

8. POST- MORTEM PROTOCOL

(John Trupkiewicz)

8.1. GENERAL COMMENTS

Those individuals who may be charged with performing a necropsy examination should formulate a plan of action in the case of the sudden, unexpected death of a rhinoceros. Due to their large body size, the internal temperature will be retained for an extended period post-mortem, so autolysis will proceed rapidly. Also, the animal will have most likely died in its exhibit or holding area, thereby limiting the amount of time available for the post-mortem examination. Even in the case of planned euthanasia, transporting the animal to a necropsy laboratory may not be practical.

All necessary equipment and supplies to perform the post-mortem examination must be readily accessible and prepared for transport to the animal's enclosure. Consideration should also be given in advance to identifying additional pathologists, veterinarians, or other staff to assist with the necropsy – the more assistants, the better. Although one individual will need to oversee the entire post-mortem (generally the institutions pathologist or chief veterinarian), it may also be helpful to divide additional staff into separate teams to assure that all systems are examined and the proper samples collected. For example, 2 or 3 individuals may be assigned to examine the digestive tract, one individual to examine the heart and lungs, one for the urogenital tract, etc. This 'team' approach will reduce the likelihood that samples are overlooked or improperly collected.

All staff should be briefed on research protocols and other samples to be collected prior to the start of the necropsy. The relevant medical history and other historical information should be reviewed and recorded.

In general, the 'standard procedures' of pathology should be followed. Body weight should be collected, if possible, and an external exam performed. Note any evidence of trauma, skin lesions, tags or other distinctive markings, musculoskeletal condition, ectoparasites, discharges from body orifices, etc. If possible, the body should be placed in left lateral recumbency prior to opening the abdomen with a ventral midline incision from mandible to pelvis. Note amount and appearance of fluid accumulations and obtain a swab for bacterial culture and / or a sample for cytologic examination. Note the appearance, size, color, and position of all abdominal viscera. Open the diaphragm, and enter the thoracic cavity by cutting the ribs, again assessing the volume and appearance of thoracic fluid, and collect samples for culture. Collect samples of heart blood for bacterial cultures. Additional samples should be saved at minus 70° C as whole blood and serum. Internal organs are removed and examined systematically.

Every effort should be made to collect accurate weight and size measurements for internal organs. Currently, there is limited information on organ size and weights in Indian rhinoceros, and additional information is needed.

Samples of all internal organs and tissues, along with representative samples of all lesions, should be collected for histopathologic examination, as well as samples for culture, virus isolation, electron microscopy, toxicology, and other studies as appropriate. It is advisable to freeze samples of heart, lung, liver, spleen, kidney, adipose tissue, intestine, and brain (if possible) at minus 70° C for future research. Small samples of these organs and all lesions can also be minced (1 mm cubes) and

placed in Trump's fixative, 2% glutaraldehyde, or other fixative for electron microscopic examination. Multiple samples of heart muscle should be saved, to include left and right ventricular free walls, papillary muscles, valve leaflets, and interventricular septum.

If the skull is to be saved for a museum or educational specimen, then the brain cannot be examined - removal of the brain will likely result in extensive damage or destruction of the skull. If the brain is removed, it should be placed in 20% Formalin. Eyes may be placed in Bouin's fixative (preferred) or the globe may be incised and placed in Davidson's fixative or (least preferred) 10% Formalin.

Samples for histopathology should be placed in 10% Formalin (volume of Formalin 10 times the volume of tissue collected). Tissue samples should be not more than 0.5 cm thick to assure adequate fixation.

Additional samples from neonatal animals should include examination and sampling of the placenta and fetal membranes, if available. The fetus should be measured, (weight, crown-rump length). The umbilicus should be examined and described, and sample of the umbilicus saved for histopathologic examination. Examine the oral cavity for cleft palate, and the heart for septal defects, persistent ductus, or other anomalies. Stillbirth can be determined by placing a sample of lung tissue in Formalin – if the lung sinks, the animal is likely stillborn, and did not breathe.

