TAPIR FIELD VETERINARY MANUAL

IUCN/SSC TAPIR SPECIALIST GROUP (TSG)
Veterinary Committee

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1. Veterinary Medicine in Tapir Conservation

Animal populations of many wild species are declining at an alarming rate. In some cases a species has disappeared without the scientific community being able to adequately learn about their basic natural history, ecology, physiology or behavior. Several species have had their conservation efforts severely threatened by the occurrence of disease epidemics, such as the black footed ferret, the Serengeti lion, and several Central America amphibians.

In some cases the diseases that affect wild animals have not been defined. Such is the case for the tapirs. It is still, as yet, unknown if disease (and which diseases) play a major role in tapir population dynamics. Little is known about the biology and medicine of tapirs. Most of the information available is condensed into few references and sporadic case reports.

Unfortunately, the term "free-ranging" hardly exists anymore, especially for tapirs that, in most countries, only remain in protected areas. These areas limit the natural movement of animals, which can increase the prevalence of diseases. In addition, the restriction of tapir population sizes (some threatened by extinction) in isolated reserves, often surrounded by domestic animals, makes them susceptible to outside health menaces. It is important to involve disease specialists that are equipped to foresee the health-related problems of such interactions. Veterinarians are trained in epidemiology and animal health and thus are the best qualified professionals to tackle such problems. Many diseases can be introduced by human activities, as a result of the growth of communities with consequent habitat loss and changes in land use, a situation that can force restricted tapir populations to have contact with domestic animals, chemistry, physic and noise pollution, and multiple stressor and pathogenic agents. In order to foresee and perhaps prevent such situations, baseline information must be gathered on these populations. Such information may include:

a) Health menaces to which such a population is susceptible.

b) The types of etiologic agents that normally cause clinical disease.

c) What role diseases normally play in population dynamics?

d) What, if any, domestic animal diseases can affect tapirs.

e) Evaluate if tapirs could be reservoir for domestic animal diseases.

f) Methods for predicting, preventing and/or controlling such diseases if necessary.

The different uses of habitats and ecotourism offer the potential for expanding conservation efforts; however, often their effects on free-ranging wildlife are overlooked. Field projects have transformed into multidisciplinary projects as a necessity to meet the demands for maximizing the amount of information gathered through any one event. It is assumed that the veterinarian who will participate in such projects has experience with field methods for capture, immobilization and disease investigation. The relationship between the field researcher and the veterinarian should be established prior at the start of the project. This will allow the veterinarian to research the needs of the project given the specific genus, the conditions for chemical immobilization, the size of the population, the regional differences in disease, and the diseases that affect local livestock.

The involvement of veterinarians in field research enriches the scientific data gathered during such projects and provides the following advantages:

1. A veterinarian is a person with specialized knowledge and training in capture and chemical immobilization.

2. A veterinarian is familiar with the regional diseases that affect other ungulates and tapirs. A veterinarian is able to evaluate and monitor epidemiological or endemic population health challenges, and elaborate disease control strategies.

3. A veterinarian could provide appropriate collection, handling and storage needed for the necessary diagnostic tests of biological samples for disease, genetic and other priority scientific investigation.

4. A veterinarian is trained to interpret the results of diagnostic exams which can often be a source of confusion for lay people.

5. A veterinarian is trained in the areas of anatomy and physiology and therefore is a valuable consultant in projects which will include any aspect of nutrition, reproduction and behavior.

6. A veterinarian is able to train field personnel (biologists, para-biologists, zoo personnel etc) on capture, immobilization, sample collection/handling/storage, identification of disease based on clinical signs, physical examination of the living animal, nutritional deficiencies and post-mortem examination.

7. In the case of re-introduction, translocation, or populations restoring, the veterinarian's participation becomes an absolute necessity. The veterinarian should be in charge of evaluating the health of all animals intended for release, to avoid the introduction of novel pathogens, and to protect the population intended for reintroduction.

The formation of multidisciplinary teams is fundamental to improve conservation projects and maximize their yield. The role of the veterinarian in the conservation of the tapir species is oriented towards solving problems related to capture, immobilization, the recognition of disease and methods for predicting and controlling its effects on populations, if needed. However, the veterinarian can offer much more as a crisis manager, such in cases of epidemics or natural disasters, a facilitator, such as when communicating with other specialists (*e.g.* geneticists), as a consultant in areas such as nutrition, and as a trainer.

2. Tapir Anatomy: General Information

The internal anatomy and physiology of tapirs is similar to that of the domestic horse and other Perissodactyla. When specific data is not available for tapirs, it is recommended to adapt the doses and therapeutics from protocols used for equids and rhinos.

The tapirs have a solid and massive body structure, and their body weight is around 150-300 kg, or above 300 kg for the Malayan tapir (Further details on **TABLE 1**). Females tend to be larger than males, but there is no evident sexual dimorphism. Tapirs have a proboscis derived from muscle and soft tissues from the nose and upper lip. The proboscis is highly mobile and sensitive to touch, and is very important for the manipulation and ingestion of food. Lowland tapirs have an exuberant crest on the back of the neck, which is derived from fat and soft tissues and covered by very long black hair.

TABLE 1. Body Weight of Different Tapir Species

SHOEMAKER, A.H. *et al.* Linhas Mestras para Manutenção e Manejo de Antas em Cativeiro. IUCN/SSC Tapir Specialist Group (TSG).

Species	Male (kg)	Female (kg)
Tapirus bairdii	180-270	227-340
Tapirus indicus	295-385	340-430
Tapirus pinchaque	136-227	160-250
Tapirus terrestris	160-250	180-295

Tapirs have pharyngeal guttural pouches similar to that of the domestic horse, but no important affections of these structures have been reported. The parietal and visceral pleura are normally thick and prominent, but only Malayan tapirs have anatomic fibrous connective tissue between the lung and chest wall that might be mistaken for pathological adhesions. The jugular vein is found deeply in the laterals of the trachea.

The dental formula of adult tapirs is 2x (I-3/3, C-1/1, PM-4/3, M-3/3) for a total of 42 teeth. Males and females have similar teething. The upper third incisors are large and well developed, while the upper canines are reduced and separated from the incisors by a narrow diastema. The lower third incisors are reduced and the lower canine is well developed, occluding with the canine-like upper third incisors. There is also a large diastema between canines and premolars in both jaws.

Tapirs have three hoof-like nails in the rear feet and four in the front feet, the fourth nail is less developed and does not touch the ground. The digits are frontally covered by thick and resistant nails. The weight of the body is divided between an elastic cushion under the feet and the central digits, which becomes evident in the tapir footprints.

The tapir's digestive system presents a small gut, a well developed cecum and colon, and lacks a gallbladder. The kidneys are not lobulated and, as in other water-associated ungulates, its cortex represents about 80% of the renal mass in the adult.

RECOMMENDED LITERATURE

Janssen, D.L.; Rideout, B.A. & Edwards, M.S. 2003. *Tapiridae*. *In*: Fowler, M.E. Zoo and Wild Animal Medicine 5th Edition. London: W.B. Saunders.

Padilla, M. & Dowler, R.C. 1994. *Tapirus terrestris*. Mammalian Species, 481:1-8.

3. Capture Methods

The capture technique(s) to be adopted should be planned very carefully in order to minimize stress and injure hazard to the animals. It should assure safety for the animal and personnel involved in the operation. Moreover, it should be adequate to the procedures that determined the need for capture, such as biological sample collection, marking, radio-transmitter placement, transport, translocation etc. Data such as the species to be captured, local environmental conditions, crew and equipment transport, and the field assistant capabilities, should be regarded while choosing the capture method. Whatever the method, best results are achieved after placing baits to attract the animals, salt or native forest fruits usually are functional options.

In order to capture and chemically restrain free-ranging tapirs it is absolutely vital that the personnel involved is well-trained and prepared to operate as a team. The experience of local hunters and ranchers can be most useful. Capture stress and traumas are intrinsic risks of the handling of wild tapirs, however a well-planned capture method and the selection of a safe chemical restraint protocol can significantly reduce these risks.

3.1. Anesthetic Dart Shooting

In some instances it is possible to capture tapirs by shooting the animals with darts containing anesthetic solutions, from a platform built near a spot where the bait is placed, or from the ground. Compressed air or carbon oxide guns should be used to rush the darts. The bait should be placed at up to 10 meters away from the platform, which should prevent trajectory errors. Fire systems are not recommended, for the noise would startle the animal. Long waiting periods should be expected. The success of the method is influenced by the species activity period. Tapirs are often active during dawn and dusk, when poor lightning lowers the precision of both the shot and body weight estimation. Light bulbs for supplementary lightning may be of use. Additionally, anesthetic drugs usually demand up to 15 minutes for the induction. During this period, an escaping animal is more prone to suffer trauma due to the effects of the anesthetic, and may eventually go somewhere it cannot be found. Alternatively, a transmitter dart can be used. The advantages of this method are the possibility of capturing the individual again later, requirement of few field assistants, and logistic feasibility. **This method was successfully used by Charles R. Foerster and Sonia Hernandez-Divers to capture Baird's tapirs in Corcovado National Park, Costa Rica. This method was also used to capture/recapture 5 lowland tapirs in Morro do Diabo State Park and surrounding Atlantic Forest fragments in São Paulo State, Brazil (Patrícia Medici and Paulo Rogerio Mangini, IPÊ - Instituto de Pesquisas Ecológicas).**

Further details about this capture method are provided in:

Capture and Immobilization of Free-Living Baird's Tapirs (*Tapirus bairdii*) for an Ecological Study in Corcovado National Park, Costa Rica - 2001 - Sonia Hernández-Divers and Charles R. Foerster - Zoological Restraint and Anesthesia, D. Heard (Ed.) - International Veterinary Information Service (www.ivis.org), Ithaca, New York, USA.

3.2. Pitfall

A pitfall for capturing tapirs consists in a 220-cm deep, 150-cm wide and 240-cm long hole in the ground, covered with roofing tiles, and camouflaged with forest debris. Pitfalls less than 200 cm deep may allow the tapirs to escape. Pitfalls with these dimensions were used to capture lowland tapirs, and may not be adequate for other species of tapirs. It is important to emphasize that the pitfalls should be dug in frequently visited paths or bait stations. This technique is very controversial. Fracture hazard, catching more than one animal at a time, manipulation of the captured animal inside the hole, habitat disturbance and local geologic conditions must be considered. Some advantages can be pointed out. The traps are unnoticeable, and the same animal can be repeatedly captured. Also, after the animal is caught, there is time enough so the animal can be manipulated at the most suitable moment. The animals usually remain calm. It is easier to estimate the body weight and shoot anesthetic darts precisely. The impossibility of escape after the first shot allows the design of safer protocols, with correct administration of pre-anesthetic drugs and capability to hold the animal until recovery is complete. Captured tapirs can be easily darted through the use of a dart pistol or a blowpipe. The procedure to release a tapir captured in a pitfall implies one of the pitfall walls to be tumbled down until a slope is formed, so that the animal can walk out of the pitfall as soon as it has completely recovered from the chemical restraint. **This method has proven very successful and safe for the capture/recapture of 14 lowland tapirs in Morro do Diabo State Park and surrounding Atlantic Forest fragments in São Paulo State, Brazil (Patrícia Medici and Paulo Rogerio Mangini, IPÊ - Instituto de Pesquisas Ecológicas).**

Further details about this capture method are provided in:

Medici, E. P. & Mangini, P. R. 1998. Avaliação da Utilização de Trincheiras para Captura de *Tapirus terrestris* em Vida Livre. In: *Book of Abstracts of the XXI Annual Conference of the Brazilian Association of Zoos*. Salvador, Bahia, Brazil.

Medici, E. P. & Mangini, P. R. 2001. Evaluation of Different Methodologies used to Capture Wild Lowland Tapirs (*Tapirus terrestris*) at the Pontal do Paranapanema Region, São Paulo State, Brazil. In: *Book of Abstracts of the First International Tapir Symposium*. IUCN/SSC Tapir Specialist Group (TSG), American Zoo and Aquarium Association (AZA) Tapir Taxon Advisory Group (TAG), and Tapir Preservation Fund (TPF). San Jose, Costa Rica.

Medici, E. P.; Velastin, G. O. & Mangini, P. R. 2004. Avaliação da Utilização da Metodologia de Trincheiras para a Captura de *Tapirus terrestris* em Vida Livre. In: *Book of Abstracts of the XXIII Annual Conference of the Brazilian Association of Zoos*. Rio de Janeiro, Rio de Janeiro, Brazil.

