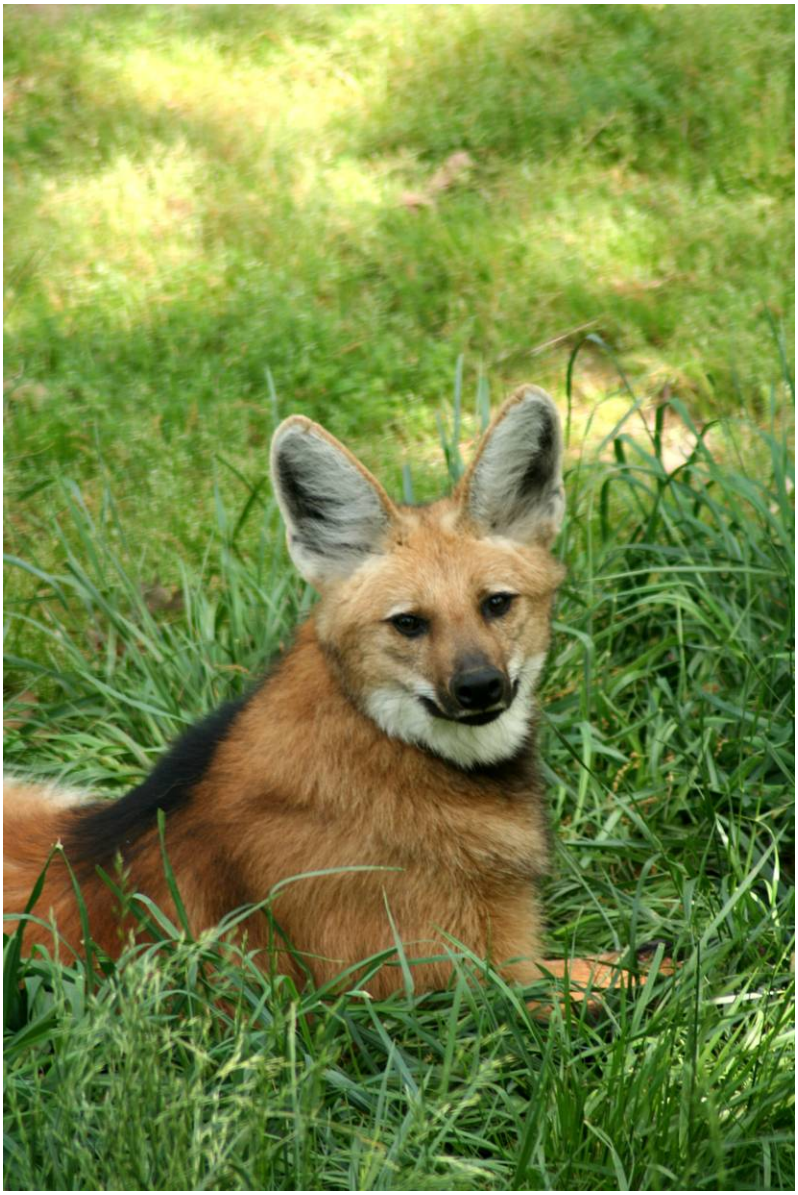
Maned Wolf Husbandry Manual
2007 Edition



Maned Wolf SSP©

INTRODUCTION and ACKNOWLEDGMENTS

When the Maned Wolf SSP was formed in 1985, one of the first goals was to produce husbandry guidelines for managing the species in captivity. The original MWSSP Husbandry Manual was prepared by Mike Blakely and Melissa Rodden in 1987 and revised in 1995 (Norah Fletchall, Steve Taylor & M. Rodden, eds.). Contributors to previous editions of the MWSSP Husbandry Manual include:

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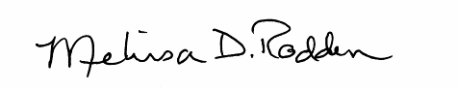
The 2007 edition presents new information about several aspects of maned wolf biology and management. It is a living document and will be updated as new information becomes available. My sincere gratitude to the many individuals who contributed their time and expertise to the 2007 edition of the Maned Wolf SSP Husbandry Manual.



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CHAPTER ONE
NATURAL HISTORY

TAXONOMY

Maned wolves (*Chrysocyon brachyurus*) are members of the canid family which encompasses 15 genera and 35 species (Sheldon, l992). Representing the largest canid species in South America and the fourth largest canid worldwide, the maned wolf was first described by D'Azara in 1801. Maned wolves are commonly known by several names in their native habitat, including: lobo guará, boroche, aguará guazú and lobo de crin.