A copy of the necropsy report, and (if possible) re-cuts of all slides (or replicate samples of all tissues fixed in Formalin) should be sent to:

In Europe:

Dr. Friederike von Houwald
Veterinary Advisor Rhino Tag
Basel Zoo
Binningerstrasse 40
CH-4054 Basel, Switzerland
vonhouwald@zoobasel.ch

In the USA:

Dr. John Trupkiewicz
Director of Pathology
Philadelphia Zoo
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Philadelphia, PA, 19104, USA
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Commonly affected organs in Rhinoceros

The stomach may be affected by ulcers, which may be related to stress, and can serve as an indicator of husbandry conditions.

Liver, Gallbladder, Gastrointestinal tract: parasitism is common, especially in newly imported animals or those housed on larger enclosures.

Fungal pneumonia, e.g. chronic interstitial pneumonia caused by *Micropolyspora faeni* (Farmer's lung), can stand in correlation with bad quality hay fed to the animals.

Vaginal and Uterine leiomyomas have been frequently identified in rhinoceros. Ovarian cysts are also common.

Chronic pododermatitis in captive Indian rhinoceros has been described at several institutions, and careful examination and description of the feet, with photographic documentation of any lesions, is suggested.

The Post-mortem procedures for wildlife veterinarians and field biologist, written by Woodford, Keet & Bengis (2000), give also valuable information concerning pathological procedures and preparation of the examination.

8.2. NECROPSY REPORT FORM (compressed version)

Institution: _____	Date of Death: _____
Address: _____	Necropsy Date: _____
_____	Necropsy # : _____
_____	Prosector: _____
Animal ID: _____	Contact Phone _____
Sex: _____ Age: _____	or e-mail: _____

Clinical History (Diet, captive born vs. wild caught, prior movements or relocations, prior medical history, recent clinical signs, pertinent laboratory results, treatments, circumstances of death, etc.):

Gross Necropsy Examination:

Weight: _____ kg / lb Actual / Estimated

External exam: Nutritional Status, Skin, Condition of Body, Wounds, Tags

Musculoskeletal: Bones, Joints, Muscles

Body Cavities: Fat Stores, Abnormal Fluids

Hemic-Lymphatic: Lymph Nodes, Spleen, Thymus, Bone Marrow

Cardiovascular: Heart, Pericardium, Aorta, Vessels

Respiratory: Nasal Cavity, Larynx, Trachea, Lungs

Digestive: Oral Cavity, Teeth, Tongue, Esophagus, Stomach, Intestines, Cecum, Colon, Liver, Pancreas

Urinary: Kidneys, Ureters, Bladder, Urethra

Reproductive: Gonads, Uterus, Vagina, Penis, Prepuce, Prostate, Accessory Glands, Mammary Glands, Placenta

Endocrine: Adrenal, Thyroid, Parathyroid, Pituitary Glands

Nervous: Brain, Spinal Cord, Peripheral Nerves

Special Senses: Eyes, Ears

Organ Weights:

Heart: _____ g / Kg LAV: _____ mm / cm RAV: _____ mm / cm

PULM: _____ mm / cm AOR: _____ mm / cm

L Wall: _____ mm / cm R Wall: _____ mm / cm

Septum: _____ mm / cm

Lungs: left: _____ g / kg right: _____ g / kg

Kidney: left: _____ g / kg right: _____ g / kg

Adrenal: left: _____ g / kg right: _____ g / kg

Liver: _____ g / kg

Brain: _____ g / kg

Thyroid: left: _____ g / kg right: _____ g / kg

Tissues and Samples Saved:

In Formalin ()

In Electron Microscopy Fixative ()

Freezer / Ultracold ()

Embedded in Paraffin Blocks ()

Microscope Slides ()

Photographs ()

Tissue Collection Checklist:

Skin		Liver		Gonad	
Muscle		Pancreas		Uterus	
Nerve		Tongue		Vagina	
Diaphragm		Esophagus		Prostate	
Lymph Nodes		Stomach		Accessory Glands	
Spleen		Duodenum		Eye	
Thymus		Jejunum		Brain	
Heart		Ileum/Cecum		Spinal Cord	
Aorta		Colon		Adrenal	
Trachea		Kidney		Thyroid/Parathyroid	
Lung		Ureter		Pituitary	
Other		Bladder		Bone	

Samples for Laboratory Analysis:

Gross Diagnosis: