafreeze) and fixation (in buffered 10% formalin).

Thoracic organs should be carefully evaluated for early stages of TB as follows:

- After removal of the lungs and trachea, locate the bronchial nodes at the tracheal bifurcation. Use clean or sterile instruments to section the nodes. Freeze half of the lymph node to be submitted for mycobacterial culture (NVSL or another experienced laboratory is recommended). Culture should be done **even if no gross lesions are evident**. Submit sections in formalin for histopathology.
- Carefully palpate the lobes of both lungs from the apices to the caudal borders to detect any firm B-B shot to nodular size lesions. Take **NUMEROUS (5 or more)** sections of any suspicious lesions. In the absence of visible or palpable lesions, collect 4 representative sections of lung for histopathology (2 sections from each side including one cranioventral and one caudodorsal section).
- Open the trachea and look for nodules or plaques and process as above. Regional thoracic and tracheal lymph nodes should also be examined and processed accordingly.
- Split the trunk from the tip to its insertion and take samples of any plaques, nodules or suspicious areas.
- Look for and collect possible extra-thoracic TB lesions, particularly if there is evidence of advanced pulmonary TB.

For further information on laboratories performing diagnostic tests for TB, consult the current version of the **Guidelines for the Control of Tuberculosis in Elephants and Recommendations for the Diagnosis, Treatment and Management of Tuberculosis (*Mycobacterium tuberculosis*) in Elephants in Human Care (2017)**.

INTERNET SITES

These guidelines and other elephant protocols are available on the internet at the following sites:

1. http://www.aphis.usda.gov/animal_welfare/index.shtml
2. www.aazv.org
3. www.elephantcare.org
4. http://www.aphis.usda.gov/animal_welfare/downloads/elephant/Postmortem%20Exam%20Procedures.pdf – Elephant Postmortem Examination
5. <http://www.osha.gov/SLTC/tuberculosis/standards.html> - OSHA TB standards and rules
6. <http://www.eehvinfo.org> – Information on Elephant Endotheliotropic Herpesvirus
7. http://www.nasphv.org/Documents/ElephantTB_NASPHV.pdf - Information on Elephant TB; updated 2017

EQUIPMENT CHECKLIST

1. At least 6 quality large necropsy knives, knife sharpener, diamond, steel, and/or stone
2. Standard large animal necropsy instruments, multiple scalpel handles, duplicates or triplicates of other instruments, extra box of scalpel blades
3. Sterile instruments for culture collection
4. 10% neutral buffered formalin (at least 2 gallons)
5. Field acid-fast staining kit (to determine the presence or absence of Mycobacteria sp.)
6. Gluteraldehyde, 2.5-4% (at least 100mls)
7. Containers for sample collection; cylindrical plastic tubes
8. Culture swabs, sterile urine cups, glass slides
9. Serum tubes for blood and urine collection
10. Aluminum foil and plastic bags for freezing tissues (whirl-paks of various sizes)
11. Labels and waterproof marking pens
12. Scale for obtaining organ weights
13. Tape measure (metric), at least 2 meters long
14. Chain saw, axe, or reciprocating saw to cut through the cranium
15. Hammers, chisels and handsaws
16. Small hand meat hooks x 6
17. Hoist/crane/small tractor
18. Heavy straps, chains, ropes
19. Carts on rollers to move heavy parts
20. Coveralls, boots, gloves, caps, masks, protective eye and head gear, face shields (waterproof disposable suits are ideal)
21. Accessible water supply with hose
22. Camera and size reference (ruler)
23. First aid kit
24. Surgical masks approved for TB exposure
 - OSHA/CDC guidelines require N,R, or P-type particulate filter respirators with at least 95% efficiency (ie. N95,N99,N100; R95,R99,R100; P95,P99,P100) (example: 3M model N95)
 - Positive air pressure respirators (PAPRs)
25. Biohazard bags (red bags)
26. Styrofoam or other leak-proof boxes
27. Disinfectant solution (tuberculocidal)
 - Approved tuberculocidal disinfectants should list Mycobacteria sp. as susceptible on the label and are classified as “intermediate-level” disinfectants. Numerous products are commercially available.

LOGISTICS AND NECROPSY TIPS

The necropsy of an elephant should proceed in the same manner as the necropsy of any smaller mammalian species. A review of elephant necropsy techniques and tips is available at http://www.aphis.usda.gov/animal_welfare/downloads/elephant/Postmortem%20Exam%20Procedures.pdf. Although the size and scope of an elephant necropsy may seem intimidating, the procedure can be accomplished in 8-10 hours (sometimes less) by a team of dedicated prosectors and assistants. The necropsy should be performed with the elephant in left lateral recumbency. An external examination is performed to evaluate body condition and lesions. The oral cavity should be closely examined for evidence of lesions consistent with **endotheliotropic herpes virus infection**. The trunk should be examined according to above guidelines in the **tuberculosis** section.

Heavy equipment may be necessary to move a dead elephant. For an on-site necropsy, chains and a tow truck may be sufficient to reposition the animal or to move it a short distance. If the animal must be transported to a remote site, a truck with a hoist will be needed. It may be easier to manipulate the animal onto a flatbed trailer. Vehicles must be able to handle these approximate weights: female Asian: 2,300 - 3,700 kg; male Asian: 3,700 - 4,500 kg; female African: 2,300 - 4,000 kg; male African: 4,100 - 5,000 kg. Trucks can generally be rented. If a flatbed carrier is used, the animal will need to be strapped to the bed and covered with a tarp. If transportation will be delayed, the carcass can be covered with ice (800-1000lbs of ice can be laid on top of and next to the carcass and will preserve the carcass quite well even in summer heat).

Assigning specific tasks to team members will help the necropsy proceed in an orderly manner. For example, a team may be assigned to each of these areas: head, fore limbs, hind limbs, abdominal region. One person should oversee the collection, labeling, and processing of research materials and any communication concerning research requests. It may be helpful to designate a media spokesperson. One of the most important tasks to be assigned is the task of knife sharpener. One person with knife sharpening experience should be assigned to be continually sharpening knives and cycling sharpened knives to prosectors. Removal of the legs, head, skin, and rib cage is made easier through the use of chain hoists or a small tractor or backhoe. This equipment should be used to lift the very heavy body parts in the interests of safety and efficiency and to preserve the strength of primary prosectors.

Dissection of the head is best completed after separating it from the body. A good portion of the cranium must be damaged to remove the brain intact; a chain saw, large axe, and chisels are needed to penetrate the thick cranium. A battery operated reciprocating saw with a replaceable metal cutting blade may be safer and easier to handle. A posterior approach to brain removal can be made by 3 connecting deep cuts with a chain saw in the margins of the flattened triangle formed at the base of the elephant skull. The bony plate is then removed in chunks with a curved crow-bar. The brain itself is removed in portions (i.e. the cerebellum and brainstem, then one cerebral hemisphere followed by the other). Use of a chain saw on bone can be hazardous and cause shrapnel-like fragments to be launched. Protective eye, head and face gear should be worn by the chain saw operator and personnel in the immediate area.

During examination of an elephant with unknown, suspicious, or positive **TB test history**, dissection of the thoracic cavity should always be performed last, and should be done by two people with proper (at least N-95) face masks and other protection against *Mycobacterium* sp.. All other personnel should be dismissed from the area before the thoracic cavity is entered. After the abdominal viscera have been removed, the diaphragm can be cut from its costosternal attachments. The lungs are then palpated from a caudal approach for tuberculous nodules, as the lobes are being separated from the closely adhered visceral and parietal pleura. The heart, lungs, and associated structures may then be removed “en bloc”.

CARCASS DISPOSAL AND DISINFECTION

Options for disposal include incineration, tissue digestion, rendering, and burial (the most common option). Few institutions possess an on-site incinerator, but a bio-hazardous waste company may be of assistance in locating incineration services. Incineration often requires that the carcass be broken down into manageable pieces (50-100lbs) for transportation and is a very difficult and time consuming process. Tissue digesters, more and more popular for human biohazard waste disposal, are uncommon except in a few veterinary schools around the country. Some veterinary schools may be willing to dispose of carcasses for a fee (especially smaller carcasses). Rendering may be available in some states once it has been determined that no infectious disease agents are present. Burial is the option most commonly used and is the easiest option logistically. Ideally, the necropsy should be performed adjacent to a hole large enough to contain the carcass and deep enough to prevent odors and excavation by scavenging animals. In the event of a **TB suspect necropsy**, it is ideal for the hole to be large enough that the entire procedure can occur in the hole to eliminate the chances of contamination of the surrounding area.

Please be aware that special permissions or permits may be required from city, county, or state government for burial of a carcass and may be especially important in the event of burial of a TB suspect animal.

ELEPHANT NECROPSY PROTOCOL GROSS EXAMINATION WORKSHEET

Institution/Owner _____

Address _____

Species _____ Accession/ISIS# _____ Studbook# _____
Name _____

Birth date/Age _____ Sex _____ Weight (Kg) _____
Actual Estimate

Death date _____ Death location _____

Necropsy date _____ Necropsy location _____
Post mortem interval _____

Captive Born Wild Caught

History (clinical signs, circumstances of death, clinical lab work, diet & housing)

GROSS EXAMINATION

(If no abnormalities are noted, mark as normal or not examined (NE); use additional sheets if needed)

General Exam (physical and nutritional condition, skin, body orifices, superficial lymph nodes). **Skin nodules have been associated with EEHV in African elephants* (samples for fresh/frozen/formalin should be saved).**

Musculoskeletal System (bones, marrow, joints, muscles)

Body Cavities (fat stores, pleura, thymus, lymph nodes)

Spleen (capsular fibrous tags and siderotic plaques are common, age-related incidental lesions)

Respiratory System (trunk passages, pharynx, larynx, trachea, bronchi, lungs, regional lymph nodes; submit lung lesions for TB culture; bronchial lymph nodes should be cultured for TB even if normal in appearance). **Lymphoid nodules in lungs may be associated with EEHV infections*** (samples for fresh/frozen/formalin should be saved).

Cardiovascular System (heart, pericardial sac, great vessels, myocardium, valves, chambers, **be sure to closely examine abdominal aorta for subtle or obvious aneurysms**)

Digestive System (mouth, teeth, tongue, esophagus, stomach, small intestine, cecum, large intestine, rectum, liver, pancreas, mesenteric lymph nodes)

Urinary System (kidneys, ureters, bladder, urethra)

Reproductive System (testes/ovaries, uterus & cervix, penis/vagina, urogenital canal, prostate, seminal vesicles, bulbo-urethral gland, mammary gland, placenta). **Examine endometrium for changes consistent with polyploid and/or cystic endometrial hyperplasia. Uterine masses/tumors are extremely common in Asian cows and multiple tumor types may be present – please record number and size of masses as well as gross description. Ovarian and uterine serosal fibropapillomas also occur frequently in Asian and African cows. Mucosal nodular lesions in the distal urogenital tract have been associated with EEHV infections* (samples for fresh/frozen/formalin should be saved).**

Endocrine System (thyroids, parathyroids, adrenals, pituitary)

Central Nervous System (brain, meninges, spinal cord)

Sensory Organs (eyes, ears)

Additional Comments or Observations:

Prosector: _____ Date: _____

Summarize Preliminary Diagnoses:

Laboratory Studies: Please attach results of cytology, fluid analysis, urinalysis, serum chemistries, bacteriology, mycology, virology, parasitology, x-ray, photographs, or other data collected.

TISSUE CHECK LIST

Freeze 3-5 cm blocks of tissue from lesions and major organs (brain, heart, lung, liver, kidney, spleen) in small plastic bags. Freezing at -70 degrees Celsius in an ultra-low freezer is preferred. If this is unavailable, freezing at conventional temperatures is acceptable (use a freezer without an automatic defrost cycle if possible).

Any lesions noted in the lungs should be submitted to NVSL or other qualified mycobacterial laboratory for mycobacterial culture (i.e. National Jewish Diagnostic Lab, Colorado). Bronchial lymph nodes should be cultured for TB even if normal in appearance. Preserve as many of the tissues listed below as possible in 10% buffered formalin at a ratio of approximately 1 part tissue to 10 parts solution. Tissues should be no thicker than 0.5 to 1.0 cm. Fix diced (1x1 mm) pieces of kidney, liver, spleen and lung in a suitable EM fixative (glutaraldehyde base e.g., Trump-McDowell fixative) if possible. **NOTE: There is generally no need to fix and label each tissue separately with the exception of lymph nodes.** Ideally, tissues should be processed for histopathology as soon as possible following 24-48 hours of formalin fixation. Send tissues required for diagnosis to a primary pathologist. Diagnostic histopathology is available through the University of Illinois Zoological Pathology Program for a fee; contact the SSP pathologist, Dr. Jaime Landolfi for submission instructions and information. Also, freeze post mortem serum (from heart), urine and any abnormal fluid accumulations. Consult the **Research Tissues/Samples Requests** (at the end of this document) for specific project sample requests.