3.3. Box Trapping

Box traps consist of wooden or metal boxes with two doors located on opposite sides. As the tapir attempts to go through the open box, a trigger is pulled and the doors fall simultaneously, restraining the animal inside. The traps are placed on natural tapir paths, with bait placed inside to attract the animals. The main advantage of this technique is that the animal is close enough to be easily reached, manipulated, or injected with anesthetic drugs. It prevents the animal from escaping and is a very practical method for relocation. However, it could be ineffective when the box is small, and in addition, tapirs may be reluctant to enter the box, even with both of the doors held open.

3.4. Capture Pen (Corral)

Corrals should be preferably built with wooden pillars wider than 10-cm and wooden boards thicker than 2.5-cm. The walls, as for the pitfalls, should be at least 220-cm high to avoid escapes. The lateral dimensions can be about 350 x 200 cm, preventing the captured individual from moving in excess. An automatic trigger placed in the very bottom of the trap closes the door. For this capture technique it is necessary to use bait. Captured tapirs can be easily darted through the use of a dart pistol or even a blowpipe. **This method has proven very successful and safe for the capture/recapture of 16 lowland tapirs in Morro do Diabo State Park and surrounding Atlantic Forest fragments in São Paulo State, Brazil (Patrícia Medici, Paulo Rogerio Mangini, and Joares A. May Jr., IPÊ - Instituto de Pesquisas Ecológicas).**

3.5. Hunting Dogs

The use of hunting dogs is an alternative in rough terrains, where tapirs might find proper corners to hide from the chasing. Once cornered, the tapir might be hit by anesthetic darts through the use of a dart pistol or even a blowpipe. The method is safe, but eventually the dogs might cause superficial skin lesions to the captured animal, and eventually they might also be wounded. These risks, however, are reduced as the dogs are better trained. The stress in this capture method must be carefully considered, and the method used only when other alternatives are not feasible. **This method has proven very successful and safe for the capture of 7 mountain tapirs in Los Nevados National Park, Colombia (Diego J. Lizcano and Paulo Rogerio Mangini).**

Further details about this capture method are provided in:

Mangini, P. R.; Lizcano, D. & Cavalier, J. 2001. CHEMICAL RESTRAINT OF TWO WILD *Tapirus pinchaque* IN THE CENTRAL ANDES OF COLOMBIA. In: *First International Tapir Symposium*, San Jose, Costa Rica, Book of Abstracts. IUCN/SSC Tapir Specialist Group. V. 1, p. 17-18.

Lizcano, D.; Cavalier, J. & Mangini, P. R. 2001. Use of GPS Collars to Study Mountain Tapirs (*Tapirus pinchaque*) in the Central Andes of Colombia. In: *First International Tapir Symposium*, San Jose, Costa Rica, Book of Abstracts. IUCN SSC Tapir Specialist Group. V. 1, p. 9-9.

4. Chemical Restraint

Several anesthetic protocols for captive tapirs have been compiled by Janssen *et al.* (1996), Janssen (2005), Nunes *et al.* (2003), and Mangini (2006). However, some of the anesthetic protocols used in captive animals may not be suited for free-ranging tapirs. Currently, several tapir field projects utilize anesthetic protocols which have not been published in scientific literature that is readily available, but that have been exhaustively tested in free-ranging tapirs in many different areas. Therefore, the following information was collected from those veterinarians currently employing these protocols in the field in order to provide other veterinarians and biologists with a variety of alternatives. It is assumed that any individual who uses this information has consulted with a veterinarian prior to implementing the protocol in the field. In addition, the conditions under which these protocols are successful should be carefully explored and taken into account when attempting to apply them to different situations. It is highly recommended that the veterinarian who has experience with each of these protocols be contacted for further consultation. A short guide to the drugs described in this chapter and their effects on animal physiology is available on **APPENDIX 1**.

4.1. Recommended Protocols

Butorphanol / Xylazine

DVM Sonia Hernandez-Divers

Baird's Tapir *Tapirus bairdii* - Corcovado National Park, Costa Rica

Protocol: A total dosage for a 200-300 kg animal composed by 40-50 mg of Butorphanol Tartarate (Turbogesic®) plus 100 mg of Xylazine in the same dart. Additional Ketamine 187±40.86 mg/animal, administered IV the majority of times, to extend the anesthetic period.

Reversal: Naltrexone (50 mg) mixed with 1200 mg of Tolazoline in the same syringe, IM, given no sooner than 30 minutes from the last administration of Ketamine.

Comments: This protocol was administered to animals from a tree blind via dart. The animals had been habituated to come to bait (ripe bananas) for several days and thus were relatively calm when darted.

Further details about this protocol are provided in:

Butorphanol/Xylazine/Ketamine Immobilization of Free-Ranging Baird's Tapirs in Costa Rica - 2000 - Sonia Hernández-Divers, James E. Bailey, Roberto Aguilar, Danilo Leandro Loria, and Charles R. Foerster - *Journal of Wildlife Diseases,* 36(2), pp. 335–341

Capture and Immobilization of Free-Living Baird's Tapirs (*Tapirus bairdii*) for an Ecological Study in Corcovado National Park, Costa Rica - 2001 - Sonia Hernández-Divers and Charles R. Foerster - Zoological Restraint and Anesthesia, D. Heard (Ed.) - International Veterinary Information Service (www.ivis.org), Ithaca, New York, USA.

Cardiopulmonary Effects and Utility of a Butorphanol/Xylazine/Ketamine Anesthetic Protocol for Immobilization of Free-Ranging Baird's Tapirs (*Tapirus bairdii*) in Costa Rica - 1998 - Sonia Hernández-Divers, James E. Bailey, Roberto Aguilar, Danilo Leandro Loria, and Charles R. Foerster - Proceedings American Association of Zoo Veterinarians (AAZV).

Etorphine / Acepromazine

DVM Alberto Parás Garcia & DVM Iván Lira Torres

Baird's Tapir *Tapirus bairdii* - Mexico

Protocol: The total dosage for a 200-250 Kg animal is a mixture of 1.96 mg Etorphine hydrochloride plus 5.90 mg of Acepromazine maleate, in the same dart (Fowler 1986; Janssen *et al.* 1996; Parás & Foerster 1996; Kreeger 1997).

Reversal: Diprenorphine hydrochloride (Revivon Large Animal, C/Vet limited) - 5.88 mg.

Comments: This protocol has been designed given the particular conditions of the Sierra Madre of Chiapas, Mexico. This region has a highly accented topography with pronounced slopes of more than 60 degrees of inclination. For this reason, induction times must be minimized, in order to avoid fatalities.

Further details about this protocol are provided in:

Immobilization of Free Ranging Baird's Tapir (*Tapirus bairdii*) - 1996 - Alberto Paras-Garcia, Charles R. Foerster, Sonia Hernández-Divers, and Danilo Leandro Loria - Proceedings American Association of Zoo Veterinarians (AAZV).

Butorphanol / Medetomidine

Researcher Patrícia Medici
Applied by DVM Joares A. May Jr., DVM Paulo Rogerio Mangini & DVM George Ortmeier Velastin

Lowland Tapir *Tapirus terrestris* - 15 immobilizations
Morro do Diabo State Park and surrounding forest fragments, São Paulo, Brazil

Protocol: Butorphanol Tartarate (Turbogesic®) 0.15 mg/Kg mixed with Medetomidine (Domitor®) 0.03 mg/Kg plus Atropine (0.025-0.04 mg/Kg), IM, in the same dart (5 ml).

Reversal: Atipamezole 0.06 mg/Kg + Naltrexone 0.6 mg/Kg in the same syringe, IV (slowly).

Comments: Adequate for tapirs captured in pens or pitfalls. This protocol produces adequate chemical restraint for procedures such as radio-tagging and biological sampling. The average induction time is 15 minutes for this protocol. It is important to keep in mind that Medetomidine is commercialized in different concentrations, and whenever possible it is advisable to use higher concentrations.

Further details about this protocol are provided in:

Velastin, G. O.; Mangini, P. R. & Medici, E. P. 2004. Utilização de Associação de Tartarato de Butorfanol e Cloridrato de Medetomidina na Contenção de *Tapirus terrestris* em Vida Livre - Relato de Dois Casos. In: *Book of Abstracts of the XXIII Annual Conference of the Brazilian Association of Zoos*. Rio de Janeiro, Rio de Janeiro, Brazil.

Tiletamine-Zolazepan, Alpha-2-Adrenergic, Ketamine, and Atropine

DVM Paulo Rogerio Mangini & Researcher Patrícia Medici

Lowland Tapir *Tapirus terrestris* - 6 immobilizations

Morro do Diabo State Park and surrounding forest fragments, São Paulo, Brazil

These protocols that use an anesthetic mixture in a single dart were designed to provide anesthetic safety for animals with corporeal weight between 200 and 300 kg. These protocols are applied when using the darting from distance capture method, producing a short induction time, and adequate chemical restraint for the animal's manipulation.

In all these protocols all the anesthetic drugs are managed in a single mixture, with the use of only one dart. The Ketamine and Alpha-2 agonist are used to dilute Tiletamine-Zolazepan lyophilized powder. In some individuals it is necessary to administer supplementary doses, which are given with Ketamine and the same Alpha-2 agonist used in the original mixture. The average induction time for this protocol is 5 minutes.

1) Detomidine - 1 dart

Detomidine - 0.06-0.04 mg/kg
Ketamine - 0.62-0.41 mg/kg
Atropine - 0.025-0.04 mg/kg
Tiletamine-Zolazepam - 1.25-0.83 mg/kg

2) Romifidine - 1 dart

Romifidine - 0.05-0.03 mg/kg
Ketamine - 0.62-0.41 mg/kg
Atropine - 0.025-0.04 mg/kg
Tiletamine-Zolazepam - 1.25-0.83 mg/kg

3) Medetomidine - 1 dart

Medetomidine - 0.006-0.004 mg/kg
Ketamine - 0.62-0.41 mg/kg
Atropine - 0.025-0.04 mg/kg
Tiletamine-Zolazepam - 1.25-0.83 mg/kg

Comments: Best results on immobilization, cardio-respiratory parameters and recover are obtained with Medetomidine, followed by Romifidine. Short apnea episodes are observed more frequently using Detomidine protocol. All these protocols are able to knockdown lowland tapirs in a short period of time. The veterinarian in charge of the immobilization is responsible for deciding if the use of Atropine is appropriate or not according to his/her professional experience. It is recommended to associate Atropine in anesthetic protocols that use Alpha-2-agonists, in order to control the cardiac depression and excessive secretions.

Tiletamine-Zolazepan, Alpha-2-Adrenergic, and Atropine

DVM Paulo Rogerio Mangini & Researcher Patrícia Medici

Lowland Tapir *Tapirus terrestris* - 15 immobilizations

Morro do Diabo State Park and surrounding forest fragments, São Paulo, Brazil

These protocols are used to immobilize tapirs captured in pitfalls and box traps, using two (2) darts: First 1 dart with pre-anesthetic drugs (Alpha-2 + Atropine) followed by a second dart containing the Tiletamine-Zolazepam association. The 2-dart protocols calculated doses for animals with corporeal weight between 150 and 350 kg. The average induction time for this protocol is 20 minutes. The reversal of all of the protocols was made with Atipamezole or Yohimbine in the doses of 3 to 5 times plus the Alpha-2 agonist doses used, providing less agitated recovering time.

1) Medetomidine - 2 darts
Medetomidine - 0.01-0.008 mg/kg
Atropine - 0.04 mg/kg
Interval of 10 minutes
Tiletamine-Zolazepam - 4.11-5.6 mg/kg

2) Romifidine - 2 darts
Romifidine - 0.11-0.09 mg/kg
Atropine - 0.04 mg/kg
Interval of 10 minutes
Tiletamine-Zolazepam - 4.11-5.6 mg/kg

3) Xylazine - 2 darts
Xylazine - 0.56-0.42 mg/kg
Atropine - 0.04 mg/kg
Interval of 10 minutes
Tiletamine-Zolazepam - 4.11-5.6 mg/kg

Comments: The protocols are based in the association of dissociative anesthetics, Alpha-2 agonist, benzodiazepines, and atropine. Dosages were calculated using inter-specific allometric scaling. Medetomidine was the most used drug, producing the best results obtaining good muscular relaxation and more stable cardio pulmonary parameters, Xylazine produces the worst results with poor muscular relaxation and analgesia. It is important to provide space to patients to recover, usually disturbed with standing and falling periods. Antagonist drugs could provide better recover.

Further details about this protocol are provided in:

Mangini, P. R. & Medici, E. P. 1998. Utilização da Associação de Cloridrato de Medetomidina com Cloridrato de Tiletamina e Cloridrato de Zolazepam na Contenção Química de *Tapirus terrestris* em Vida Livre - Relato de Dois Casos. In: *Book of Abstracts of the XXI Annual Conference of the Brazilian Association of Zoos*. Salvador, Bahia, Brazil.

Mangini, P. R. & Medici, E. P. 1999. Aspectos Veterinários do Estudo de *Tapirus terrestris* em Vida Livre na Região do Pontal do Paranapanema - Estado de São Paulo - Brasil. In: *IV Congresso Internacional de Manejo de Fauna Silvestre en Amazonia y Latino America*, Assunción. Programa y Libro de Resumenes do IV Congresso Internacional de Manejo de Fauna Silvestre en Amazonia y Latino América. Assunción: La Fundación Moisés Bertoni, 1999. v. 1, p. 101-101.

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4.2. Important aspects to be considered

- The success of the chemical restraint of free-ranging tapirs depends on careful planning, which should consider:

    1. Basic characteristics of the anatomy, metabolism and physiology of the captured species;
    2. The environmental conditions of the location where the capture will take place;
    3. The capture method that will be applied;
    4. The available equipment that might be used during the capture process;
    5. Estimates of the time required to carry out the biological sampling and clinical procedures during the animal's manipulation;
    6. If there is need to translocate the animal from the original capture site;
    7. The possibility of unexpected events interrupting or interfering with the chemical restraint;
    8. Detailed knowledge of the pharmacology, adverse effects and counter-indications of the drugs that will be used for the chemical restraint.

- The determination of the individual's exact corporal weight is one of the obstacles in the chemical restraint of free-ranging tapirs. A wide safety margin is of major importance since it is impossible to determine the exact body weight of the animals to be captured. The calculation of predetermined doses for body weight estimates at 50 kg intervals is usually safe enough for adult tapirs. The experience of the team with captive animals might prove useful for more accurate body weight estimates.

- Chemical restraint should be performed during the milder parts of day, and the animal must be monitored until it has fully recovered. After the manipulation, the animals should be capable to perform all of their ecological functions. It is also necessary to predetermine protocols for possible emergencies, as well as the destination of animals that may eventually became wounded or present some critical clinic situation during the capture process.

- The intramuscular administration of anesthetic agents can be applied on the side of the neck or on the gluteal musculature, while subcutaneous administration is easier on the abundant subcutaneous tissue behind the insertion of the ears, or on the dorsum between the scapulae.

- Once the anesthetic agents start to take effect, the head of the tapir should be positioned below the body level to avoid aspiration in case of regurgitation. Tracheal tubing is difficult as the head is long and narrow and the glottis is not visible, however is advisable to avoid aspiration of gastric reflux. Blind intubation is possible with experience. Direct observation of the larynx is possible with long laryngoscope blade. Tracheal tubes must be 10 to 14 mm for juveniles and 16 to 24 mm for adults.

- The capture and immobilization procedures must be carried out in an isolated place avoiding excessive noise and unnecessary personnel. As soon as the animal falls under the effect of the anesthesia its eyes must be covered in order to protect them from excessive sunlight and to minimize stress.

- When dealing with wild animals, it is generally impossible to have a proper health evaluation prior to the restraint. Most of the times, it is only possible to have a rough evaluation of body condition, skin lesions and deformities. The conditions of the circulatory and respiratory system will be unknown until the animal is already immobilized, which might become an important risk to the anesthetic and chemical restraint procedures.

- The handling of extremely stressed animals should be avoided, as the acute stress can have serious effects on the cardio respiratory system and metabolism, jeopardizing the desired effect of anesthetic agents and even risking the life of the animal.

- It is important to make sure that during the anesthetic induction or recovery the animal does not have access to water and rocky or uneven terrain, to avoid severe injury or even lethal accidents.

- The accessibility to the animal (depending on the selected capture method) and the volume of anesthetics to be administered are decisive in the process of choosing the most appropriate equipment to administer the drugs (syringe, dart pistol, blowpipe etc.).

- In order to administer the chemical restraint agents, special darts can be hand-made or purchased (*e.g.* Dan-Inject, Telinject, Pneu-Dart etc.). For animals in box-traps or pitfalls, blowpipes, injection sticks or syringes can be applied if the handler is agile.

- The ideal anesthetic protocol to be used in the capture of wild animals should be effective in a single dose, forcing the animal to fall and giving a sufficient handling time for all the desired procedures, and should be easily and safely supplemented by additional drug administration if there is a need to extend the handling period.

- Some anesthetic protocols are not available for veterinarians, given the difficulty in acquiring certain drugs in certain countries. In some cases there are special legal restrictions for the use of certain types of drugs, such as opioids in Colombia. For this reason, it is necessary to use alternative protocols for chemical restraint of tapirs in these countries. However, these protocols must be tested by qualified personnel under well-defined research designs.

- The most common emergencies during tapirs captures are hypothermia, hyperthermia, bradicardia and apnea. The continuous monitoring of body temperature is essential to the safety of the chemical restraint, as anesthetic drugs tend to interfere in the thermoregulation functions of the animal. Monitoring should be twice as careful in particularly cold or hot days. The animal should not be exposed to cold air streams, wet surfaces, direct sun or environments with poor air circulation and increased temperature. Due to their large body mass and low 'body surface/mass' proportion, tapirs are more prone to develop hyperthermia than hypothermia. Animals in hypothermia should be exposed to heat and/or protected with thermo isolants, while animals in hyperthermia should be bathed with fresh water and, if possible, transferred to ventilated places.

- It is also necessary to carefully monitor the physiological parameters of the animal under anesthesia. Auscultation of the heart and lungs, monitoring body temperature, mucosa coloration and indirect blood pressure measurements (such as capillary refill time) are the basic parameters to be monitored. The respiratory rate, type and amplitude are the most important parameters to monitor anesthetic depression. Monitoring of blood oxygen saturation with pulse oxymetry is also recommended, especially with anesthetic protocols that may involve episodes of short apnea. It is important to keep in mind that tapirs have physiological apnea for swimming. Therefore, short periods of apnea during the chemical restraint tend to be less compromising for these species.

- The field veterinary in charge of a tapir capture and chemical restraint should be fully acquainted with the physiology of stress and its medical consequences for the capture of an animal in the field, as it is one of the most important factors that affect the physiology and response to anesthetics in wild animals. It must be considered that different tapir species and individuals can respond differently to the same types of stressing events. All captures should be carefully planned to reduce noise and other stimuli to which the captured animal will be exposed to a minimum. The team should be prepared to minimize noise and unnecessary activity near the animal. Ultimately, unnecessary noise also affects the concentration and efficiency of the team members, raising the risks of human failure during the procedures.

- It is preferable to use anesthetic drugs for which there is a reversal drug. The use of reversal drugs along with the capture conditions might be definitive to determine the safety of the chemical restraint and the team's ability to capture a larger number of animals.

- The most common adverse effects during the induction or recovery from chemical restraint in tapirs are apnea, arterial hypotension and agitation/ataxia. However, the associations of Medetomidine or Romifidine tend to produce more stable cardio respiratory patterns than other Alpha-2-agonists.

- It is essential to keep a detailed record of the anesthetic doses and physiological monitoring during each capture. The results of these records, their success and failures must be published or somehow made available to other field researchers, to help improving our knowledge on the chemical restraint of these species. On **APPENDIX 3** we present a model of spreadsheet for recording and monitoring chemical restraints in the field.

RECOMMENDED LITERATURE

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5. Clinical Evaluation

The clinical evaluation of the animal begins right after first sighting of the animal inside the trap (or during the pursuit for capture), where the apparent health of the tapir, its nutritional condition, skin and hair, locomotion ability and estimated body weight can be observed. In the case of apparently unhealthy animals, with several skin lesions, bad nutritional condition, evident difficulty in locomotion etc., the veterinarian should reevaluate the anesthetic protocol to be used, choosing more appropriate drugs or even deciding not to chemically restrain the animal.

The skin of wild tapirs may present several cuts and scars (most likely from intra-specific aggression), which can be used to identify individuals. Vesicular dermatitis has been reported as an important disease of captive tapirs, and should be carefully investigated in wild animals. The presence of pigmentation spots and the condition of the dermic glands should also be evaluated. Wild mountain tapirs frequently present large areas of alopecia on the back, which is probably due to rubbing on trees as a territorial marking with dorsal glands.

There should be a careful ophthalmic examination, as degenerations such as corneal opacity and abnormal corneal pigmentations are reported as common in captivity, and seem to be frequent in wild animals. The inflammation of peri-ophthalmic glands has also been reported in wild lowland tapirs. It has been reported that some wild lowland tapirs show white external rings on their iris, which could be due to senility.

The degree of dental wearing may provide tips as to the age of the animal, although diet is another important factor that should be taken into consideration when making that estimate. The small opening of the mouth also makes it hard to evaluate more carefully the mouth and dental conditions. A key to estimate the age of wild tapirs from dental evaluation is currently under development, and should be available in a few years.

The integrity and function of the legs should be evaluated. Current and consolidated fractures should be noted, as well as the erosion of nails and lesions on the feet cushions.

Normally, wild tapirs are heavily infested with ticks (mostly *Amblyomma* and *Ixodes*). Whenever possible, the researcher should try to quantify this infestation, comparing the degree of infestation with hematological parameters. Wild lowland tapirs have been reported to have abdominal jigger (*Tunga penetrans*). The ectoparasites tend to concentrate on the abdomen, ears, mammary glands, vulva/penis and rear legs.

It has been reported that wild lowland tapirs wearing radio-collars for prolonged periods of time have deformations on the crest, with alopecia and skin hardening under the collar. In some cases, the radio-collars cause chronic skin lesions by friction, which might predispose to local myiasis.

The clinical evaluation of females should include the inspection for vaginal flows and vulvar lesions and the evaluation of the mammary glands. For males, the exposure of the penis is observed during chemical restraint, especially when Alpha-2-agonists are employed.

6. Collection, Handling and Storage of Biological Samples

Information about the health status of tapir populations is still incipient. Little information has been produced, and is scattered in sporadic case reports. Baseline data is still needed to evaluate reference values of complete blood cell counts, biochemical analysis and susceptibility to disease agents of free-ranging tapirs. A review of diseases that commonly affect captive tapirs was provided by Ramsay & Zainuddin (1993), and Janssen, Rideout & Edwards (1998). However, there is little information on diseases that affect free-ranging tapirs. As a result, the TSG Veterinary Committee encourages veterinarians and other individuals working with tapirs to collect the biological samples mentioned below. It is of utmost importance that veterinarians planning to collect biological samples consult with the diagnostic laboratory that will perform the analysis prior to the collection of samples to avoid inappropriate sample collection, handling or storage. Given that most commercially available diagnostic tests have been designed and tested for domestic animals, it is highly recommended that a veterinarian consults with specialists in the different areas (microbiologists, virologists etc.) to determine the appropriate test to use and its adequate interpretation. In some cases, the use of commercial tests may be inappropriate and may mean resources wasted on meaningless results. In all cases, and given the rapid advances of diagnostic medicine, the veterinarian is highly encouraged to develop a storage system and maintain samples for future analysis, as resources for new diagnostic tests become available.

6.1. Sampling Procedures

All biological samples collected should be accompanied with the marking of the animal, the date and time of collection, the location where the sampling has been collected, and if possible the geographic coordinates. The season of collection (which might affect the prevalence of some diseases), and a detailed history describing the conditions under which the samples were collected (sedation, general anesthesia, necropsy etc.), and any relevant anatomic features (*e.g.* blood collection site, ectoparasite collection site) must be added. In order to provide a checklist of samples to be collected and notes to be taken, we recommend the use of the spreadsheets provided on **APPENDIX 3**.

Finally, tapirs are listed by CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and for instance, the transport of any biological product deriving from them falls under CITES regulations. Whenever transporting samples out of the country of origin, in addition to other import/export permits, a CITES permit is required. The veterinarian is strongly encouraged to become familiar with the legislation in their country that limits the movement of biological products from tapirs.

6.1.1. Blood

For each sampling procedure, the area must be properly disinfected with 1:1 povidine iodine/ethanol 70% solution or chlorhexidine, given that tapirs have semi-aquatic behavior and their skin can be highly contaminated.

The venipuncture can be easily made on the saphenous or cephalic veins or in their carpal/tarsal derivates, on medial access, where the skin is thinner. The jugular vein is deep and not always easy to access, but is an important alternative when large blood volumes are necessary or when the other veins are collapsing after puncture. For the case of young animals, the jugular vein tends to be the easiest access. The caudal auricular vein which runs along the center of the back of the ear may also be used.

The use of vacuum sampling systems (*e.g.* Vacutainer®) is recommended for the collection of blood samples, as it avoids the contamination of the samples and allows collecting multiple samples from a single vascular puncture, reducing vascular trauma.

6.1.1.1. Blood with Anticoagulant

For hematology, the blood must be collected with EDTA given its property of preserving the cell size and shape, and a smear in a slide is recommended to be performed immediately. Try to fill the tube with the proper blood volume, otherwise there will be dilution alterations, and the cell count will be not realistic. The sample must be refrigerated until its processing in the laboratory. Heparin retards blood coagulation for up to 8 hours, and its use is recommended for cytogenetic studies in tapirs.

The blood collected with anticoagulant should be homogenized soon after collection, with slow and continuous movements to mixture the blood and the anticoagulant. The blood should then be refrigerated to reduce hemolysis, possibly with a thermo box with ice.

6.1.1.2. Blood without Anticoagulant

For serum analyses for biochemistry studies, samples must be collected without anticoagulant, and serum must be analyzed immediately, or stored in cryovials in liquid nitrogen for further analysis. Samples must be refrigerated until processed in the laboratory through the first 24 hours.

Samples are collected without anticoagulant, in vacuum tubes with or without gel. Serum amount obtained per blood sample depends on the conditions of the animal, and is generally 50% or less. Hemolysis must be avoided, so the samples must be managed with care and protected from direct sunlight. After a short period of rest the blood should be refrigerated to reduce hemolysis, possibly with a thermo box with ice.

6.1.1.3. Handling and Storage of Blood

Once in the laboratory, a fraction of the blood with anticoagulant should be used for hematology, and another fraction should be frozen for posterior analysis. The remaining of the sample should be centrifuged and its components, plasma, leukocytes and red blood cells, must be separated and stored separately.

The blood is centrifuged at 1500 rpm for 5 minutes. Aliquots of 1ml each can be stored in cryovials of 2ml, and stored in -20ºC freezers or in liquid nitrogen. Never exceed half of the capacity of the cryovial, because it may explode when put into the liquid nitrogen.

Some serum and plasma samples may present a lipidic aspect, which may be considered normal due to several physiological aspects.

6.1.2. Blood Smear

Blood smears are recommended for the evaluation of blood parasites. For the purpose of preparing the smears, blood should be collected from peripheral vessels, such as auricular veins. Collect the blood with a small syringe or heparinized capillary, and place a small drop on a microscopy slide. With another slide inclined on 45º, spread the blood over the microscopy slide, and let it dry at ambient temperature, protected from insects. Transport in a slide box, at ambient temperature. On the laboratory, fixate the slide with heat or ethanol 70% and use the proper stains for microscopic evaluation.

6.1.3. Swabs for Microbiological Analysis

The collection of microbiological samples for bacterial cultures can be made with sterile swabs and proper nutritive/transport culture medium. Sampling techniques vary depending on the type of microorganism, being necessary to use swabs as transport media in bacterial samples whereas fungi do not require them. A thorough aseptic process is required in order to avoid undesired contaminations, and the use of sterile containers is strictly required. Prudence is required during the manipulation of the samples, in order to avoid accidental human infections, so this process must be carried out by trained staff. The different techniques currently used for bacteria and fungi areas follows:

- Swabs from skin and mucosa such as conjunctiva, auricular cavity, oral cavity, nasal cavity, anal cavity, prepuce and vagina are stored in transport media such as Stuart's swab, trypticase-soy broth, nutritive broth or thioglycolate broth. The sample may be refrigerated.

- For labile bacteria, special enriched media such as hemine or yeast extract are required, and the processing of the sample must be carried out immediately, without refrigeration.

- Washes of the foreskin are generally made with 30cc of saline solution or thioglycolate broth. The aspirates are collected in sterilized tubes by using a rubber hosepipe connected to a syringe, before a careful massage of the area.

- In the case of an abscess, an incision is made into the external wall after previous disinfection, and the pus is drained. The sample is collected by rubbing the swab on the internal face of the wall.

- In the case of hematoma, or edema or fluids from the joints, samples are collected by puncture and aspiration of the fluid, previous disinfection of the external wall.

- Skin lesions and wounds can be sampled, previous cleaning of the area, removing scares and washing with saline solution.

- Hemocultures are recommended in cases where the occurrence of hematuria, hemoglobinuria, jaundice or septicemia are considered to be a possibility. Samples are collected in 0.05-0.25% sodium polyanetosulfonate (SPS). Ammonium oxalate, sodium citrate and EDTA are not recommended because they inhibit some bacteria.

- Fecal swabs are collected directly from the rectum, previous disinfection of the perineal region. Samples are transported in Stuart's broth, green brilliant broth, brain-heart infusion or thioglycolate broth.

- Fungi For the study of saprophyte fungi, the skin may be cleaned with ethanol 70%, and once dry, the sample is collected by rubbing the surface with a piece of sterile gauze. For pathogenic fungi in the skin, a sample is collected by scraping the periphery of the lesion with a blade, together with the collection of hairs from the affected area. In both cases, the samples are collected in sterilized bags, and stored without refrigeration in a dry, fresh and dark place until the processing in the laboratory.

6.1.4. Fecal Samples

Fecal samples are used for the study of fecal parasites, hormones, and genetics. Whenever possible, feces should be obtained directly from the rectum. Feces should be stored in 5% formaldehyde solutions (human fecal sample kits are most effective) for subsequent analysis (1 part formaldehyde to 4 parts feces).

6.1.4.1. **Parasites:** Two methods are most successful at yielding parasites in the field: flotation and sedimentation. Neither of these methods can guarantee the identification of endoparasites down to species, rather the endoparasites (ova or larvae) collected can be placed in general families. If identification of specific species is needed, it is necessary to consult with a veterinary specialized in parasites for methods of culturing ova and/or larvae in the field, storage and handling.

a. **Flotation Method:** 3-5 g of feces are placed in a small container (10-15 ml) and mixed with a solution of a greater specific gravity than water, which will encourage the "flotation" of parasitic ova, cysts and some larvae. A super-saturated solution of sugar can be made by mixing table sugar and water. This solution is not ideal, as it can lead to the rupture of some ova, but is effective in the field when other solutions are not available. The container is filled with the mixture of feces and fecal flotation solution to form a positive meniscus and covered with a clean microscope slide. This is allowed to sit for 10-15 minutes, at which time the slide is removed and presumably the ovum that have floated to the top and thus have "stuck" can be examined with a light microscope.

b. **Sedimentation Method:** This method allows the sedimentation of heavy parasitic ova that typically are not found with the flotation method (*e.g.* trematode eggs). 1 g of feces is thoroughly mixed with 5 ml of acetic acid. This is allowed to rest for 1 minute and then strained into a centrifuge tube. An identical volume of ether is added to this tube, mixed thoroughly and centrifuged for 1 minute at 1,500 RPM. The consequent sediment should contain the parasitic ova. The top most layers in the tube contain ether and acetic acid and should be appropriately discarded. The sediment should be mixed with a couple of drops of warm water and mixed thoroughly. This mixture is aspirated with a pipette and a couple of drops are placed on a clean microscope slide and examined with a light microscope.

Note: When fresh endoparasites are found on the feces, they should be washed in fresh water and fixated with alcohol 70% (nematodes) or AFA solution, Alcohol-Formalin-Acetate (flat worms).

6.1.4.2. **Hormones:** For the dosage of hormonal metabolites, samples should be frozen and sent to specialized laboratories. Fecal samples for hormonal analysis have to be as fresh as possible. Once the sample is collected it can be dried frozen or extracted in the field. The extracted sample can be stored until it is processed in an endocrinology laboratory.

6.1.4.3. **Genetics:** See details about collection, handling and storage of fecal samples for genetics studies in the **IUCN/SSC Tapir Specialist Group (TSG) Manual of Sampling Techniques for Genetic Analysis**. This manual was developed by the TSG Genetics Committee and is available online on the TSG Website (www.tapirs.org) in English, Spanish and Portuguese. Further information can be obtained on the Genetics Committee WebPages:

http://tapirs.org/committees/genetics/index.html

6.1.5. Tissue Samples

6.1.5.1. **Genetics:** See details about collection, handling and storage of tissue samples for genetics studies in the **IUCN/SSC Tapir Specialist Group (TSG) Manual of Sampling Techniques for Genetic Analysis**. This manual was developed by the TSG Genetics Committee and is available online on the TSG Website (www.tapirs.org) in English, Spanish and Portuguese. Further information can be obtained on the Genetics Committee WebPages:

http://tapirs.org/committees/genetics/index.html

6.1.6. Hair

6.1.6.1. **Genetics:** See details about collection, handling and storage of hair samples for genetics studies in the **IUCN/SSC Tapir Specialist Group (TSG) Manual of Sampling Techniques for Genetic Analysis**. This manual was developed by the TSG Genetics Committee and is available online on the TSG Website (www.tapirs.org) in English, Spanish and Portuguese. Further information can be obtained on the Genetics Committee WebPages:

http://tapirs.org/committees/genetics/index.html

6.1.6.2. **Tricological Analyses**: The hair should be collected preferably from the back of the animal, carefully pulling both rough and thin hair manually. Hair samples should be transferred to a dry envelope or recipient, and kept away from humidity and excessive heat. If collected and stored properly, hair samples will remain intact for years.

6.1.7. Milk

If lactating females are captured, it is advisable to collect milk for bromatological analysis, so that appropriate replacement milk may be developed for abandoned captive neonates. The milk should be collected in sterile flasks, protected from light and frozen as soon as possible, and sent to the appropriate laboratory.

6.1.8. Urine

The collection of urine by cystocentesis or urethral probing is not common in the field. The collection is usually made when the animal voluntarily urinates during the chemical restraint, due the relaxation caused by the anesthetic drugs. The urine should be collected in a sterile graduated screw capped flask, kept under refrigeration during transport, and frozen until laboratorial analysis. Standard urinalysis and urine sediment analysis are recommended. Urine urinary strips can also be applied on the field, for a rapid evaluation of possible metabolic/urinary diseases. A fraction of the urine should be transferred to Eppendorf-like flasks or cryotubes for epidemiological analysis. For leptospirosis diagnosis, the urine should be transferred to a saline 0.85% on the 1:9 proportions, and 0.5ml of this mixture will be transferred to the appropriate culture medium.

6.1.9. Ectoparasites

The ticks should be removed carefully, rotating it in order to avoid pulling up the bucal apparatus, which is critical for its microscopic identification. For cases when it is necessary to store ticks for longer periods of time samples should be preserved in Ethanol 70%.

To determine if the interaction parasite-wild host - parasite - domestic host implies epidemic risk, all the plethoric females may be gathered and submitted to a laboratory for larval cultures. To determine the parasitic load of an individual, all the ticks bigger than 4.5 mm of diameter present in half of the body of the animal are counted, and the given number multiplied by two.

The mites producing scabies are collected from scrapings and hairs from the periphery of the affected area of the skin, and stored in sterile tubes with glycerin. Fleas can be collected directly from the body of the animal, and conserved in ethanol 70%.

6.1.10. Vaginal Cytology

Vaginal cytology is a tool to access the reproductive health of the females. Hygienize the vulva and insert a clean swab into the vagina (without touching the vulva), rotate the swab on the vaginal walls, remove the swab and roll it over a microscope slide. The fixation of the slide on the field is recommended, with alcohol or commercial sprays, then leave the slide to rest at ambient temperature and protected from insects. On the laboratory, the Fast Panotic or Giemsa stains can be used for microscopic analysis.

6.1.11. Other Cytological Samples

Diagnostic clinical cytology (cythopathology) is an aid for the diagnostic clinical bacteriology, because it consists of the direct analysis of the liquid obtained from punctures and aspirations. It allows the identification of the dominant cell type in a given inflammatory process, as well as the status of the cells from the affected tissue, and in some cases the identification of the etiologic agent. It is generally a relatively simple technique that can be practiced in the field.

TABLE 2. Collection, Handling and Storage of Biological Samples on the Field.

Sample	Material	Collection Method	Handling	Storage
Non-Clotted Blood	flask with anticoagulant	venipuncture	homogenize and leave to rest	refrigeration
Clotted Blood	flask without anticoagulant	venipuncture	leave to rest	refrigeration
Blood Smears	microscopy slides	venipuncture	dry at ambient temperature	slide transport box, at ambient temperature
Skin / Tissue	Jigger, scissors and flask	ear	ethanol 90%	ambient temperature, protected from light
Feces	flask	rectum	-	refrigeration
Urine	flask	spontaneous miction	-	refrigeration
Hair	flask or envelope	hand pulling	-	ambient temperature
Milk	sterile flask	hand milking	-	refrigeration
Microbiological Sampling	sterile swab	nostrils, mouth, ears, genitals, anus	nutritive/transport media	ambient temperature
Vaginal Cytology	swab	rotation of the swab on the vaginal canal	Microscopy slide, chemical fixation	slide transport box, at ambient temperature
Ectoparasites	perforated flask	manual collection, hand pulling	-	ambient temperature

6.2. Basic Equipment and Supplies for the Collection of Biological Samples:

- 5-10 ml Vacutainer® plastic or glass tubes for blood collection with anticoagulants (EDTA, heparin, sodium citrate);
- 5-10 ml Vacutainer® plastic or glass tubes for blood collection without anticoagulants (with or without coagulation gel);
- Two Vacutainer® needle adapters;
- Vacutainer® needles 21G1 and 20G1½;
- 2 ml cryovials;
- Eppendorf-like 2 ml conic propylene micro tubes;
- One tube-stand;
- Sterile swabs and flasks of nutritive media, for microbiological sampling;
- Sterile and non-sterile polyproline graduated screw capped 10 ml flasks;
- Microscopy slides;
- 19G, 20G, 21G and 22G scalps;
- One bistoury and several blades;
- Disposable syringes of 1, 3, 5, 10 and 20 ml;
- Disposable needles 18G, 19G, 20G, 21G and 22G;
- Hemoculture flasks;
- Disposable latex gloves;
- Nipper and scissors for the collection of a skin fragment;
- Envelope for the storage of collected hair;
- Plastic perfored flasks for the storage of ectoparasites.

6.3. Biosecurity and Health-Protection Equipment

During the collection and manipulation of biological samples, the use of disposable latex gloves, protection glasses and clothes is highly recommended. Even after the collection in the field, all samples should be treated as a biological risk until a complete immunological screening has been made to access what infectious agents the animal might have carried thought its blood, feces or other samples.

7. Hematology and Blood Chemistry

Blood analyses are a valuable tool for field researchers, because they provide information about the physiology and health status of the animal. These analyses permit the establishment of mean hematological and serum values and also the diagnosis of infectious, anemic and nutritional processes, hemoparasites and internal organ malfunctions. Basic hematology can be carried out directly in the field, with the assistance of a trained veterinarian. Other analyses such as enzyme analyses, levels of glucose, lipids, cholesterol, vitamins and minerals are more difficult to develop in the field, but serum samples can be collected, stored and submitted to a laboratory.

The blood analyses can be carried out by human laboratories, which will often be more accessible than veterinary clinical laboratories. Make sure the cell counts are made manually and not by automatic equipments, and investigate the technique to be employed in the blood chemistry, otherwise the results might be biased.

The interpretation of blood chemistry results should take into account the possible metabolic alterations, the clinical conditions of the animal at the moment of the chemical restraint, the method of blood collection and the capture method. For several parameters, the results represent only an instant picture of the biochemical condition of the blood at the moment of the capture, and cannot be considered to represent the whole physiological conditions of the animal. Capture-related stress can deeply modify some hematological and biochemical values.

It is extremely important to understand the results of the exams crossing them with the available information on the environment in which the tapir lives, the likely human interferences, and the serological findings of the tapir and other species (including domestic animals) that might be in indirect contact with tapirs.

The pollution of waters with dejects from humans and domestic animals, farming pesticides, mining products and other pollutants may have cumulative effects on the environment and on the tapirs, affecting the biochemical and blood parameters in various ways.

The results of hematology and blood chemistry exams can be compared with the reference values developed for captive tapirs available in **Physiological Data Reference Values for Tapir Species - International Species Information System (ISIS)** published in 2006 and made available online on the website of the IUCN/SSC Tapir Specialist Group (*www.tapirs.org*).

TABLE 3. Expiration of Samples for Blood Chemistry under Different Storage Temperatures (Santos 2006).

Exam	Type of Sample	Expiration of the Sample
Glucose	serum	immediate use only
Total Protein	serum or plasma	3 days 2-8°C, 1 week -10°C
Albumin	serum	3 days 2-8°C, 1 week -10°C
Amylase	serum or plasma (EDTA or heparin) or urine	24 hours 15-25°C, 2 months 2-8°C
AlkP	serum or plasma (heparin)	6 hours 2-8°C, several months -10°C
AST	serum or plasma (EDTA or heparin)	4 days 2-8°C, 1 week -10°C
Cholinesterase	serum or plasma (EDTA or heparin)	1 week 2-8°C
CK	serum or plasma (EDTA or heparin)	24 hours 15-25°C, 1 week 2-8°C
GGT	serum or plasma (EDTA or heparin)	2 weeks 2-8°C, 6 months -10°C
Lipase	serum or plasma (heparin)	24 hours 15-25°C, 3 weeks 2-8°C
SDH	serum or plasma (EDTA or heparin)	4 days 2-8°C, 1 week -10°C
Fibrinogen	plasma (citrate)	4 hours 2-8°C
Total Lipids	serum or plasma (EDTA)	10 days 2-8°C
Triglycerides	serum or plasma (EDTA or heparin)	3 days 2-8°C, 1 month -10°C
Cholesterol	serum or plasma (heparin)	1 week 2-8°C, 3 months -10°C
Creatinine	serum or plasma (EDTA or heparin) or urine	1 week 2-8°C
Total Bilirubin	serum	4 days 2-8°C safe from light, 3 months -10°C
Uric Acid	serum or plasma (EDTA or heparin) or urine	3 days 2-8°C, 1 week -10°C e 6 months -20°C
BUN	serum or plasma (EDTA or heparin) or urine	12 hours 15-25°C, 1 week 2-8°C, 3 months -10°C
Na	serum or plasma	1 week 2-8°C
K	serum or urine	1 week 2-8°C
Ca	serum or plasma (heparin) or urine	1 week 2-8°C, 2 months -10°C
Cl	serum or plasma (heparin) or urine	1 week 2-8°C, several months -10°C
P	serum or plasma (heparin)	2 days 15-25°C, 1 week 2-8°C
Mg	serum or plasma (heparin) or urine	24 hours 15-25°C, 2 weeks 2-8°C

AlkP = alkaline phosphatase
AST = aspartate aminotransferase
CK = creatine phosphokinase
GGT = gamma-glutamyltransferase
SDH = sorbitol dehydrogenase
BUN = blood urea nitrogen

8. Immunological Screening (Serology)

Serology data on both free-living and captive wildlife is a widely used and valuable tool for field researchers. In populations where frequent interactions between wild and domestic animals exist serum screening to detect specific pathogens antibodies is an important evaluation in both groups. In these cases, mutual transmission of pathogens can occur, and this condition in some cases directly affects human populations. These studies also help with the identification of the role played by wildlife species in some diseases, and provide an important scientific baseline for the implementation of control measures in the case an epidemic disease arises.

In order to plan for any serological investigation and point out the most important diseases in the region where the tapir captures will be taking place, it is recommended to contact local governmental agencies and other epidemiology and sanitary agencies, as well as human and animal health organizations. It is always recommended to compare the results obtained with those of other species, especially domestic animals and humans, in order to understand more deeply the importance of the tapir in the epidemiological chain.

The screening for diseases of compulsive notification (either to the OIE - World Organization for Animal Health - or local agencies) should be carefully considered by the veterinarian in charge, taking into account the economic and social consequences of such decision.

The interpretation of results should always take into account the sensibility and specificity of the employed laboratory technique, as well as characteristics of the infectious agent and of the tapir species.

TABLE 4. Suggested Serological Tests for Tapirs.

Agents	Serological Tests
Viral	Indian Vesicular Stomatitis New Jersey Vesicular Stomatitis Bluetongue Infectious Bovine Rhinotracheitis Foot and Mouth Disease Equine Herpesvirus Equine Influenza Eastern Equine Encephalitis (EEE) Western Equine Encephalitis (WEE) Venezuelan Equine Encephalitis (VEE) Rabies Avian Influenza Equine Rhinovirus Bovine Viral Diarrhea (BVD) Bovine Viral Leucosis Aujeszky's Disease Swine Parvovirus Johnes Disease Parainfluenza 3 Equine Infectious Anemia West Nile Virus
Parasites	*Trypanosoma* spp. *Leishmania* spp. *Babesia* spp. *Toxoplasma* spp. *Erlichia* spp. *Anaplasma* spp.
Bacterial	*Brucella* spp. *Salmonella* spp. *Mycobacterium bovis / tuberculosis / avium / paratuberculosis* *Chamydophyla* spp. *Leptospira* spp.

TABLE 5. List of *Leptospira interrogans* serovars.

pomona	*hebdomadis*	*autumnalis*	*tarassovi*
hardjo	*copenhageni*	*castellonis*	*mini*
icterohaemorrhagiae / copenhageni	*javanica*	*bataviae*	*guaicurus*
grippotyphosa	*panama*	*butembo*	*ballum*
canicola	*pyrogenes*	*whitcombi*	*sejroe*
bratislava.	*wolffi*	*cynopteri*	*szwajizak*
andamana	*shermani*	*sentot*	*saxkoebing*
australis	*patoc*		

PREVIOUS STUDIES

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Mangini, P. R. & Medici, E. P. 2001. Sanitary Evaluation of Wild Populations of *Tapirus terrestris* at the Pontal do Paranapanema Region, São Paulo State, Brazil. In: *Book of Abstracts of the First International Tapir Symposium*. IUCN/SSC Tapir Specialist Group (TSG), American Zoo and Aquarium Association (AZA) Tapir Taxon Advisory Group (TAG), and Tapir Preservation Fund (TPF). San Jose, Costa Rica.

Hernández-Divers, S.; Bailey, J. A.; Aguilar, R.; Loria, D. L. & Foerster, C. R. 2005. Health Evaluation of a Radiocollared Population of Free-Ranging Baird's Tapirs (*TAPIRUS BAIRDII*) in Costa Rica. In: *Journal of Zoo and Wildlife Medicine* 36(2): 176–187.

9. Reproduction

9.1. Brief Reproductive Physiology Review

Male and female tapirs reach their sexual maturity when they are approximately two years old. In the wild, occasionally the one year-old calves are sighted accompanied by their mothers.

The adult males have a small and pendulous scrotum, and the testicles are located near the perineum. To urinate they move the extremity of the penis backwards, in order to hurl the urine far away. Like in the domestic horse, the tapir's urethra finishes with a small prominence in the lower side of the gland. From the penis morphology in erection, it may be deduced that the ejaculation occurs inside the uterus, like in equines.

The females have a pair of mammary glands on the inguinal area and the uterus presents two horns, the placenta is epiteliocorial. The vaginal mucous produces a lipid secretion that provides vulvar lips adherence, making the vaginal environment not only isolated from the external medium but protected when the animal stays in the water.

The tapir estrus is very difficult to determine. In general, female tapirs are annual polyestrical and the estrus, in general, lasts 1-4 days and is repeated each 28-32 days. Fertile estrus is possible 9-27 days after the calf birth. However, the estrous cycle for tapirs should be approached individually for each species as well as the gestation length.

9.2. Hormones during Estral Cycle and Gestation

Hormone screening is used to monitor the estrous cycle and hormonal status in both captive and free-living animals. Because of the stress produced by immobilization, blood samples are not reliable for these studies, so fecal, urine and salivary samples are the best choices, because the collection of these samples is much less invasive for the animals and the hormonal concentration can be measured more accurately given that the animals are not stressed out by the capture process. The most widely employed technique is the radio-immunoassay, for the detection of the hormone metabolites. For the determination of pregnancy in captive tapirs, samples must be collected at least every week, in order to project the fluctuations of progesterone serum levels.

In captivity, animals can be trained for the collection of saliva and urine. Fecal samples are the best choice for field studies, but the collection must be performed right after the defecation. The samples can be stored in a container with ethanol 90% and the precise time of collection must be recorded. The sample can be dried in an oven, sunlight, or a lyophilizeror extracted in the field as mentioned earlier.

Studies with the serum progesterone concentrations of captive Baird's tapirs made by Dr. Janine Brown (1994) in the United States indicate that the duration of the estrous cycle is about 25-38 days, with a luteal phase length of 18.1 ± 0.4 days (range 15-20 days). The interluteal period is relatively long, comprising approximately 40% of the estrous cycle. Females resume cycling 16.2 ± 2 days after parturition and might become pregnant during the first postpartum estrous.

Studies carried out with captive lowland tapirs in the Fundación Temaikén, Argentina, showed that the serum concentrations vary between 17.2-35.1 ng/ml for estrogens, and between 0.78-1.64 ng/ml for progesterone. The male serum testosterone concentrations vary between 0.12-1.73 ng/ml, a value of 0.2 ng/ml was registered in the copulation period in a male of lowland tapir.

