

Guidelines for collection of biological samples for giant otters (*Pteronura brasiliensis* Gmelin, 1788) and Neotropical otters (*Lontra longicaudis* Olfers, 1818)

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Giant (*Pteronura brasiliensis*) and Neotropical (*Lontra longicaudis*) otters are semi-aquatic mustelids of the Brazilian fauna. As one of the top predators of aquatic systems' food web, they play an important role in ecosystem balance. The giant otter is globally classified as Endangered (Rodrigues *et al.*, 2013; Groenendijk *et al.*, 2015), whereas the Neotropical otter is considered Near Threatened (Rheingantz and Trinca, 2015). In Brazil, both species occur currently in the Amazon, Cerrado and Pantanal biomes; the Neotropical otter also occurs in the Atlantic Forest and Pampas. The main anthropic threats to the species are hunting, habitat fragmentation, human conflicts, and contamination of water bodies (Groenendijk *et al.*, 2015; Rheingantz and Trinca, 2015). Several studies on the ecology and conservation of these species have been carried out in recent decades (Koepfli and Wayne, 1998; Astúa *et al.*, 2010; Cabral *et al.*, 2010; Oliveira *et al.*, 2011; Rheingantz *et al.*, 2011; Pickles *et al.*, 2012; Leuchtenberger *et al.*, 2018). However, many knowledge gaps regarding basic information about giant otters remain, and even more so for Neotropical otters.

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In 2010, one of the strategies by the Ministry of the Environment (MMA) for the conservation of Brazilian fauna (Ordinance ICMBio n° 43/2014) was to develop the first National Action Plan (NAP) for giant otters, which also included Neotropical otters (ICMBio/MMA, 2010). The NAP Giant Otter recognized the need to standardize protocols for collection, processing, and storage of otter biological samples to improve and optimize capture, handling, and necropsy efforts of both species of otters. Standardized animal care and veterinary guidelines for otters in zoos, aquariums, rehabilitation, and wildlife centers and a *postmortem* protocol for otters have previously been described (Simpson, 2001; AZA Small Carnivore TAG, 2008; Myers, 2012).

Developed by veterinarians with experience in wildlife, members of the Technical Advisory Group of the National Action Plan for Conservation of Giant Otters (TAG/NAP Giant Otter) coordinated by the Centro Nacional de Pesquisa e Conservação dos Mamíferos Carnívoros (ICMBio/CENAP) and reviewed by other otter specialist members of the Group, these protocols are intended for biologists, field veterinarians and other professionals involved in conservation who are working with these species. The use of these guidelines will be helpful to optimize the health evaluation of otters in the wild and under human care, understand the epidemiology of diseases in otters, guarantee the quality of the information collected, and maximize the efforts in research projects.

Two protocols were developed: 1) collection, processing, and storage of biological samples from live giant and Neotropical otters, and 2) collection of biological material during necropsy. We emphasize that each step mentioned in these protocols must be properly performed to maintain the quality of the biological samples until they arrive in laboratories for analysis.

1) Protocol for collection, processing, and storage of biological samples from live giant and Neotropical otters

The biological samples described here can be collected from anesthetized individuals (Figs 1a, 1b and 1c) or directly from the field (e.g. feces and hair). Table 1 presents samples and suggested quantity to be collected, equipment needed, the most appropriate collection method, processing, storage, and laboratory