The karyotype of maned wolves is similar to *Canis* but the diploid number is 76 for *Chrysocyon* and 78 for members of the genus *Canis* (Dietz, l984). Maned wolves are believed to have evolved in the Central Highlands of Brazil during the Pleistocene era from an ancestral canid (Wayne & O'Brien, l987), and taxonomists postulate that the maned wolf is more closely related to the bush dog (*Speothos*) than to the South American foxes (Wayne *et al.*, 1997).

PHYSICAL CHARACTERISTICS

Maned wolves are atypical canids in appearance. Their legs are long and stilt-like allowing them to see above and move through the tall grasses of their native habitat. Rusty red fur covers most of the body; the muzzle and the lower legs are dark brown or black. A “mane” of relatively long black fur runs from the nape down the midline of the back. The fur on the shoulders and neck is sometimes raised during agonistic encounters, hence the "maned" wolf. The throat, inside of the ears and the tail tip are white. There is little variation in these patterns between individuals. The walking gait of the maned wolf is, to our knowledge, unique among canids. It is best described as “pacing”, in which the front and hind legs on one side move in unison, giving a rolling appearance to the walk, similar to pacing horses seen at the harness racing track. It is possible that the pacing movement is a more energy efficient way to move through long grasses in the native habitat.

There is no significant difference in size of appearance between adult males and females.

Height at the shoulders=73-90cm
Head and body length=95.1-145.5 cm
Tail length=32-50.9 (Paula *et al.*, in prep)

Weight: Subadult (N=5 male + 3 female): 22.5 ± 1.2 kg
Adult male (N=7): 28.0 ± 0.8 kg
Adult female (N=13): 26.7 ± 0.9 kg (May *et al.*, in prep)

RANGE AND HABITAT

Maned wolves range from northeastern Brazil (except coastal areas) to northern Argentina, Paraguay, eastern Bolivia and west to the Pampas del Heath in Peru; a total of ~5 million km^2 (Dietz, 1985). Preferred habitat includes grasslands, cerrado, and scrub forest (Redford & Eisenberg, l992).

STATUS IN THE WILD

Accurate estimates of the wild maned wolf population have been difficult to obtain due to the secretive nature of the species. A Population Habitat and Viability Assessment workshop was held in Brazil in October 2005.

Field biologists working with population models concluded that there may be up to 25,000 maned wolves left in the wild; the vast majority live in Brazil (~21,000). The grasslands (cerrado) of Brazil are undergoing rapid conversion to agriculture, primarily soybean monoculture. Recent studies have discovered that maned wolves utilize disturbed habitat, although they are found primarily in undisturbed areas along the edges of agricultural zones (Paula, pers. comm.).

Maned wolves are listed on Appendix II of CITES, and as Near Threatened on the IUCN Red List. The species is classified as Endangered by US Fish & Wildlife Service. Maned wolves are protected in most range countries, although enforcement of the laws is often problematic. Habitat loss and fragmentation, road kills, and disease transmission from domestic pets are the primary threats facing the wild population.

DIET

Maned wolves are omnivorous, opportunistically feeding on a variety of different items. Seasonal changes in food availability result in changes in dietary components. Animal material consumed includes small mammals (rodents, armadillos), reptiles, gastropods and bird eggs (Silveira, 1968), as well as amphibians and insects (Bestelmeyer 2000). Bestelmeyer and Westbrook (1998) documented that maned wolves also successfully hunt pampas deer. Wolves are known to kill domestic poultry for which they may be shot by farmers.

In Brazil, the plant portion of the diet is primarily *Solanum lycocarpum*, commonly called lobeira ("Fruit of the Wolf"). This everbearing fruit is a consistent part of the wolves' diet throughout the year. Lobeira, a member of the Solanaceae family, resembles a large tomato, which turns yellow when ripe. Wolves are believed to consume the fruit before it is ripened (Dietz, l984). Matera (1968) reported that the fruit plays a role in the treatment of giant kidney worm, a common parasite in maned wolves, although there is no scientific evidence to support this theory.

Dietz's scat analysis of free ranging wolves in Serra de Canastra National Park in Brazil showed "58% … was lobeira, 28% was small mammals and 2.3% was birds." (Dietz, l984). Using direct observations rather than scat analyses, Bestlemeyer (2000) observed maned wolves (where, in Brazil?) consuming 7 categories of food of animal origin (small mammals, birds, insects, armadillos, amphibians/reptiles, deer, and unidentified animal) and 6 categories of food of vegetable origin (*Solanum lycocarpum* fruit, *Anadenanthera colubrina* fruit, three unidentified fruit types, and grass). Bestlemeyer observed fewer small mammals and birds being consumed and had a much larger proportion of unidentified prey than Dietz (1984). Other studies confirm the maned wolf's omnivorous habits, reporting consumption of a wide variety of plant and animal material, with about 50% of the diet composed of plant material and 50% animal (Lilienfeld, 2000; Jacomo, 1999; Rodrigues, 2002; Motta-Junior *et al.*, 1996; Carvalho & Vasconcellos, 1995).