- | | | | |
|---|---|--|---|
| <input type="checkbox"/> Adrenal | <input type="checkbox"/> Kidney | <input type="checkbox"/> Penis | <input type="checkbox"/> Thymus |
| <input type="checkbox"/> Blood * | <input type="checkbox"/> Large intestine | <input type="checkbox"/> Pituitary | <input type="checkbox"/> Tongue |
| <input type="checkbox"/> Bone with marrow | <input type="checkbox"/> Liver (x2) | <input type="checkbox"/> Prostate | <input type="checkbox"/> Trachea |
| <input type="checkbox"/> Bulbo-urethral gland | <input type="checkbox"/> Lung (x 4 at least) | <input type="checkbox"/> Salivary gland | <input type="checkbox"/> Trunk cross section |
| <input type="checkbox"/> Brain (entire) | <input type="checkbox"/> Parathyroid | <input type="checkbox"/> Temporal gland | <input type="checkbox"/> Seminal vesicles |
| <input type="checkbox"/> Cecum | <input type="checkbox"/> Mammary gland | <input type="checkbox"/> Skin | <input type="checkbox"/> Ureter |
| <input type="checkbox"/> Diaphragm | <input type="checkbox"/> Muscle | <input type="checkbox"/> Small intestine | <input type="checkbox"/> Urinary bladder |
| <input type="checkbox"/> Esophagus | <input type="checkbox"/> Nerve (sciatic) | <input type="checkbox"/> Spinal cord | <input type="checkbox"/> Vaginal/urogenital canal |
| <input type="checkbox"/> Eye | <input type="checkbox"/> Ovary/testis | <input type="checkbox"/> Spleen | <input type="checkbox"/> Uterus/cervix |
| <input type="checkbox"/> Hepatic bile duct | <input type="checkbox"/> Epididymis | <input type="checkbox"/> Tonsillar lymphoid tissue | |
| <input type="checkbox"/> Heart/aorta | <input type="checkbox"/> Pancreas | <input type="checkbox"/> Stomach | <input type="checkbox"/> Thyroid gland |
| <input type="checkbox"/> Hemal node | <input type="checkbox"/> Lymph nodes (tracheobronchial, submandibular, tonsillar, mesenteric) | | |

* Collect post mortem blood, separate serum and freeze for retrospective studies.

Primary Pathologist (Name): _____

Lab _____

Address _____

Phone _____

Please send a copy of the final report including significant gross as well as histopathologic findings and pertinent digital or color slides to:

Jaime Landolfi, DVM, PhD, Diplomate ACVP; SSP Pathology Advisor, Elephants

Zoological Pathology Program, University of Illinois

c/o Chicago Zoological Society, 3300 Golf Road, Brookfield, IL 60513

W (312) 585-9043; Cell (708) 305-0611

Email: jaimeland@gmail.com; landolfi@illinois.edu

INDIVIDUALS INTERESTED IN PARTICIPATING IN NECROPSY PROCEDURES

The following people may be available to participate in necropsies. If you are interested, please contact them as soon as possible after an animal dies or before euthanasia.

| Name/Location | Phone Numbers | Email | Availability |
|--|---------------------------------------|---|--|
| Jaime Landolfi, DVM, PhD, DACVP Chicago, IL | W: 312-585-9043 Cell: 708-305-0611 | jaimeland@gmail.com; landolfi@illinois.edu | On site participation and phone consultation |
| Rita McMannon, DVM Athens, Georgia | W: 678-429-3134 | ritamcm@uga.edu | On site participation and phone consultation |
| Susan Mikota DVM Hohenwald, TN | W: 931-628-5962 Cell: 931-628-5963 | smikota@elephantcare.org | On site participation and phone consultation |
| Scott Terrell, DVM, DACVP Orlando, Florida | W: 407-938-2746 Cell: 321-229-9363 | scott.p.terrell@disney.com | Phone consultation |
| Dee MacAloose, DVM, DACVP Bronx, NY | W: 718-220-7105 Cell: 646-852-4962 | dmcaloose@wcs.org | Phone consultation and local/regional on-site participation |

April 2019 jl

RESEARCH REQUESTS

Institutional reminder - all requests made are conditional and not automatic, and may require the researcher's presence if they want detailed measurement info and/or complicated samples that are difficult to obtain and ship. Please contact the researchers in advance if you would like help in the collection of more complicated/labor intensive samples.