The copulation can take place both in land or in the water. Lowland tapir gestation period vary from 395 to 399 days, being shorter for Malayan tapir and Baird's tapir. The gestation is not evident in a physic or visual test, even on the final stage. Gestation suspicions should be confirmed by ultrasound or analyses of hormonal concentrations in serum, urine or feces. Little is known on the vaginal cytology of tapirs, but the present experiences suggest that it might be possible to differentiate the stages of the estrus cycle stages or to diagnose gestation.

Progesterone concentrations higher than 2.5 ng/ml are suspicious of pregnancy, although it should be confirmed doing 3 tests during 15 days to confirm or discard it. If the values increase in the consecutive tests then a pregnancy could be securely diagnosed and there will be almost 13 months to control the gestation evolution.

In pregnant females of lowland tapirs, the progesterone serum concentrations show increases and decreases along the whole gestation, registering minimum values of 2.67 ng/ml on the first period of the gestation, and maximum values of 22.6 ng/ml on the last period. On the same period, the estrogens serum concentrations show uniform behavior, with values of 20-30 pg/ml. In lowland tapirs it has been shown that in 7-10 days before the parturition, both hormones reach a maximum level, and then decrease drastically some hours before the parturition. A similar behavior has been described in Baird's tapirs, with estrogens values considerably higher, of 85-131 pg/ml.

In lowland tapirs the cortisol at the end of the gestation does not seem to play an important role in initiating parturition, since its serum concentrations do not show significant changes. The values registered during pregnancy varied from 2.52 ng/ml in the first period of gestation to 3.19 ng/ml 48 hours before the parturition. A similar behavior was described in Baird's tapirs, as the cortisol values registered in the early stage of the gestation varied between 6.9-10.2 ng/ml and, in the late stage, the values were between 9.5-10.8 ng/ml.

The use of ultrasound would certainly be most interesting on field studies, providing gestation diagnosis and information on the development and viability of the fetus. The recommended measurements to determine the fetal development would be the biparietal and thoracic diameter and the total length of the fetus. Studies with 3-months old lowland tapir fetus showed a biparietal diameter of 2.35 cm and body length of 15 cm. At 6 months, the biparietal diameter was 3.02 cm, the thoracic dorsum ventral length was 6.5 cm and the total length was 20 cm. At the end of the pregnancy the fetus had 75 cm of length, a biparietal diameter of 11 cm and a thoracic diameter of 40 cm.

9.3. Recommended Research Topics

Several groups are currently attempting to develop reproductive research with little baseline data. Given that captive reproduction will undoubtedly serve to enhance any conservation effort, the veterinary committee has listed research in the area of reproduction as a major priority. The main areas to be studied are:

1. Monitoring of reproductive hormones through non-invasive methods;
2. Electro-ejaculation and sperm handling and storage, with studies of spermatozoid viability;
3. Artificial insemination protocols;
4. Collection, preservation and viability analysis of oocytes;
5. Monitoring fetal viability by ultrasound studies when it is possible (could be in tapirs in captivity under operant conditioning);
6. Nutritional requirements for the pregnant female during different periods of pregnancy;
7. Analyses of the nutritional composition of milk (including colostrums), in the four tapir species. This knowledge will be very useful if should be necessary.

RECOMMENDED LITERATURE

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Janssen DL, Rideout BA, Edwards ME, 2003. Tapiridae. In Fowler, M.E. Zoo and Wild Animal Medicine 5th Edition. London: W.B. Saunders Company, Philadelphia.

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Quse,V.B.; Francisco E.; Gachen G.; Fernandez J.P., 2004. Hormonal and Ultrasonography Studies During the Pregnancy of Lowland Tapir. Second International Tapir Symposium. 10-16 January, 2004. Symposium Abstracts: 47. Panama City, Republic of Panama.

10. Necropsy

Field necropsies are valuable sources of information for wild tapirs' medicine, however they are rare opportunities and should never be wasted. It is more common to find carcasses in advanced decomposition rather than fresh corpses, and in these cases it is still preferable to make a limited necropsy evaluation rather than ignoring the carcass. It is hard to refrigerate or freeze adult tapir's carcasses, so with field necropsies it is important to perform the process very rapidly.

It is recommended to use the proper individual protection equipment during the necropsy, such as disposable latex gloves, mask, and protection glasses, clothes and boots.

The necropsy is essentially an observation and description exercise, and should involve little interpretation unless the performer of the necropsy is an experienced pathologist. The performer of the necropsy should make the most accurate and detailed description of the appearance and texture of the tissues, making descriptions even more detailed when there is a doubt whether what is being observed is normal or not. Photographs are important tools, as they allow the later re-evaluation of data and information exchange between pathologists.

The objective of a necropsy is to identify all the pathologic processes that developed in the animal, both those that led to the death of the animal and all the others that occurred concurrently. For that reason, all tissues and organs should be carefully observed and sampled for histopathology, even when they do not seem to be involved in the cause of death. The collection of gastric content, parasites, genetic samples etc. is useful to provide a basis of comparison with other animals with unknown cause of death and to provide basic data on the biology of the species.

The notes taken should avoid subjective or colloquial terms (a lot, much, few, huge etc.) and should preferably use objective descriptions (precise measurements). The use of necropsy protocols may be useful to provide a checklist so that the performer of the necropsy does not forget any evaluation or sample collection. In **APPENDIX 3** we provide a spreadsheet and a simple checklist for field necropsies. We also hope the use of this spreadsheet will standardize the information collected by different tapir research projects, allowing comparisons on the causes of death of tapirs in different locations.

The necropsy classically is divided in three phases:

1. External exam (skin, mucosa, natural orifices, apparent health)

2. Structural organization of viscera (compression, volvulus, dystopias, cavitary liquids)

3. Individual evaluation of the organs

All organs should be analyzed and have their external (size, form, location, surface, color, symmetry) and internal (structure, consistence, content, thickness, parasites, cutting surface, internal color, symmetry, nodules) characteristics meticulously described and carefully compared to the anatomical normality.

The necropsy provides the opportunity of collecting a series of samples for posterior laboratorial exams, as summarized in **TABLE 6**.

RECOMMENDED LITERATURE

Almosny, N. R. P. & Santos, L. C. 2001. Laboratory Support in Wild Animal Medicine. In: Fowler, M. E. & Cubas, Z. S. (eds). *Biology, Medicine and Surgery of South American Wild Animals*. Iowa: Iowa State University Press.

Matushima, E. R. 2006. Técnicas Necroscópicas. In: Cubas, Z. S.; Silva, J. C. R. & Catão-Dias J. L. *Tratado de Animais Selvagens: Medicina Veterinária*. São Paulo: Roca.

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TABLE 6. Collection, Handling and Storage of Samples from Necropsies.

Analysis	Objective	Sample	Collection and Handling	Storage
Histopathology	Complements the necropsy, identifying pathological processes and the cause of death.	All organs should be collected, altered or not.	Fragments should be no larger than 1 cm^3, always including a fraction of normal tissue. Use a clean flask with formol 10% (formalin 4%) in a volume 8-10 times greater than the samples.	Keep the flasks well closed and safe from light, at ambient temperature. These samples will be valid for years.
Microbiology	Identify bacterial or viral agents involved in infectious processes.	Collect only samples from tissue/liquids suspected of infection, soon after the death.	Puncture (1-3mL) for liquids or swab for tissues and abscesses. The assepsy of the procedure is essential.	Keep in sterile flask (or inside the syringe used for puncture) or in nutritive transport media (*e.g.* Stuart) under refrigeration. Send to laboratory within a few hours.
Toxicology	Identify if the animal was exposed to a toxin (environmental contamination, poisoning).	All the viscera (or at least brain, lungs, liver, kidneys and bone marrow), stomach content, hair, fat and cardiac blood should be sampled.	Large fragments (~100g) of the tissues and stomach content, heart blood puncture (~50mL) and hair (store in envelope).	Keep the flask under refrigeration or freezing. Send to laboratory within a few days.
Ectoparasites	Identify the ectoparasites.	Any parasites found on the skin, 5-20 individuals of each apparent species.	Transfer the parasites to a perfored flask (for longer periods, add leaves or wet cotton) or to ethanol 70%.	Keep the flask at ambient temperature. Send to laboratory within a few days (perfored flask) or weeks (ethanol).
Endoparasites	Identify the endoparasites.	Any parasites found on the viscera, 5-20 individuals of each apparent species.	Wash the parasites in water and transfer them to ethanol 70% (cylindrical worms) or AFA (flat worms).	Keep the flask at ambient temperature. Send to laboratory within a few weeks.
Stomach Content	Identify the feeding habits of wild animals.	Stomach content.	Transfer all the stomach content to a bucket, homogenize and collect several small samples to a total of 500mL or 1L.	Keep at ambient temperature or under refrigeration. Use filtration, decantation or greenhouse to dry the sample.
Testicles and Ovaries	Store gametes for assisted reproduction techniques or germplasm banks.	Testicles or ovaries, only from very fresh corpses (<6-18 h).	Collect the intact gonads, do not dissecate them from their serous membranes.	Refrigerate or freeze with maximum urgency, depending on the technique to be applied. Send to laboratory with maximum urgency.
Genetic Analysis	Check IUCN/SSC Tapir Specialist Group (TSG) Manual of Sampling Techniques for Genetic Analysis			
Taxidermization	Consult local museums and taxidermists or the appropriate literature to obtain specific recommendations on the preparation of taxidermized animals or parts. Consult also the local laws of transport and use of tapir parts and the current CITES regulations.			
Other Analysis	The other samples described on the chapter “Collection, handling and storage of biological samples” such as corporal measurements, hair, feces, urine and other, may also be collected following the same recommendations.			

11. Interventions in Individual and Population Health

The intervention in wild animal population health is a very controversial topic. As any therapeutic or prophylactic intervention, it should be considered in the light of the ecosystem balance, the conservation of the species and on-going evolutionary processes. In the last analysis, there is no single rule for whether a veterinary should or should not intervene in the health of a wild animal. However, whenever the choice is taken to intervene, the veterinarian must make sure that this action will imply no risk to the survival of the rest of the population or to the stability of the ecosystem (*e.g.* live vaccines, resistant bacteria selection etc.).

It is consensual to treat lesions that were caused by the capture or the handling of the animal, when the animal gets hurt while in the trap, hunting dogs, chronic lesions from radio-collars etc. The treatment of non-capture-related lesions, however, is much more controversial. One might argue that the treatment of these lesions implies in interference on the natural processes of mortality and evolution, while one of the pillars of the conservation philosophy is to make sure that the evolutionary process continues in its natural balance. On the other side, however, one might argue that most of these traumas are probably indirect consequences of the population stress due to human interference, and that treating these lesions would be exactly to minimize that interference. Another argument is that in reduced populations, where the death of a single individual might have important consequences on the population, the emergency situation justifies the veterinary assistance of the few individuals that are left.

Vaccination protocols, if necessary, should be applied carefully, using only inactivated vaccines, or vaccines that have been previously validated for tapirs. Some diseases where vaccines might be used include tetanus, Infectious Bovine Rhinotracheitis, and Equine Encephalomyelitis.

The removal of individuals of high genetic value may also be considered during high epidemic risk situations. These individuals may be transferred to captivity or low risk areas, following the recommendations from the **IUCN/SSC Tapir Specialist Group (TSG) Experimental Protocols for Tapir Re-Introduction and Translocation**.

APPENDIX 1 - General Information about Agents Commonly Used for the Chemical Restraint of Tapirs

Alpha-2-Agonists: Medetomidine, Romifidine, Detomidine, and Xylazine

Reversal Drugs: Atipamezole, Yohimbine, Tolazoline

These drugs produce depression of the Central Nervous System (CNS), being classified as sedatives and soft analgesics, with myorelaxation properties. The use of these drugs in tapirs should consider their capability of depressing the thermoregulation. In many species, these drugs produce emesis, however this does not seem common in tapirs. On blood pressure, there is an initial increase followed by a long depression. There are no studies on the blood pressure of tapirs with these drugs, but the experience has shown that the later drop might difficult blood collection from peripheral veins, which may be corrected with the use of atropine. Other circulatory effects include bradicardia and arrhythmias. Short apnea and exposure of the penis have also been reported as common with these drugs. The isolated use of Alpha-2-agonists has proven efficient during a series of chemical restraint procedures. In particular Romifidine has shown the best results, due to the low volume required, low costs and stable cardio respiratory parameters. In general, Alpha-2-agonists have been considered fundamental in the developing of simple and safe anesthetic protocols for tapirs. They have been successfully associated with dissociative drugs, producing deeper anesthesia both in field and captivity. They have also been associated with opioid derivates, producing safe chemical restraint and deep sedation for field capture and handling.

Opioid Derivates: Butorphanol Tartarate, Carfentanil, Etorphine

Reversal Drug: Naloxone

The opioid derivates have been classically used on the restraint and anesthesia of tapirs both in the wild and in captivity. They have been associated with Alpha-2-agonists and/or Ketamine, producing stable cardio respiratory parameters and good analgesia. The anesthetic recovery is smooth and fast, being accomplished naturally or with the use of Naloxone.

Dissociative Drugs: Ketamine, Tiletamine

No specific reversal drugs

The dissociative drugs, derivates of ciclohexamine, may produce amnesia and catalepsy, providing an uncomfortable anesthetic induction and recovery, with ataxia, falls and pedaling movements (especially with Tiletamine = Telazol, Zoletil). The associations of Tiletamine with Alpha-2-agonists in tapirs may produce periods of anesthetic respiratory depression. Sometimes the periods of apnea may require to be reversed by respiratory massage and respiratory stimulants. When Alpha-2 reversal agents are not used, the anesthetic recovery might be uncomfortable, with oscillations between consciousness and depression.

Atropine

In low doses, Atropine inhibits excessive salivation and respiratory secretions. In moderate doses, Atropine may be used to increase the hearth rate. Excessive doses, however, may reduce gastrointestinal and urinary motility. One of its most important uses in tapir anesthesia is to reduce hyper secretion and reverse the blood pressure drop due to Alpha-2-agonists or dissociatives, which hampers blood collection.

Emergency Drugs

It is highly recommended to predetermine the dosage of emergency drugs while planning for the chemical restraint of wild tapirs, so that these drugs are promptly available if needed. The use of Doxapram may be prophylactic in protocols using Alpha-2-agonists, opioids or Telazol/Zoletil, to prevent respiratory depression.

APPENDIX 2 - Selected Diseases

Bacterial Diseases

1. ***Salmonella* sp.** Salmonellosis has been reported in tapirs in captivity. *Salmonella tiphimurium* was associated with fatal septicemia in lowland tapir, and *S. poona* has been isolated from a neonatal Baird's tapir with acute gastrointestinal distress. The occurrence of Salmonellosis in zoos coincides with the rainy season. The diagnosis of *Salmonella* may be carried out as a routine bacterial culture on an enteric medium such as Selenite medium or hectone enteric agar (Ramsay & Zainuddin 1993).

2. ***Mycobacterium* sp.** Mycobacteria sporadically affects captive tapirs (Janssen *et al.* 1996). It is unknown whether these agents are endemic in free-ranging populations, the prevalence of this disease and whether it has a significant affect on free-ranging populations. With the design of new, less-invasive testing methods for Mycobacterium (DNA-based testing, ELISA, BTB tests etc.) the TSG Veterinary Committee encourages individuals working with free-ranging tapirs to investigate methods to test animals that are handled as part of a field project for *Mycobacterium* sp. As has been the case with other free-ranging mammals that come into contact with domestic livestock, there may be public pressure in the future for determining what role, if any, tapirs play in the epidemiology of tuberculosis of domestic animals.

3. ***Bacillus anthracis.*** Although there are no official reports of anthrax in tapirs, a non-official report of the disease in Andean tapirs from Colombia was made by Hernández-Camacho (Downer, pers. comm.). In general, perissodactiyls present sudden death after severe diarrhea, with foamy mucous discharge from mouth and nostrils and eventual rectal prolapse (Ramsay & Zainuddin 1993). This disease and its impact on the wild tapir populations must be investigated in endemic regions.

4. ***Leptospira* spp.** Serological antibody titers against *Leptospira* in absence of clinical signs have been reported in wild tapirs (Hernández-Divers *et al.* 2002; Mangini 2000). The relationship between the tapirs and these bacteria and its specific serovars, together with their role of tapirs as carriers of the disease must be studied. There are recommendations for other bacterial diseases such as clostridial infections and brucellosis that have not been demonstrated in tapirs yet, however, research must be carried out in order to define their status as potential pathogens for tapirs.

5. **Mandibular swellings.** The tapirs are particularly prone to develop mandibular abscess or "lumpy jaw" both in captivity and in the wild. Although the condition is considered to be similar to that seen in domestic cattle, its pathogenesis in tapirs is unknown. The microorganisms isolated from the lesions are *Corynebacterium pyogenes,* ß-hemolytic *Streptococcus, Actinomyces, Necrobacillus, Escherichia coli* and *Mycobacterium*. No viruses have been associated with this disease, but research should be conducted. The lesion may involve the bone leading to osteomyelitis and frequently ends in death because of systemic involvement. This condition must be reported in free-ranging animals and samples must be collected, in order to identify the pathogens involved.

Viral Diseases

1. **Herpesvirus.** There is one report of mortality in Malay tapirs as a result of herpesvirus (Janssen *et al.* 1996). However, the type of herpesvirus was not determined. Little is known about the epidemiology of this disease, even in captive populations**.** Recently, a new gamma2 herpesvirus has been partially sequenced in a captive lowland tapir but nothing is known about its potential pathogenicity. As a latent DNA virus, herpesvirus should be common and widespread in populations, but stress and/or immuno-supression (*e.g.* the effects of fragmented populations, inadequate captive conditions) may reactivate the virus, and lead to clinical (and sometimes lethal) symptoms (de Thoisy, pers. comm.). The Veterinary Committee encourages veterinarians to consult with virologists that specialize in herpesvirus for the appropriate sample collection, analysis and interpretation.

2. **Encephalomyelitis (including West Nile Virus; EEE - Eastern Equine Encephalitis; VEE - Venezuelan Equine Encephalitis; and WEE - Western Equine Encephalitis).** There are no scientific reports that confirm that tapirs are susceptible to encephalitis. However, several zoos vaccinate tapirs for these diseases and a recent health survey demonstrated serological titers to VEE in a small population of free-ranging Baird's tapirs in Corcovado National Park, Costa Rica, Central America (Hernández-Divers *et al.* 2002). Additionally, a long-term lowland tapir research project in Morro do Diabo State Park, São Paulo State, Brazil, found positive serum titles for both EEE and WEE. It is recommended that pre- and post-vaccination titers are performed to determine the efficacy of such vaccines. In addition, any evidence of viral encephalomyelitis should be reported. There are anecdotal reports of West Nile Virus clinically affecting rhinos. Therefore, some captive collections of tapirs are currently vaccinated with the equine West Nile Virus vaccine (Ft. Dodge). It is important to obtain pre and post-vaccination titers to West Nile to determine the efficacy of this vaccine. Any tapir that dies as a result of West Nile Virus should be reported.

3. **Foot and Mouth Disease.** An outbreak of FMD in the Paris Zoo, France, which affected lowland and Malay tapirs was described by Urbain *et al.* (1938). The clinical findings were limited to only interdigital lesions. However, at the Mountain Tapir Population and Habitat Viability Assessment (PHVA) Workshop carried out in Colombia in October 2004, Peruvian field biologist Jessica Amanzo reported two FMD outbreaks in the northern Peru that produced a high mortality of mountain tapirs. The first outbreak occurred 50 years ago and the second 25 years ago. Although this information has not been confirmed, tapir researchers must be alert to this disease, and serologic surveys specific to FMD must be carried out, especially in mountain tapirs.

Non-Infectious Diseases

"Vesicular Dermatitis". Research of the condition termed "vesicular dermatitis" currently occurring in captive tapirs. A syndrome termed "vesicular dermatitis" was first described by Finnegan *et al.* (1993). Although the syndrome continues to affect captive tapirs, its etiology has not been identified yet. Ideally, skin biopsies of affected areas should be collected and preserved in 10% buffered formalin. A histopathologic examination of the samples is necessary to diagnose this syndrome.

Iron Storage Disease (in captive tapirs). There is some evidence that the iron levels in captive tapirs are significantly higher than in their free-ranging counterparts (Dr. Don Paglia, pers. comm.). This may potentially be the same situation for black rhinos. In order to elucidate whether this is a pathologic condition in captive tapirs, histopathologic evaluation of tapir's liver is recommended.

Parasitic and Rickettsial Diseases

Ectoparasites. The identification of ectoparasites such as ticks and flies in free-living tapirs may permit the establishment of the interaction between tapirs and livestock. If this interaction occurs, then the risk of mutual transmission of diseases may exist and sudden outbreaks of unusual diseases in both tapirs and livestock that may lead to high mortalities may occur. It is also possible to identify those parasitic genera that naturally infest tapirs. Furthermore, their analysis may help in the establishment of the role of tapirs as potential reservoirs of some diseases.

Endoparasites. As with the ectoparasites, endoparasites may be studied in order to identify those that naturally infest tapirs, and differentiate between those that were acquired from livestock. In the same way there are some parasites potentially highly pathogenic, in which cycles the tapirs may play an important role. This is the case of *Toxoplasma* sp., whose high prevalence has been reported in free-ranging ungulates from French Guiana. Since prevalence was significantly linked to ground-dwelling behavior of animals (de Thoisy *et al.* 2003), tapirs may be infected.

Rickettsial Diseases and Hemoparasites. Free-ranging tapirs are parasitized with several species of ticks known to be vectors of a wide variety of rickettsial and hemoparasitic diseases. To date, there are no reports of rickettsial disease in tapirs; however, given that the importation of tapirs from Latin America may be needed to improve the genetic stock of the North American captive population, it would be imperative to study these issues to avoid any inadvertent disease introduction and to predict and prevent potential morbidity/mortality from such diseases during periods of stress due to shipping.

APPENDIX 3 - Spreadsheets

www.tapirs.org

IUCN/SSC Tapir Specialist Group (TSG)

CHEMICAL RESTRAINT AND CLINICAL EVALUATION

Species: _____ Identification: _____

Sex: ( ) Male ( ) Female Age: _____ | ESTIMATED BODY WEIGHT: | REAL BODY WEIGHT:

Location: _____ GPS: _____ Date: ___/___/_____

Team: _____

Nutritional Condition: ( ) Caquetic ( ) Lean ( ) Good ( ) Obese

Apparent Health: ( ) Good ( ) Regular ( ) Bad

Behavior: ( ) Depressed/apathy ( ) Calm ( ) Alert ( ) Excited ( ) Aggressive

Capture Method: ( ) Captivity ( ) Capture pen ( ) Pitfall ( ) Dart Shooting ( ) Hunting dogs

Weather: ( ) Dry ( ) Open ( ) Partially cloudy ( ) Cloudy ( ) Rainy

Temp. (°C): _____

Humid. (%): _____

Drug	Conc. (mg/ml)	Dose (ml)	DS	Via	Time
					:
					:
					:
					:
					:
					:
					:

DS = Delivery System = DP (dart pistol), S (syringe), BP (blowpipe) etc. **Via** = SC, IM, IV etc.

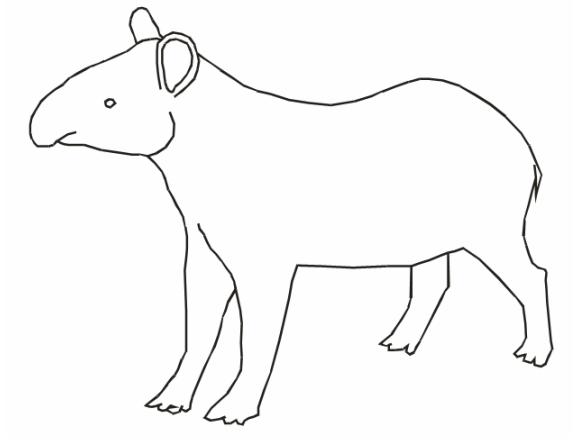
Time	RR	RR type	HR	%O_2	T°C	CFT	RELAX	Comments
:								
:								
:								
:								
:								
:								
:								
:								
:								
:								
:								
:								
:								
:								
:								
:								
:								
:								

RR = Respiratory rate (mpm), **HR** = Hearth rate (bpm), **%O_2** = blood oxygen saturation (%), **CFT** = Capillary refill time (s), **RELAX** = Muscular relaxing (none = 0, soft = 1, incomplete = 2, complete = 3), **RR type** = Respiratory rate type (costal = C, abdominal = AB, costal/abdominal = CAB, superficial = S, deep = D).

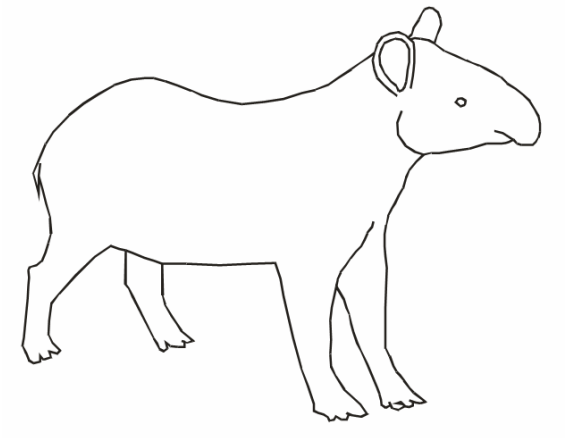
GENERAL CLINICAL EXAM

Skin/Hair: ( ) Good ( ) Regular ( ) Bad **Comments:** _____
Ectoparasites: ( ) Few ( ) Regular ( ) Excessive **Which?** _____
Consolidated fractures: ( ) Yes ( ) No **Where?** _____
( ) Mouth/Teeth/Nostrils: _____
_____
( ) Eyes/Ears: _____
( ) Vagina/Penis/Testicles: _____

EXTERNAL LESIONS (cuts, abscesses, nodules, scars etc.)



LEFT



RIGHT

Description of the lesions: _____
_____
_____
_____
_____

Identify and number the lesions on the drawings, and describe them in detail on the space provided above.

COLLECTION OF BIOLOGICAL SAMPLES

( ) Blood with anticoagulant, volume: _____ ml ( ) Blood without anticoagulant, volume: _____ ml
( ) Blood smears, no. of plates: _____ ( ) Vaginal cytology, no. of plates: _____
( ) Genetic samples, which? _____ ( ) Feces from the rectum
( ) Ectoparasites, no. of flasks: _____ ( ) Hair
Swabs: ( ) Mouth ( ) Nostrils ( ) Eyes ( ) Ears ( ) Vagina ( ) Prepuce ( ) Rectum ( ) Lesions ( ) Eyes
Other samples (urine, milk etc.): _____

Notes: _____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

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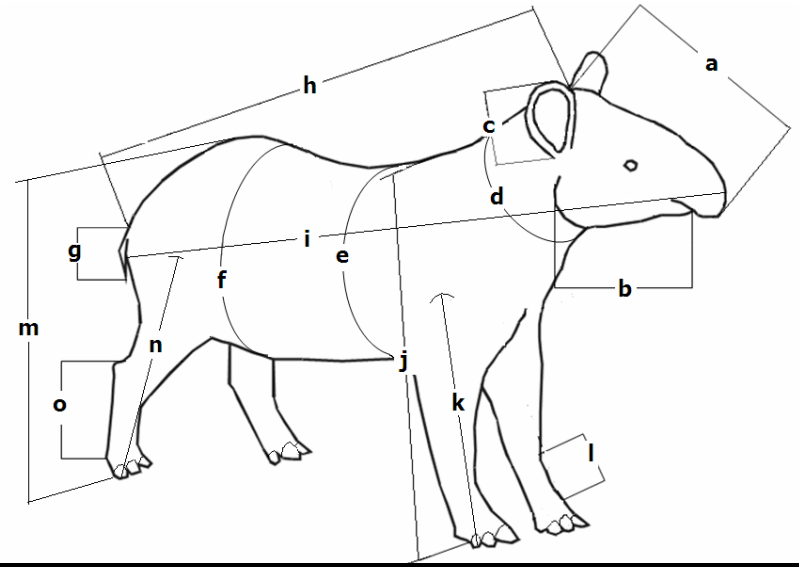
CORPORAL MEASUREMENTS

Species: _____ Identification: _____
Sex: ( ) Male ( ) Female Age: _____ Body weight: ____ ( ) ESTIMATED ( ) REAL

Location: _____ GPS: _____ Date: ___/___/_____

Photographs: ( ) FACE ( ) FULL BODY ( ) MOUTH/TEETH ( ) GENITALS ( ) PARTICULAR SIGNS



a. Head Length: _____	f. Abdomen Circ.: _____	k. Front Leg Length: _____
b. Jaw Length: _____	g. Tail Length: _____	l. Carpal Length: _____
c. Ear Length: _____	h. Trunk Length: _____	m. Rear Height: _____
d. Neck Circ.: _____	i. Full Length: _____	n. Rear Leg Length: _____
e. Thorax Circ.: _____	j. Front Height: _____	o. Tarsal Length: _____
Distance between eyes: _____	Left Testicle Length: _____	Right Testicle Length: _____
Vulva Length: _____	Left Testicle Circ.: _____	Right Testicle Circ.: _____

NOTE: All measurements should be taken with measure tape along the body, following its natural curves.

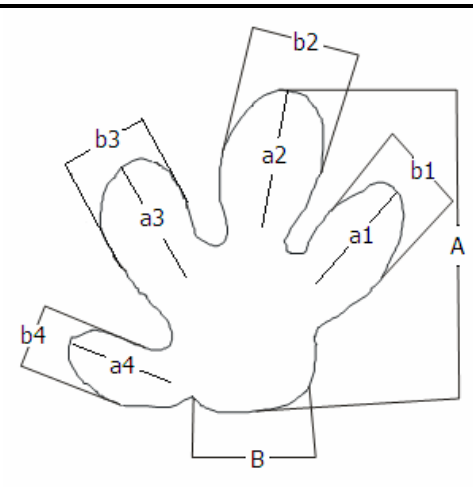
DENTITION (measurements and fractures)

Upper Left											Upper Right										
M3	M2	M1	P4	P3	P2	P1	C	I3	I2	I1	I1	I2	I3	C	P1	P2	P3	P4	M1	M2	M3

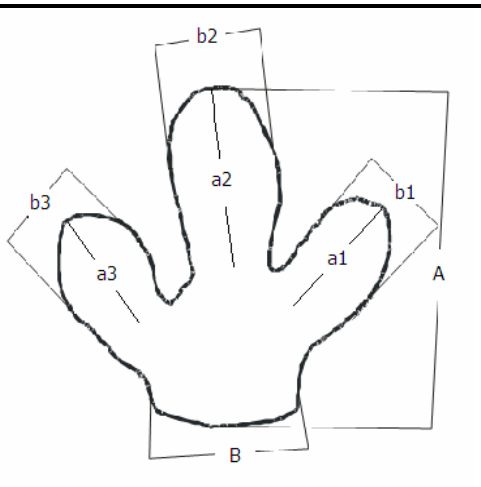
Lower Left											Lower Right										
M3	M2	M1	P3	P2	P1	C	I3	I2	I1		I1	I2	I3	C	P1	P2	P3	M1	M2	M3	

NOTE: Measure the length of incisors and canines, for other teeth mark a cross when a tooth is fractured or missing.

Notes: _____



ANTERIOR FEET

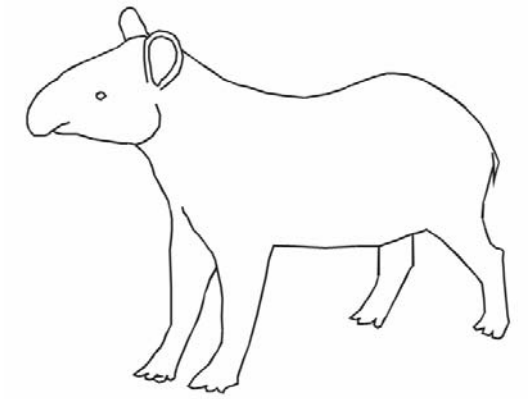


POSTERIOR FEET

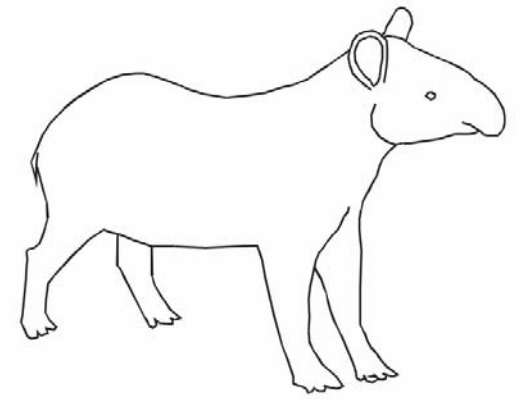
Left Anterior Foot	a1: _____	a2: _____	a3: _____	a4: _____	A: _____
	b1: _____	b2: _____	b3: _____	b4: _____	B: _____
Right Anterior Foot	a1: _____	a2: _____	a3: _____	a4: _____	A: _____
	b1: _____	b2: _____	b3: _____	b4: _____	B: _____
Left Posterior Foot	a1: _____	a2: _____	a3: _____		A: _____
	b1: _____	b2: _____	b3: _____		B: _____
Right Posterior Foot	a1: _____	a2: _____	a3: _____		A: _____
	b1: _____	b2: _____	b3: _____		B: _____

NOTE: The digits are counted from the interior to the exterior of the feet.

PARTICULAR SIGNS (pigmentation spots, scars etc.)



LEFT



RIGHT

Description of the signs:_____
_____
_____
_____
_____

NOTE: Identify and number the particular signs on the drawings, and describe them in detail on the space provided above.

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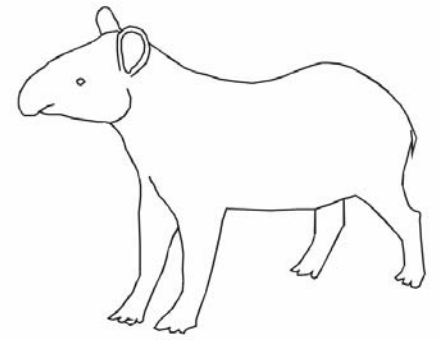
NECROPSY

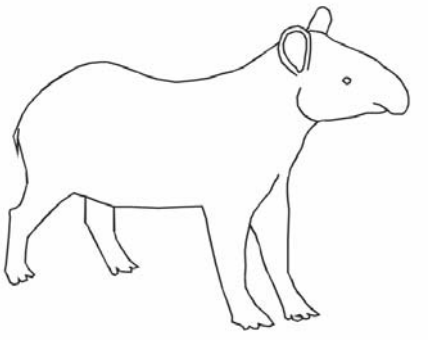
Performer of the necropsy: _____ Institution: _____
Address: _____
Location (region, city, province, country): _____

Species: _____ Identification: _____
Sex: ( ) Male ( ) Female Age: _____ Body weight: ____ ( ) ESTIMATED ( ) REAL
Date of death (estimated): ___/___/_____ Date of necropsy: ___/___/_____
Location: _____ GPS coordinates: _____

Known history of animal / Circunstances of death:



LEFT



RIGHT

External exam (skin, scars, ectoparasites, natural orifices, nutritional condition):

Body cavities (peritoneum, pleura, pericardium, visceral positioning, cavitary liquids, fat stores):

Respiratory system (nasal cavity, pharynx, larynx, trachea, bronchi, lungs, regional lymph nodes):

Cardiovascular and hemolymphatic systems (heart, great vessels, spleen, lymph nodes, thymus):

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):

Nervous system and sensory organs (brain, meninges, spinal cord, peripheral nerves, eyes, ears):

Endocrine system (thyroids, parathyroid, adrenals, pituitary):

Musculoskeletal system (bones, bone marrow, joints, tendons, muscles):

Preliminary Diagnosis:

Samples collected for histopathology:

( ) Skin	( ) Trachea	( ) Lungs	( ) Myocardium
( ) Spleen	( ) Thymus	( ) Esophagus	( ) Stomach
( ) Small intestine	( ) Large intestine	( ) Cecum	( ) Liver
( ) Pancreas	( ) Kidneys	( ) Ureters	( ) Bladder
( ) Testes/Ovaries	( ) Male sexual glands	( ) Uterus	( ) Vagina
( ) Brain	( ) Meninges	( ) Spinal cord	( ) Pituitary
( ) Thyroids	( ) Adrenals	( ) Bone marrow	( ) Muscle

Other samples:

( ) Endoparasites	( ) Ectoparasites	( ) Stomach content	( ) Testes/Ovaries
( ) Toxicology: viscera, stomach content, hair, fat, cardiac blood, bone marrow.			( ) Genetic samples

( ) Microbiology (describe samples): _____

Notes: _____

APPENDIX 4 - Useful Websites

Equipment & Supplies

(Capture, Immobilization, Data Collection etc.)

Pneu-Dart - www.pneudart.com

Telinject - www.telinject.com

Dan-Inject - www.dan-inject.com

Capchur - www.palmercap-chur.com

Telonics - www.telonics.com

Televilt - www.televilt.se

Telemetry Solutions - www.telemetrysolutions.com