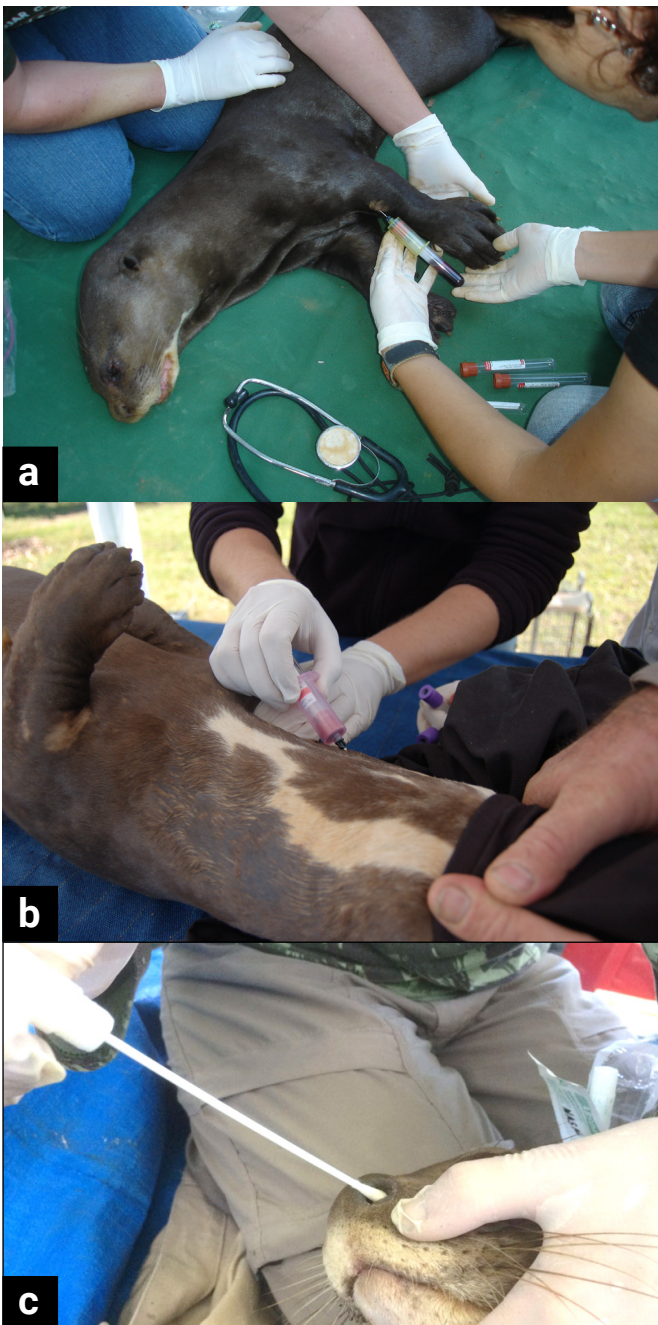


Figure 1. Collection of biological samples of live giant otter (*Pteronura brasiliensis*). a) Blood sample collection from the cephalic vein; b) Blood sample collection from the jugular vein; c) Nasal secretion collection with a sterile swab. (Photo 1a: Jaguar Conservation Fund/Instituto Onça-Pintada; Photo 1b: Caroline Leuchtenberger/ Instituto Farroupilha; Photo 1c: Grazielle Soaresini/ Giant Otter Conservation Fund).

analyses to be performed. We present an ideal situation and recognize that many factors may prevent the professional from following the full protocol; therefore, this should be viewed as a guide to be adapted according to each particular situation.

All samples must contain information on species, sex, estimated age, date of collection, capture site with geographical coordinates, identification of the animal and person responsible for collection (Fig. 2). We recommend using a system where each animal receives a unique ID number that provides a rapid code and facilitates the identification. Animal ID should be assigned as

the example in Fig. 3.

During events of captures/anesthesia of giant and Neotropical otters, the professional should strive to collect the additional information described in Table 2. Anesthesia protocols in giant otters and recommendations for anesthesia procedures are available in the literature (AZA Small Carnivore TAG, 2008; Silveira *et al.*, 2011; Myers, 2012; Duplaix *et al.*, 2015) and we recommend that it should be performed with care and responsibility by experienced veterinarians.

2) Protocol for the collection of biological material during necropsy in giant and Neotropical otters

Necropsy should be performed as soon as possible after the animal's death is confirmed. In the latter case, the carcass can be refrigerated at 4 – 8 °C for up to 24-36 h. Freezing should be performed only when the necropsy is unfeasible in a timely manner, as it causes the physical rupture of most cell membranes. Protective clothing such as latex gloves, protective mask, and coveralls should be used to perform the necropsy. Photographing external and internal lesions provides a record of the changes observed in the examined organs (Figs 6a, 6b and 6c).