These requests are not a requirement for completion of a detailed diagnostic necropsy.

The following table summarizes all current research requests. Specific details regarding protocols/procedures, shipping information, and/or other special instructions are addressed in the following text section.

Research tissues summary:

| Samples | Requesting facility | Purpose | Preservation | Transport | Special instructions |
|--|---|----------------|---------------------|-----------------------------------|-----------------------------|
| - Serum - Brain - Heart - Lung - Liver - Spleen - Kidney | TAG-SSP | Frozen archive | Frozen (-80C) | NA – maintain at holding facility | |
| - Serum - Whole blood (EDTA) - Placenta - Swab of herpetic lesions - Heart - Liver - Tongue - Spleen - Intestine - Kidney - Tissues with hemorrhagic lesions | Smithsonian's National Zoo National Elephant Herpes Lab | EEHV screening | Frozen (-80C) | Priority overnight | |
| - Lung - Lung lymphoid nodules - Serum - Whole blood - Tissue with hemorrhagic lesions | Johns Hopkins School of Medicine | EEHV research | Fresh, unfrozen | Priority overnight on ice packs | Transport media required |

| | | | | | |
|--|--|-----------------------|---|---|--|
| <ul style="list-style-type: none"> - Whole blood (EDTA) - Serum - Cavitory effusion - CSF - Heart - Salivary gland - Trigeminal ganglion - Lymph node - Brain - Liver - Spleen - Tongue - Skin lesions - Rectal polyps - Vaginal polyps - Uterine polyps - Tumors | Fox Chase Cancer Center | EEHV research | Fresh, unfrozen | Priority overnight on ice packs; ship within 24 hrs of collection | Wash tissues in PBS, then transport media required |
| <ul style="list-style-type: none"> - Small and large intestinal mucosal tumors, polyps or proliferative lesions | University of Southern California | Cancer research | Immediately frozen (-20C or lower) or in EDTA for 3 hrs prior to freezing | Priority overnight | |
| <ul style="list-style-type: none"> - Temporal bone | Harvard University School of Medicine | Hearing loss research | Frozen (-20C or lower) | Priority overnight | Researcher available to assist sample collection and/or offset costs |
| <ul style="list-style-type: none"> - Duodenum - Jejunum - Parathyroid gland - Skin (x2) | Cornell University | Vitamin D metabolism | Frozen (-80C) | Priority overnight on dry ice | |
| <ul style="list-style-type: none"> - Tumors - Non-neoplastic tissue adjacent to tumors - Whole blood (EDTA) | University of Utah/Huntsman Cancer Institute | Cancer research | Fresh, unfrozen | Priority overnight on ice packs | Wash tissues with PBS, then transport media required |
| <ul style="list-style-type: none"> - Small intestine - Large intestine - Cecum - Rectum - Salivary gland | Baylor College of Medicine | EEHV research | Fresh, unfrozen | Priority overnight on ice packs | Wash tissues with PBS, then transport media required |

CURRENT TISSUE/SAMPLE RESEARCH REQUESTS

1. Jaime Landolfi, DVM, PhD, Diplomate ACVP
SSP Pathology Advisor, Elephants
Zoological Pathology Program, University of Illinois
c/o Chicago Zoological Society, 3300 Golf Road, Brookfield, IL 60513
W (312) 585-9043; Cell (708) 305-0611
Email: jaimeland@gmail.com; landolfi@illinois.edu

Dr. Landolfi requests an electronic copy of the **final necropsy and histopathology report** including all pertinent culture results for inclusion in the TAG/SSP Pathology Archive.

Tissues for histopathology may be sent to the institution's primary pathology service. Diagnostic histopathology is also available through the University of Illinois Zoological Pathology Program for a fee ("at cost"; approximately \$10/block); contact Dr. Landolfi for submission instructions and information. Please check with your primary pathology service to determine their policy for retention and disposal of paraffin blocks. If your primary pathology service retains paraffin blocks in perpetuity, then no further action is needed. **If your primary pathology service disposes of paraffin blocks after a defined time period, please request that those blocks be sent the zoo or to the SSP pathologist once the case is finalized.** Paraffin blocks retain valuable diagnostic, molecular and retrospective information and are easier to ship and store than formalin fixed tissues. Formalin fixed tissues present significant long term shipping, storage and archival challenges and have little or no ancillary diagnostic/research value. **After finalization of the case**, formalin-fixed tissues may be disposed of at the discretion of the holding facility.