SOCIAL ORGANIZATION

Dietz (1984) studied maned wolves in Serra da Canastra National Park, Brazil over a two year period during which he saw five maned wolves without the aid of telemetry. He determined that pairs of maned wolves occupied home ranges averaging 30 km². Rodrigues (2002) observed home ranges averaging around 57 km² in Águas Emendadas Ecological Station, Brazil, while Silveira (1999) reported a 49 km² home range size in Emas Park. Maned wolves are primarily nocturnal or crepuscular in activity patterns (Sheldon, l992). Mated pairs do share a home range but are rarely seen together except during breeding season. Territories are marked by site-specific defecation spots (termite mounds, ant mounds, shrubs, trees, grass) and landmarks that present physical barriers (roads, rivers). Dietz (1984) reported that the territories of same-sex maned wolves in Brazil's Serra da Canastra National Park did not overlap. However current studies in the same park commonly find overlapping territories, indicating that there may be considerable plasticity in behavior depending on resource availability (Paula, pers. comm.). The same home range is thought to be occupied throughout life. When an animal dies or abandons a territory a nomadic individual usually takes up residence. Within territories animals are known to mark with scats and urine. Marking often takes place on elevated surfaces, such as termite mounds. (Dietz, l984). Maned wolves are also thought to utilize a roar-bark to announce their location within their territory. Barking may serve to "promote the spacing of individuals through avoidance" (Kleiman, l972).

Maned wolves are facultatively monogamous throughout life with pairs coming together during breeding season (April-June) for mating. Observations of captive wolves indicate males play a role in raising pups by regurgitating food (Bestelmeyer, 1999; Rasmussen & Tilson, l984) and grooming pups (Sheldon, l992).

Few actual observations of pups have been noted in the wild. Most information is anecdotal but suggests that females and possibly males defend nest or den sites for a period of time after parturition (Dietz, l984). Bestelmeyer (2000) provides the first quantitative observations of free-ranging maned wolves; her study was conducted in Emas National Park, Brazil. She was able to observe one habituated female and its pup on several occasions. The pup accompanied its mother on hunting forays at 8 weeks of age and at least through 14 weeks of age but was not observed in her company thereafter. Bestelmeyer (2000) therefore inferred that dispersal occurs sometime after this age. An adult male was seen within 1.2 km of the den site and was presumed to be the sire of the pup; however, he did not approach the den site during observations.

REPRODUCTION

Maned wolves in the wild are believed to be monestrous with estrus lasting approximately 5 days (Dietz, l984). Following a 63-67 day gestation 2-5 pups are born. Births may occur as early as February, but the majority of pups are born during the dry season from June into September (Dietz, 1984). North of the equator, the reproductive season shifts by 6 months. Although it is the fourth largest canid in body size, the maned wolf has the second smallest canid litter size (Bestelmeyer, 2000).

Females will cache food at nest sites before whelping. Most denning sites utilize some type of existing topography, such as rock crevices, abandoned termite mounds, or dry mounds in marshy tall grass areas. Dimensions of one den were 60cm wide by 100cm deep (Dietz, l984). Bestlemeyer (2000) located one active den site in grassland habitat; the den was a 16 cm depression under a 133 cm tall shrub. Near the den, she found numerous trails, bed sites, and tunnels in the tall grass. Silveira and Jacomo (unpublished data) reported that a female gave birth to 3 pups in tall marsh grass in Emas National Park. The pups were still utilizing the den at 45 days of age, at which time the 2 male pups weighed ~2.25kg and the female weighed 2.0 kg.

DISEASES

Maned wolves in the wild suffer from two primary disease processes: parasites and cystinuria (Carvalho & Vasconcellos, 1995). The most common parasite found in maned wolves is the giant kidney worm (*Dioctophyme renale*). This worm is transferred via intermediate hosts (fish and mollusks) that the wolves consume. The parasite always infects the right kidney, severely damaging or destroying the organ (Matera *et al.*, l968).

Other parasites noted in wild maned wolves include nematodes (*Trichuris, Ancylostoma, Toxocara*) and cestodes. Ectoparasites include ticks and screw worm larvae. A noticeable lack of fleas on trapped animals may be due to the lack of underfur on adult wolves (Dietz, l984). Beccaceci (1992) found evidence of tuberculosis in a wild maned wolf in Argentina.