Biological materials must be stored according to the type of research to be conducted. It is important to note that tissues preserved in formaldehyde must not be frozen, and samples for infectious or toxicological diseases must be frozen or refrigerated. The samples must be collected in clean (or sterile), sealed and identified vials/containers (Fig. 7). The necropsy should preferably be performed following the steps described below (Matushima, 2007; Catão-Dias and Miranda, 2014).

a) General examination

Assess the physical condition of the animal: observe the presence of lesions, parasites, signs of predation, bruises, fractured bones, and signs of trauma that could have been the cause of death. Differentiate between *postmortem* injuries caused by scavengers. Assess the nutritional status and dental arch of the animal. Include a detailed photograph of the dental arch.

b) Collection of skin ectoparasites

Through manual rotation. Store in vial or tube with 70% alcohol at room temperature.

c) Aseptic collection of cardiac blood

After opening the chest cavity, perform aseptic collection of cardiac blood with a syringe and needle. The blood must be transferred to a culture bottle and refrigerated.

d) Collection of material for microbiological examination

Samples for microbiological analysis must be collected before manipulating the organs. Tissue fragments of 3 cm³ should be collected with sterile material as swabs or sterile syringes. Samples must be kept refrigerated and Stuart or Amies transport medium can be used. To improve the research, samples for microbiome can also be collected from the organs in a sterile swab, stored in an Eppendorf tube and frozen.

e) Collection of material for histopathological examination

Fragments of a maximum of 2 cm³ from all organs must be collected with a scalpel or a sharp knife. Preferably, samples

Table 1. Protocol for collection, processing, and storage of biological samples from live giant and Neotropical otters.

| SAMPLE | MATERIAL SUPPLIES | NBR SAMPLES (QUANTITY) | METHOD OF COLLECTION | PROCESSING | STORAGE | ANALYSES |
|--------------------------------|--|--------------------------|---|--|--|---|
| Blood | Tube with anticoagulant (EDTA) (purple top tube)* | 12 ml | Venous puncture | Homogenize and keep at room temperature up to 12h preferably, but no longer than 24 h, then refrigerate. | Up to 24 hours after collection | Blood count Hematocrit |
| | | | | | Aliquot in 0.6 ml Eppendorf, freeze at -20 °C or -80 °C when available | Pathogens detection |
| Blood | Tube without anticoagulant (red top tube) | 30 ml | Venous puncture | Keep at room temperature up to 12h preferably, but no longer than 24 h, then refrigerate. | 1:1 Easy Blood Buffer. Homogenize, aliquot in 0.6 ml Eppendorf, freeze at -20 °C | Genetic analysis |
| | | | | | Separate the serum and aliquot in 0.6 ml Eppendorf; freeze at -20 °C | Serosurvey Hormonal analysis Biochemical analysis |
| Blood | Filter paper or tube with ethanol | 1-2 drops | Blood from the syringe and needle used for blood collection | Drip one or two drops of blood on the filter paper or inside the tube with ethanol | Room temperature | Genetic analysis |
| Blood smear | Microscope slide | 2 | Venous puncture (from tubes with or without anticoagulant) | Dry Preserve with methanol | Room temperature Box of slides or wrapped in tissue paper (room temperature) | Hemoparasite detection |
| Hair | Plastic bag | 1 | Manual traction, no need to come with the bulb | - | Freeze at -20 °C | Heavy metals |
| | | 1 | Manual traction, the hair needs to come with the bulb | - | Freeze at -20 °C | Genetic analysis |
| Urine | 15 ml sterile vial | 1 | Catheter | - | Up to 24 hours after collection Aliquot in 1.5 ml Eppendorf, freeze at -20 °C | Urinalysis Pathogen detection |
| Feces | Universal collector or 50 ml Falcon tube | 1 | Directly from the rectum | - | 1:1 potassium dichromate 2%. Refrigerate or room temperature | Endoparasite detection |
| | | | | | Freeze at -20 °C | Pathogen detection |
| | | | | | 1:1 potassium dichromate 2%. Refrigerate or room temperature | Endoparasite detection |
| | For diet: airtight plastic bag | 1 | Directly from the rectum | - | Merthiolate-Iodine-Formaldehyde (MIF) (when parasitological examination is performed later than 24 h after collection) | Parasitological analysis |
| | | | | | Absolute alcohol or 1:3 silica. Refrigerate or freeze as soon as possible (only fresh feces) | Molecular analysis |
| | | | | | Fresh feces or mucus – refrigerate as quickly as possible | Hormonal analysis |
| Sterile swab | 1 | Directly from the rectum | - | Room temperature | Diet analysis | |
| Freeze at -20 °C | Microbiome characterization | | | | | |
| Mucus | Universal collector or 50 ml Falcon tube | 1 | Manual collection | - | Absolute alcohol or 1:3 silica. Refrigerate or freeze as soon as possible (only fresh feces) | Molecular analysis |
| Milk | Eppendorf | - | Manual extraction | - | Freeze at -20 °C | Analysis of milk composition |
| | Sterile swab | - | Manual extraction | - | Freeze at -20 °C | Microbiome characterization |
| Microbiological samples | Sterile swab (with culture for virus, fungus and bacteria) | 1 | Rotate in mouth, ear, nose, lesion | - | Virus: freeze at -20 °C Fungus and microbiology: refrigerate | Pathogen detection |
| Vaginal cytology | Swab/ Microscope slide | 2 | Rotate in the vaginal canal | Preserve in methanol | Room temperature | Reproductive analysis |
| Ectoparasites | Vial or tube with 70% alcohol | as many as possible | Manual rotation | - | Room temperature | Ectoparasite detection |
| Biopsies | Skin, muscle, lesion | 1 | Disposable punch | - | Skin and muscle: absolute alcohol | Genetic analysis |
| | | | | | Skin and muscle: 70% alcohol Lesion: freeze/formaldehyde 10% | Microbiological analysis |