2. Elephant TAG/SSP

The elephant TAG/SSP recommends that serum be collected and stored from all elephants on a regular basis for the benefit of retrospective analyses by the holding institution or requests for future research projects. A **minimum of 4 mls serum/elephant** should be collected on an annual basis, frozen and stored by the current facility, ideally at -80°C.

In addition to tissues collected in formalin for histopathology, it is recommended that at least 50 g of the following tissues be collected and stored frozen at -80°C by the current facility from any elephant that dies or is euthanized: brain, heart, lung, liver, spleen, kidney (50 g each)

3. National Elephant Herpes Lab

Erin Latimer
Smithsonian's National Zoo
Department of Pathology
3001 Connecticut Ave. NW
Washington, D.C. 20008
Work: 202-633-4252
Cell: 703-855-9611
Email: latimere@si.edu

The following samples are requested for EEHV screening: 1. **Serum** – 2 mls; transfer to plastic screw-top tube and store at -80C or non-defrosting freezer until shipped. Ship samples overnight with ice packs or dry ice. 2. **Whole blood** – 1-2 mls in EDTA tube, then transfer to plastic screw-cap for storage at -80C freezer until shipped (for EEHV detection). 3. **Placenta** – freeze 1 inch³ piece in liquid nitrogen or dry ice, then store at -80C freezer until shipped Also, serum and whole blood from dam and baby. 4.

Suspected herpetic lesions – wet a cotton swab with small amount of sterile saline, swab lesion and place in sterile 15 ml plastic test tube; store at -80C until shipped. **5. Necropsy tissues (heart, liver, tongue, spleen, intestine, kidney, any tissue with hemorrhages, any kind of nodules – skin, lung, vestibular)** – aseptically place 3x3 cm piece of tissue in small Ziploc or WhirlPak bag. Label with type of tissue, elephant ID, date. Use separate bag for each tissue; store at -80C until shipped. **Shipping** – FedEx overnight; email tracking number to latimere@si.edu. EEHV Lab will pay for shipping of samples; contact lab for account information.

4. John Hopkins School of Medicine

Gary Hayward

Johns Hopkins School of Medicine, Viral Oncology Program

3M09, Bunting-Blaustein Cancer Research Building,

1650 Orleans St,

Baltimore, MD 21287 (Fedex)

PH 410-955-8684 Fax 410-955-8685 (work)

PH 410-821-8197 (Home/weekend)

Email: ghayward@jhmi.edu

Contact lab for FedEx information

Dr. Hayward's lab is conducting on-going research to better understand EEHV pathogenesis. Samples are requested for viral culture, PCR and sequencing.

1. Asymptomatic Wild-Born Adult Asian and African Elephants: Multiple small pieces of fresh, unfrozen lung tissue from (a) breadloaved bronchiolar (peripheral) areas; (b) lung lymphoid nodules (~2-3 mm, white and ovoid with smooth surfaces and embedded within the parenchyma). Also, Several small pieces of fresh, unfrozen lung from upper airway regions for epithelial cell culture. Transport unfrozen with ice packs in 40 ml plastic tubes containing PBS plus antibiotics.

2. EEHV-Positive Hemorrhagic Disease Cases: (a) Blood and Serum: Fresh heart blood (2ml to 4ml) and serum (20ml to 200ml in butterfly catheter; as much serum as possible please) transported on ice/refrigerated packs; serum may be frozen. (b) Fresh unfrozen tissue containing foci of hemorrhage (e.g. heart, lung, spleen, liver, kidney or tongue). Ship directly on ice in 40 ml sterile plastic tubes or with small volume of transport medium (PBS plus Pen/Str/Fungizone or similar).