*tubes with anticoagulant sodium citrate (blue top), sodium heparine (green top), sodium fluoride (gray top) can be added for coagulation, blood gas and blood glucose tests, according to the focus of the study.

Table 2. Additional information associated with collecting biological material from anesthetized giant and Neotropical otters.

| DATA | HOW TO PERFORM THE COLLECTION |
|------------------------------------|--|
| Weight | Weighing of the animal using a Pesola® spring balance |
| Biometrics | Head length, Head circumference, Neck circumference, Ear length, Ear width, Chest circumference, Body length without tail, Tail length, Height, Length and Width of hind and front paw (Fig. 4), Length of the right and left testicle (males; Fig. 5a), Width of the right and left testicle and perianal gland length (males) For females: note reproductive status and condition of the mammary glands |
| Coat pattern (giant otters) | Photo of the throat pattern (Fig. 5b) |
| Dentition | Photos of the dental arch in occlusion: front, right side (Fig. 5c), left side, right diagonal and left diagonal Photo of the open dental arch: front, right diagonal and left diagonal. Dental formula |
| Physiological parameters | During anesthesia, every 10 minutes record respiratory rate (breaths per minute), heart rate (heart beats per minute), and rectal temperature (°C) |

of normal tissues and injured tissues should be collected. The fragments must be stored in containers with 10% formaldehyde in a 1:9 ratio (fragment:formaldehyde). Never freeze.

f) Collection of material for toxicological analysis

Indicate when animal contact with toxic agents is suspected. It is recommended to collect 100 g of stomach contents, liver and kidney; and 50 ml of blood in a clean and dry container. The material can be refrigerated or frozen. If the toxic agent involved is suspected, it should be indicated to the laboratory. Bones can also be an option for toxicological analysis. It is preferable to use long bones as femur, humerus, radius, ulna or tibia, and ribs. After collection, bones should be cleaned of all soft tissues, muscular insertions and periosteum, and then washed in a saline

solution with gentamicin and stored in a tightly closed sterile plastic container and frozen at -20 °C.

g) Collection of material for parasitological analysis

Feces must be collected directly from the rectum and preserved in 10% formaldehyde. Observe the presence of gastro-intestinal parasites, collect the individuals carefully with the aid of tweezers and store them in vial or tube with 70% alcohol. A portion of the feces can also be frozen for carrying out a barcoding of some life stages of the parasites.

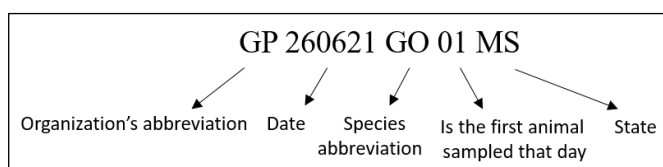
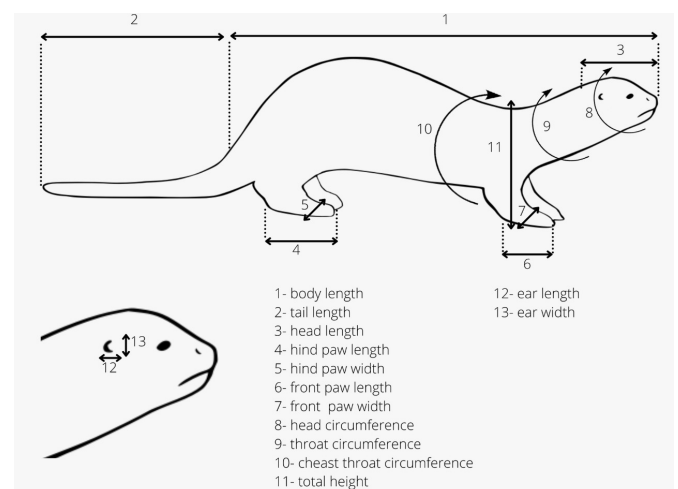
h) Collection of material for genetics analysis

Collect at least three fragments about 1x1 cm from the following organs: skeletal muscle, heart, liver, and kidney. Store in tubes with 70% alcohol.

Collect hair by manual traction and store dry in plastic bags or paper envelopes. Blood droplets are placed on filter paper or in Eppendorf tube with ethanol and stored at room temperature.

After all the necropsy steps have been carried out, the necropsy report sheet must be prepared according to the following model (Fig. 8). Upon completion of the necropsy, the remains of tissues must be buried or incinerated. Surgical instruments must be cleaned with soap and disinfected with 70% ethyl alcohol and the environment with sodium hypochlorite or bleach. The carcass must be buried for later retrieval or macerated or placed in a

Species: _____
 Date of collection: _____
 Animal identification: _____
 Sex: _____ Age: _____
 Material collected: _____
 Capture site: _____
 Geographic coordinates: _____
 Datum: _____
 Responsible for collection: _____

Figure 2. Model of label to be attached to each sample collected.**Figure 3.** Animal ID. The first two letters indicate the organization that performed the biological collection. The first six numbers indicate the date (in the format DAY-MONTH-YEAR). The next two letters designate the species, GO for giant otters and NO for Neotropical otters. The next two digits indicate the counter of animals sampled that date. The last two letters designate the Brazilian state where the collection was performed.**Figure 4.** Measuring giant and Neotropical otters. Adapted from Geraci and Lounsbury (1993; Caroline Leuchtenberger/Instituto Farroupilha).

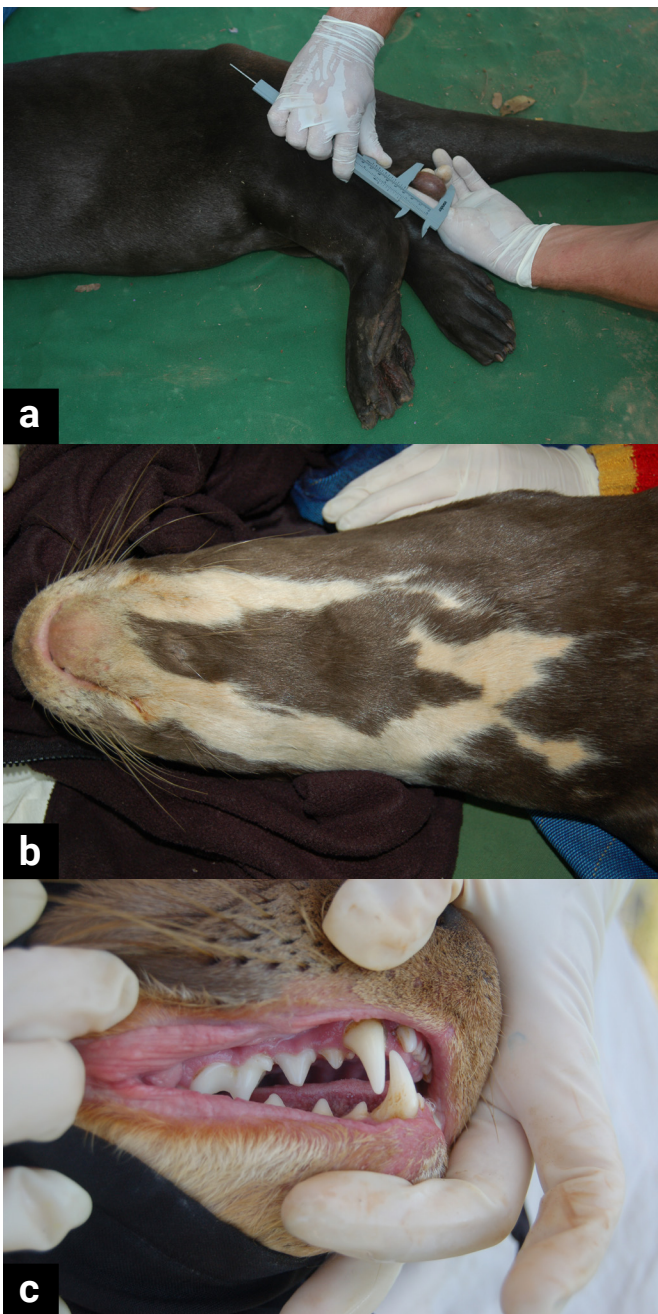


Figure 5. a) Measuring the length of the testicle in a live male giant otter (*Pteronura brasiliensis*); b) Photo of throat pattern; c) Photo of the right side of the dental arch. (Photo 5a: Jaguar Conservation Fund/Instituto Onça-Pintada; Photos 5b-c: Caroline Leuchtenberger/Instituto Farroupilha).

dermestid beetle bed such as to clean the skeleton for accession in a museum or research collection.

The use of standardized protocols suggested here for giant and Neotropical otters can contribute to expand the knowledge on health of the species and allow data comparison by different researchers/professionals in different locations. Researchers should be aware that any procedure with otters in the wild or under human care requires an environmental permit for handling, collecting, and transporting the biological material. The health evaluation of otter populations, and the knowledge on the cause of death of individuals will allow