5. Fox Chase Cancer Center

Virginia R. Pearson, Visiting Scientist

Rall Laboratory

Fox Chase Cancer Center

333 Cottman Ave,

Philadelphia, PA 19111

mobile 215-816-5734, Lab phone 215-728-3677, FAX 215-728-2412

virginiarpearson@gmail.com

Sample shipping address: Virginia Pearson

701 West Gravers Lane,

Philadelphia, PA 19118

home 215-247-1287

Dr. Pearson's lab is working to culture EEHVs and EGHVs on elephant primary and immortalized cell

lines and to establish EEHV tissue tropism. **For EEHV/EGHV-suspect cases**, requested samples include: **unfrozen** whole blood in EDTA, serum, ascites, thoracic, CSF and pericardial fluid collected as soon as possible after (or prior) to death and shipped on wet ice within 24 hours of death by overnight next morning delivery. **For ALL necropsies (including EEHV suspect cases), the following tissues are requested and may be frozen or sent fresh on wet ice for overnight delivery:** 1" cubes of heart, salivary gland, trigeminal ganglion, brain, lymph node, spleen, liver, tongue if hemorrhaged, open skin lesions, wart-like skin growths, rectal and vaginal polyps, uterine polyps, cancerous growths, eyelid lesions, any hemorrhaged organs. Please advise in advance of any planned euthanasia.

6. **University of Southern California Norris Cancer Center**

Darryl Shibata
Norris Cancer Center
1441 Eastlake Ave, NOR2424
Los Angeles, CA 90033
(323) 865-3298
dshibata@usc.edu

Dr. Shibata is requesting fresh tissue samples from any small or large intestine mucosal tumors, polyps or proliferative lesions. No more than 5 mm square of tissue is required, and the sample could be collected after tissue is collected for diagnostic histopathology. Samples should be immediately frozen at -20C or below or placed in EDTA solution (provided by Dr. Shibata) for 3 hours with gentle agitation at room temperature prior to freezing. Collected frozen samples should be shipped on dry ice to Dr. Shibata. Containers, EDTA solution and shipping instructions will be provided upon request.

7. **Harvard University School of Medicine, Caitlin O'Connell-Rodwell, PhD**

Attn: Xiyang Guan Rm 411
Sunil Puria Lab, Eaton Peabody Lab
Massachusetts Eye & Ear Infirmary
243 Charles Street
Boston, MA 02114

Dr. O'Connell-Rodwell is requesting temporal bones as part of a study to understand the mechanics of low frequency hearing in elephants. Knowledge of ossicular motion in a low frequency ear may inform improved hearing prosthetics for patients with hearing loss.

It is requested that a block of tissue that would include the temporal bone is collected using whatever saw available for this purpose (chain saw or reciprocating saw). Depending on what the necropsy facility has on hand, the smaller the block the easier to freeze and handle in order to access the temporal bone. Sample should be stored frozen and shipped priority overnight. It is very important that **NO FIXATIVE** be used in the sample preparation as this will alter the behavior of the middle ear.

Please contact Dr. for further collection details and arrangement of shipping. **NOTE – Dr. O'Connell-Rodwell may be available to come and perform/assist with on-site sample collection and/or support the cost of challenging sample preparation:**

Ceoconnell@stanford.edu
(650) 868-2251

8. **Cornell University**

Dr. Sara Childs-Sanford
Cornell University

930 Campus Road, Box 33
Ithaca, NY 14850

Dr. Childs-Sanford is requesting fresh tissue samples for on-going research into vitamin D metabolism and parathyroid activity in Asian elephants in managed care.

For this research, samples of duodenum (1 x 1 cm), jejunum (1 x 1 cm), parathyroid gland (whole tissue), dorsal skin (1 x 1 cm), and ventral skin (1 x 1 cm) are requested. Fresh sections should be placed in a plastic sample bag (e.g. whirl pak) and frozen (preferably at -80C) immediately. Samples should be shipped priority overnight on dry ice.

Please contact Dr. Childs-Sanford or Lisa Scanlon with any questions and/or to arrange shipping. Dr. Childs-Sanford can cover shipping costs.

Sec15@cornell.edu
(607) 257-7530

Lmt2@cornell.edu
(607) 279-0436

9. University of Utah/Huntsman Cancer Institute, Lisa Abegglen and Joshua Shiffman

Lisa Abegglen/Schiffman Lab
University of Utah/Huntsman Cancer
Institute 2000 Circle of Hope
Salt Lake City, UT 84112

Drs. Abegglen and Shiffman are requesting samples of elephant tumors and matched non-neoplastic tissue from the affected organ as well as samples of whole blood from affected animals to aid in on-going investigations into carcinogenesis, specifically the role of tumor suppressor gene expression, in elephants.

For this research, a 2 x 2 x 2 cm sample of tumor (including tumor only and no adjacent normal tissue) along with an additional 2 x 2 x 2 cm sample of normal tissue, collected separately, from the same organ are requested. Also requested is whole blood in a single EDTA blood tube.

1. If a skin sample is being obtained, then clean area with ethanol prior to harvest. If the sample is coming from the inside of the animal and the area has become dirty, then clean with ethanol or rinse with PBS prior to harvest.
2. Take the tissue sample (try to avoid including normal tissue with tumor)
3. Drop the sample or samples into a labeled conical with cell growth media (which we will ship overnight to harvest location prior to procedure). Please label the tube with the type of tissue added to the tube and whether the sample contains tumor or normal tissue.
4. Ship the samples to our lab to arrive within a day or two of retrieval, with an ice pack (not dry ice) overnight. Please fill the conical with media prior to shipment (also will be provided). Be careful when opening the media, because it will support the growth of any microorganisms that are accidentally introduced into the tubes or bottle. Store the media at 4C.
5. EDTA blood tube can be shipped on ice packs along with tissue samples

Please contact Dr. Abegglen with any questions and/or to arrange acquisition of tissue media and sample shipping. Dr. Abegglen can cover shipping costs.

Lisa.Abegglen@hci.utah.edu; 801-213-6226; 801-718-8629

10. Baylor College of Medicine

Dr. Paul D. Ling
 Baylor College of Medicine
 One Baylor Plaza
 Department of Molecular Virology and Microbiology
 Houston, Texas 77030
 Ph: 713-798-8474
 Email: pling@bcm.edu

Dr. Ling is requesting tissue samples from the gastrointestinal (GI) tract to grow what is commonly referred to as organoid cultures. These cultures capture some of the key attributes of the tissues they were derived from and have been instrumental in allowing the culture of some viruses for the very first time. Once established, the organoid cultures will be extremely useful for two purposes: 1) they can be developed into a model system for investigating cancer in elephants, and most importantly, 2) serve as a useful system for growing EEHV. Dr. Ling's lab is working towards development of an effective vaccine against elephant endotheliotropic herpesvirus (EEHV). Successful propagation of the virus in the laboratory will greatly accelerate efforts to understand EEHV and develop an effective vaccine against it.

To comply with this research request, viable, living tissue is required. Thus, collection from humane euthanasia cases will be most feasible. It is optimal to contact Dr. Ling in advance to allow for shipment of necessary supplies.

Protocol for sample collection:

1. Collect 3 independent 30mm² tissue specimens each from the: a) small intestine; b) large intestine; c) rectum; d) cecum; e) salivary glands
2. Rinse/wash sample by pouring sterile saline or PBS (researcher will provide) to clean off debris and/or feces material. If available, sterile saline or PBS can also be poured into a disposable cup or glass and the tissue "dipped" and agitated in the solution to clean it off. Place each specimen in a 50ml tube prefilled with 35ml media (Advanced DMEM/F12, Glutamine, HEPES, Pen/Strep/Fungizone) provided by the researcher; contact researcher ahead of time to obtain (store at 4⁰C). Seal outside of screw cap with a strip of parafilm.
3. Fill shipping container with ice packs to maintain 4⁰C and pad with paper or other packing material to prevent tubes from moving around.

In the absence of media tubes provided ahead of time by the researcher, facilities may employ the following alternate protocol:

1. Collect 8mm² specimens as described for the standard protocol and then cut them into 2- to 3-mm² fragments and transfer into cryopreservation vials containing 1ml freezing medium (Dulbecco's modified Eagle medium/F12; 10% fetal bovine serum; 10% dimethyl sulfoxide)
2. Place tubes into a Mr. Frosty cell-freezing container and freeze overnight at -80C.
3. Place vials into a shipping container with plenty of **dry ice** to keep the samples frozen during shipping and ship as described above.

Ship samples overnight to the following address using the Fed Ex reference number: 342808905

FORM FOR REQUESTING ELEPHANT TISSUE/BLOOD SAMPLES

Name _____ Date of request _____

Affiliation _____

Address _____

Work phone (____) _____ Home phone (____) _____

Fax (____) _____ Email _____

Sample(s) requested _____

Purpose of study

Duration of study _____

Instructions for sample preparation – attach protocol

Shipping instructions (dry ice? Overnight? Will you pay for shipping?)

Special instructions

Attach any additional information. Send to by email to Dr. Jaime Landolfi (jaimeland@gmail.com; landolfi@illinois.edu).

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