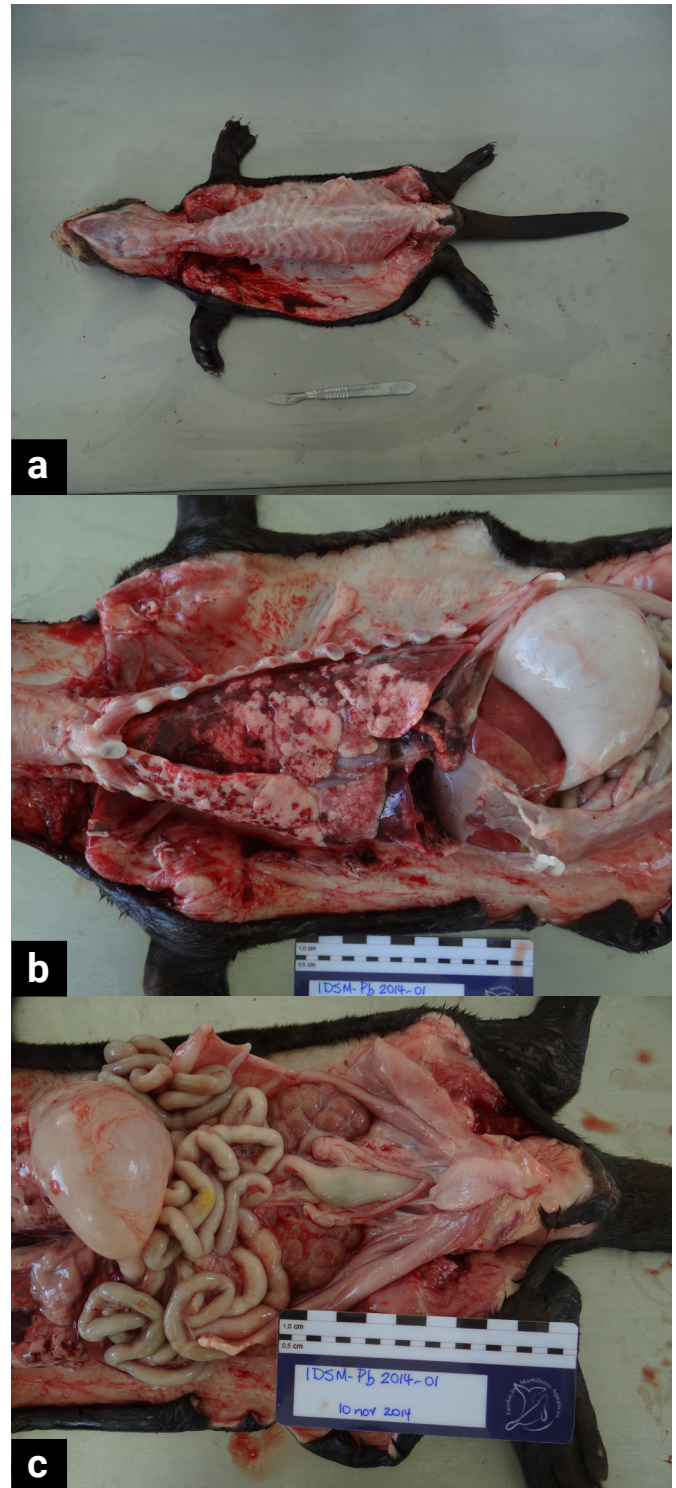


Figure 6. Necropsy of a giant otter (*Pteronura brasiliensis*) cub. a) Ventral view; b) Thoracic and abdominal cavity and major internal organs; c) Major internal organs of the abdominal cavity. (All photos: André Coelho, Instituto Mamirauá).

to identify possible pathogens circulating in the species, and in the long term, recognize which agents are endemic and how they might threaten the species. In addition, these guidelines can provide recommendations for epidemiological management strategies for the conservation of the species using standardized protocols and diagnoses obtained.

Species: _____
 Date and time of collection: _____
 Date and time of death: _____
 Animal identification: _____
 Sex: _____ Age: _____
 Material collected: _____
 Place of death: _____
 Geographic coordinates: _____
 Datum: _____
 Responsible for collection: _____

Figure 7. Model of label to be attached to each sample collected at necropsy.

Necropsy report sheet

- Animal identification
- Geographic coordinates and datum of the animals's capture site
- Conservation status of the carcass
- Clinical suspicion (if any)
- Macroscopic description of the changes observed and of the organs that did not present any apparent lesion (describe size, color, color pattern, size of the lesion, lesion distribution pattern, relationship with other organs and other characteristics deemed important)
- Macroscopic morphological diagnosis
- Preliminary cause of death
- Description of samples taken and tests to be performed

Figure 8. Necropsy report sheet model.

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