Cystinuria has also been found in a significant number of captured maned wolves (Dietz, l984, Deem, pers comm.). Maned wolves are known to excrete excessive amounts of the amino acid cystine in urine. The impact of this condition on wild populations is not yet known, although cystinuria was found in 6 of 8 wild individuals sampled (Bovée *et al.*, 1981). Studies continue in the wild and in captivity.

There is growing concern worldwide about the potential threat of disease exposure and transmission between domestic animals and their wild counterparts. In the rural areas of maned wolf range countries, domestic dogs and cats are rarely vaccinated. Recent studies by Deem and Emmons (2005) in Bolivia and Songsasen (pers. com.) in Brazil indicate that wild populations of maned wolves have been exposed to many of the diseases commonly found in domestic dogs, including parvovirus, toxoplasmosis, and leptospirosis. The Bolivian animals

had also been exposed to canine distemper, adenovirus and rabies. Studies are ongoing to further elucidate the relationship between maned wolves, domestic dogs/cats, and levels of exposure to canine diseases.

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CHAPTER TWO
CAPTIVE MANAGEMENT

Effective management of the maned wolf requires careful planning, constant review and revision of some practices and an open line of communication at all levels of care. This chapter attempts to give guidelines and direction in several areas. Managers are encouraged to use this as a base of information and to report to the SSP coordinator new experiences that may alter these guidelines. Managers are encouraged to seek advice from the Management Committee or email questions and concerns to the MWSSP Listserv for response: mwssp@lists.aza.org.

HOUSING AND ENCLOSURE REQUIREMENTS

Housing and enclosure design are critical for effective management of the maned wolf. Exhibits should be large enough and contain adequate visual barriers to provide the occupants with a certain degree of privacy and the ability to avoid both the public and each other when desired. Managers are encouraged to contact the MWSSP Coordinator and other facilities housing maned wolves when designing an exhibit and off-exhibit holding area.

Maned wolves are typically housed in male-female pairs, or pairs with young up to 1 year of age. Same-sex sibling pairs or trios can also be housed together, although trios can become problematic as adults. Attempts to introduce unrelated adults of the same sex have not been successful. Since maned wolves give birth during the winter in North America, housing requirements vary according to climatic conditions at each institution. The average minimum daily temperature for the prime birth months for maned wolves living in the Serra da Canastra National Park, Brazil, was approximately 45° F (~ 7° C) (Dietz, 1984). Institutions located in climates where daily minimum temperatures regularly fall below that level must provide adequate supplemental heat.

The following general guidelines should be heeded by institutions planning to house maned wolves. **Zoos housing breeding pairs should also read Chapter 5 carefully, as breeding animals may require additional features**:

1. In addition to the main enclosure, a backup (holding) facility must be available for separating individual members of a pair or group. Ideally, a holding facility would consist of an outdoor yard, which may be adjacent to the main enclosure, and an indoor den area.
2. Each animal in the exhibit should be provided with an indoor den area. This may consist of an individual small structure for each animal, or one large structure containing an enclosed "resting box" for each adult animal. The latter may be preferred by zoos in harsher climates where animals may need to be locked in during extremely cold weather. See "Indoor Enclosure Requirements" for recommended sizes.
3. Proximity to other animals: In their native habitat, pairs of maned wolves occupy large home ranges averaging from 15 - 50 km^2 (~6 – 20 mi^2) or greater (Rodrigues, 2002; Silveira, 1999; Dietz, 1984). Institutions intending to house more than one adult pair of animals are strongly encouraged to separate pairs by as much physical distance as possible. If adult pairs must be housed in adjacent exhibits, a solid barrier is required. Every attempt should be made to isolate pairs recommended for breeding from other adult maned wolves. Young (<2 years old) may be housed adjacent to parents, although the potential effects on the parents' subsequent breeding/pup rearing are not clearly known at this time. An individual animal may be housed adjacent to a member of the opposite sex, although visual barriers are recommended. Solid barriers are highly recommended when individuals of the same sex are housed in adjacent exhibits. Maned wolves can be housed in proximity to other canids and other taxonomic groups, although visual barriers are recommended to reduce aggression. Because there can be a good deal of

individual variation in response to neighboring species, caution should always be exercised when introducing new "neighbors".

Outdoor Enclosure Requirements

Size: Varies considerably between institutions. A l986 survey by the International Studbook Keeper of 51 facilities worldwide showed an average enclosure size of 800 m^2 (8,600 ft^2). Enclosures ranged in size from 43 m^2 (463 ft^2) to 4,000 m^2 (43,000 ft^2). A minimum size of around 930 m^2 (10,000 ft^2 or .23 acre) is recommended by the SSP for a pair or trio of maned wolves. As maned wolves are somewhat secretive and apt to hide, an exhibit that is too large may be a hindrance for public viewing. Enclosures that are overly large may also hinder observations by keeper staff. Enclosure shape, topography, substrate, plantings, and proximity to the public should all be considered when designing an exhibit. No more than 50% of the circumference of the exhibit should have public viewing access.

Structure: Enclosures may be bounded by an open dry moat or fencing (chain link, expanded metal). Diameter of mesh should be small enough to prevent wolves from becoming entangled in the fence. Fence diameter should also prevent feral domestic or indigenous exotic mammal species (raccoons, skunks, dogs, cats, etc.) from entering the exhibit. Minimum dry moat size should be 7 feet deep by 12 feet wide. Width can be reduced slightly if public viewing is at a higher elevation than the exhibit. A wet moat requires an additional barrier in most cases as maned wolves are capable of swimming. Any type of barrier must be of adequate distance from the public to prevent members of the public from placing their hands within reach of the wolves.

The fence or barrier around the perimeter of the enclosure should be at least 7’ high with an inward overhang. Maned wolves cannot easily climb chain link, however they are adept at climbing rockwork barriers. Any perimeter barrier must prevent the animals from achieving a foothold that would allow them to climb. Avoid placing natural or man-made structures near perimeter barriers to prevent animals from jumping out of the enclosure. The entire enclosure perimeter must have some type of footer to prevent the wolves from digging out. Regardless of the material used, the footer should be at least 18 inches deep or if laid flat on the ground at least 2-3 ft. wide. A good method is fencing buried at a 45° angle inward.

Water: A source of clean drinking water should be provided at all times within the enclosure. This could be in the form of a lix-it, water bowl or pool. Some wolves will defecate or urinate in standing water, water bowls or pools.

Substrate: A wide variety of substrates can be utilized for outdoor enclosures. Some type of natural substrate should make up 50% of the enclosure whenever possible. Natural substrate could be soil, grass, sandy topsoil, etc. Other potential artificial substrates include gunite or concrete. Natural substrate areas should be examined regularly for evidence of digging.

Topography: Exhibits may be terraced, sloped, contain high and low spots, etc. An exhibit with no change in elevation should be avoided unless plantings or other structures are utilized to allow wolves areas to hide or find shelter.

Plantings: Natural vegetation should be present in the exhibit to provide adequate shade and isolation. Institutions must determine which plant species are appropriate for the climate. Since wolves may chew or consume plants, vegetation should be reviewed by veterinary staff for potential toxicity. Maned wolves also scent mark on some plantings, so hardy plant species are recommended.

Temperature: Maned wolves can be exhibited in a variety of temperatures and climatic conditions. Although no absolute minimum and maximum can serve all animals, care should be taken to avoid both extremes. Minimum temperatures must take into account wind, snow and/or ice in the enclosure, age and coat condition of animal. Maximum temperatures must take into account degree of shade (shaded areas should be provided),

humidity, age and condition of individual. Animals that are transferred to different areas of the country should be slowly acclimatized to changes in climate.

Indoor Enclosure Requirements

Type: Ideally each wolf should have its own area off-exhibit constructed in such a way that each individual can be held in the area without access to the exhibit. This allows keepers to work in the exhibit safely. Minimum recommended dimensions for these areas are 16-25 ft^2 per animal. If an area houses more than one adult, each animal must have its own resting box to provide a hiding spot for more secretive individuals. Resting boxes can be made of wood but care should be taken to monitor destructive chewing of the boxes. Waterproofing wooden resting boxes is recommended. However, caution should be exercised to ensure that the treatment has a low toxicity level. Lexan viewing windows installed in resting boxes can prove beneficial when monitoring activity. As animals feel more secure in smaller spaces, a resting box measuring ~4' x 4' x 4' high is adequate. Whelping areas are discussed in a section later in this chapter and in Chapter 5-Reproduction.

Structure: Indoor enclosure areas can be made of a variety of materials. Concrete is the most predominant, but other materials can be used. Any material should be able to withstand chewing and exposure to urine, feces, disinfectants etc. Access should be provided for keepers to clean and service the area.

Substrate: Concrete, wood or natural substrates can be utilized. If concrete is used, some sort of bedding should be provided (shavings, straw, indoor-outdoor carpet), especially during colder weather. However, "wood wool" type products are not recommended for bedding material. Animals housed in concrete areas should be provided with an elevated, bedded resting bench or the floor must be covered with bedding such as hay or straw. The substrate should be able to withstand disinfectants, urine, feces, etc. Foot sores may result if animals are housed on concrete for long periods of time.

Lighting: Natural or artificial lighting should be provided for each area. Natural lighting has benefits of varying light cycles, however, on cloudy days this lighting may not be sufficient for servicing the areas. In holding areas where wolves are not exposed to natural light, timers on lights are recommended to simulate natural light cycles.

Heating: For adults without young, auxiliary heating needs to be provided if temperatures in the area will fall below 45° F (~7° C). Heat may be in the form of forced air, or electrical heaters such as space heaters, radiant heaters, heat pads or panels and heat lamps.

Ventilation: Adequate ventilation should be provided to prevent areas from being continually wet and must also provide fresh air. Care must be taken to avoid drafts, particularly in whelping areas.

Doors: Animal doors in the holding area can be composed of a variety of materials. Guillotine or sliding doors can be utilized. Domestic dog doors and vinyl freezer strips can be used in areas where heat retention in the dens is important. It should always be possible to operate access doors between the exhibit and indoor facility remotely.

Special features: It is strongly recommended that institutions housing potential breeding pairs install remote video monitoring equipment or a blind to allow keepers to observe activities without disturbing the wolves. Video cameras must be mounted out of reach of the wolves. Low light level cameras with a wide angle lens are recommended.

Mixed Species Exhibits (See Addendum I at the end of this chapter for 2 accounts)

Although large carnivores are not traditionally considered suitable candidates for mixed species exhibits, the Osnabruck Zoo in Germany successfully exhibited maned wolves with giant anteaters over 20 years ago (Druwa, 1986). More recently several zoos in the Maned Wolf SSP have successfully housed maned wolves with giant anteaters, tapir, and capybara. Zoo managers are encouraged to explore innovative ideas for exhibiting maned wolves. Mixed species groupings provide the animals with an enriching environment and offer visitors an exciting visual and educational experience.

RECORDKEEPING

The foundation of any successful management program is the establishment of information gathering and recording policies and procedures. Institutions have a variety of methods for gathering information on specimens within the collection, daily reports, unit logs, etc., but the value of this information is lost if it is not centralized in some form. The availability of ISIS/ARKS/MedARKS programs (and ZIMS in the near future) provides what should be considered the minimum format for creating a permanent centralized record for individual specimens.

Specific information which should be collected includes:

- ***Medical*** - Health status, treatments (including medication, dosage amount and duration, results, etc.), tranquilizations (type, amount, effect, etc.), diagnostic tests, etc.
- ***Reproductive Data*** - Dates and signs of breeding behavior, copulation dates and frequency, birth dates, survivorship, parent or hand-reared, etc.
- ***Diet*** - Ingredients, amounts fed, times of day fed, favorite food items, "treat" foods.
- ***Behavioral*** - Interactions with conspecifics (especially during an introduction), abnormal or unusual behavior(s), seasonal variations, what seems to constitute normal behavior for the individual.
- ***Enrichment*** - Successes and failures of items offered for behavioral enrichment, types of items, food, toys, "furniture," plant species, etc.
- ***Training*** - Successful techniques for routine husbandry and also for non-routine procedures.

The captive management of maned wolves will continue to rely on the routine movement of individuals to ensure the genetic fitness of the population. In order to make the transition from one facility to another as stress free as possible for the animal we cannot underestimate the value of the records which accompany that individual. Institutions receiving an animal must ensure that information sent with the animal reaches the appropriate staff. Information and records which should accompany an animal include:

- ISIS/ARKS specimen report
- MedARKS specimen report
- AAZK Animal Data Transfer Form
- Specimen records; copies of unit or master and veterinary

IDENTIFICATION

Permanent identification of individual specimens is an absolute necessity. A microchip transponder inserted subcutaneously between the shoulder blades is the most commonly used site. As this is evolving technology, no specific brands are recommended, however a system that can read a wide variety of transponders is a logical choice. Alternatively, the animal's studbook number can be tattooed on the inner thigh. Regardless of the method used, the permanent identification should be included in institutional records and reported to the International studbook keeper and to the SSP Coordinator, and should accompany the animal when it is shipped to a new institution.

Physically unique markings should be entered in the permanent records of each animal. Deformed or partially missing ears or tail, scars, limp, etc. must be entered as well as any uniqueness in coat pattern. Photographs may assist in individual recognition. Behavioral characteristics are also an excellent aid to individual identification as well as pertinent to the management of the individual and should be included in the permanent records.

CAPTURE AND RESTRAINT See Addendum II at end of chapter for examples.

The use of a catch box should be considered a standard management tool. Having an easily transported box, with access ports, which can be placed at routinely used animal transfer doors will facilitate many handling needs that arise with this species. Conditioning, coercing, or crowding an animal into a crate can reduce the stress associated with routine vaccinations, administration of tranquilizer, transport, or preparation for some other procedure. Catch nets, crowding boards (to pin an animal in a corner or against a fence or wall), catch poles and tranquilization (for any procedure) are other methods used with this species.

See Chapter 7 for information regarding anesthesia procedures.

TRANSPORT PROCEDURES

Crate training prior to shipment should be considered as a standard management procedure. The routine use of a catch box will aid in conditioning an animal to temporary confinement.

The primary concerns regarding a shipping crate are that it is of adequate size (animal must be able to stand, sit and lie down naturally and turn about freely) and strength, equipped with rims (to keep other cargo at least 3/4" away from crate) and handles for lifting. The crate must be leak proof, the inside free of projections or material which could cause injury. Crates must meet USDA and/or IATA (International Air Transport Association) guidelines for live animal transport. Manufactured plastic "sky-kennels" have occasionally been used for domestic shipments, as long as the two halves are securely bolted together. It is highly recommended to cut threads (via the use of a die) on the ends of the hinge rod and the locking rod and use self locking nuts to prevent the door from being forced open. Many air cargo facilities require crates meeting IATA specifications for domestic shipments and will not accept plastic kennels for shipment of this species. Managers should contact local airlines prior to designing crates.

Animals having access to food and water prior to shipment should not require feeding for 24 hours or watering for 12 hours during shipping. The delay of a shipment is always a possibility so food and water containers should be provided along with instructions.

IATA regulations must be followed for international shipments. Some specifics of note are: plastic kennels are not acceptable; food and water containers are required; a dropping tray with absorbent material must be provided.

NOTE: IATA Live Animals Regulations is an annual publication which is routinely updated and, therefore, subject to changes which can affect animal shipments. This publication should be considered as a mandatory tool for proper shipping of live animals and is ordered from www.iata.org or by contacting:

Publications Assistant
IATA
2000 Peel Street
Montreal, Quebec
CANADA H3A 2R4
(514) 985-6326

Pre-shipment physicals are discussed in Chapter 7 but one must be aware that requirements vary among institutions, states and countries, and those requirements must be met. A signed health certificate must accompany the shipment. In addition, some states require an import permit or state license be obtained prior to shipment.

Maned wolves are listed as Endangered under the Endangered Species Act and, therefore, require a USFWS export or import permit for any international shipment. Many countries also require import permits for animals entering the country, which must be obtained by the recipient prior to shipment. Maned wolves are listed as Appendix II under CITES which necessitates an export permit from the CITES representative of the country exporting the animal.

ENVIRONMENTAL/BEHAVIORAL ENRICHMENT

This area of management responsibility cannot be over emphasized and must become as routine as feeding and cleaning. The rewards of enrichment will far outweigh whatever effort is expended. Physical and psychological stimulus of an otherwise static environment will have obvious benefits to the overall well being of the individual(s). Results of a recent pilot study indicate that providing wolves with environmental enrichment significantly increased exploratory behaviors, especially when mice were hidden in the enclosure. Additionally, males exhibited increased fecal corticoid concentrations during periods of enrichment. Overall, the results suggest that environmental enrichment elicits positive effects on the behavior of captive maned wolves (Cummings *et al.*, 2007).

In 1999 Bridgette Daley, lead keeper, and Susan Lindsey, Executive Director of the Wild Canid Survival and Research Center, compiled the enrichment techniques being used for maned wolves at 27 North American institutions participating in the MWSSP (Daley & Lindsey, 2000). The survey indicated that although a wide variety of enrichment items exist for maned wolves, they are significantly underutilized by care-givers. While the rate of success with different enrichment methods varied, most facilities indicated that their wolves showed some level of interest in every enrichment item provided.

Exhibit enrichment considerations

- Varied topography
- Plantings: trees, shrubs/bushes, tall growing grasses (let a few areas grow to maturity rather than mow the whole exhibit if long grass is a specific zoo concern)
- Furniture: large rocks, stumps, brush pile, logs and branches, pile of leaves. Use items which can easily be moved in and out, or around the exhibit to provide variety
- Water features, pool, stream

Food items:

- Large bones
- Rabbit (with fur left on), whole mice or rats, chicken or quail (feathered), live crickets, and fish
- Seasonal fruits and vegetables
- Live prey?

Olfactory stimulants, (only small amounts needed):

- Common herbs and spices
- Perfume (veterinary approval should be obtained)
- Commercial "animal scents/lures" such as supplied for trappers
- Feces or urine from other species (veterinary approval should be obtained)

Toys: Encourage staff members to use their imagination

- Large bones

- Large Rawhide chew toys
- Ice blocks containing food items
- "Boomer" or other safe balls
- Coconuts
- PVC pipe with small rocks or seeds inside
- Sticks and small branches
- Feathers

CONDITIONING

Routine husbandry can be accomplished successfully through a range of management approaches. Training for both routine and non-routine management is an area that needs further exploration, as these techniques are not commonly used in maned wolves or other large canids. **Keeper staff should be carefully selected for their knowledge and temperament and must be aware of the overall purpose of managing these animals.** As with any large carnivore, maintaining a calm, assertive attitude will assist keepers in maintaining control over the animals, however institutional standard safety protocols should always be followed. A thorough knowledge of maned wolf natural history and behavior is essential to implementing a successful training regimen.

Conditioning animals, for example by placing a squeeze cage or crate in the entrance to a den or other routinely used transfer door, is an effective technique for moving animals to another enclosure, for restraining animals for routine medical procedures such as vaccinations, and also for administering anesthesia for non-routine procedures.

Blood draw at White Oak

Positive reinforcement, e.g. food reward, has proven an effective training technique for maned wolves, however hand feeding in free contact is not recommended for the safety of the keeper staff. Maned wolves have been trained through positive reinforcement to shift, separate, and stand on a scale for regular weighing. They can be trained to come to the keepers for hand feeding (through a barrier), and to target to a buoy (stationing), which can then be utilized for training non-routine husbandry, for example, positioning various body parts for inspection and treatment of wounds. They have also been trained to open their mouths to allow inspection of teeth, tongue etc. It is also possible to condition individuals for blood collection and hand injections.

INTEGRATED PEST MANAGEMENT

An obvious need for pest control exists in any animal holding facility. Feral animals such as rodents, birds, domestic cats or dogs, or other pests that have access to the maned wolf or its enclosure may serve as a source of contamination for microorganisms or parasites.

Well-maintained perimeter fencing provides an initial deterrent to larger feral animals, particularly dogs. However, climbing animals, such as feral cats, can easily defeat such barriers; therefore, areas around maned wolf enclosures should be monitored regularly for feral animal activity. Live trapping provides a method of removing feral animals. Local animal shelters may assist in removal of captured domestic animals. Removal of captured wildlife may be coordinated through state agencies or local rehabilitation groups. Trapping does not provide a total eradication of pests; therefore, the design of the maned wolf enclosures should reduce exposure to feral animals. The use of "hot wire" on the outside of enclosure fences can provide an effective deterrent to feral species intent on climbing.

Rodent Pests

Because rodents can transmit diseases (e.g. Leptospirosis) that can put maned wolves and humans at risk, rodent control is a crucial part of maned wolf captive husbandry. Rodent pests must be handled through a well-planned, supervised, continuous pest control program that is coordinated with the veterinary staff. Care must be taken in choosing compounds that are effective, yet not highly toxic in a single dose, especially when considering secondary toxicities. Several anticoagulant rodenticides are available with reduced secondary toxicity potential, e.g., diphacinone and bromadiolone. Rodenticides designed for a “quick kill” of rats should be avoided. Extreme caution should be exercised to assure that maned wolves do not consume rodenticides or rodents that have ingested rodenticides. It is important to have the rodenticide antidote (e.g. vitamin K) available in case there is accidental ingestion. It may seem obvious, but it should be emphasized that **at no time should maned wolves have primary access to any rodenticide.**

Insect Pests

Good sanitation aids in reducing insect populations, but all zoological situations experience insect pests, particularly cockroaches. Insecticide applications can be made around maned wolf enclosures with chemicals that are safe when applied in a proper manner. There are many chemicals available, both primary insecticides and newer growth regulator compounds that have low toxicity potential when used correctly. (Examples of insecticides include diazinon, piperonyl butoxide, natural and synthetic pyrethrins, carbamates, chlorpyrifos; example of a growth inhibitor is *Gencor*.) Enclosures are treated by removing the maned wolves, applying chemicals safe to use in primary enclosures, and then cleaning the enclosure to avoid exposure prior to returning maned wolves. The residual chemicals in cracks and crevices should have no contact with the maned wolves but, if so, exposure levels should be minimal. All personnel involved with the maned wolves should participate in the planning stage of the pest control program so everyone is aware of the compounds being used, where and how they are applied, and become knowledgeable of the safety of the compounds. Safety of the maned wolves is the primary concern for any pest control program.

Inadvertent use or misuse of insecticides (and herbicides and miscellaneous toxic compounds not intended for use around animals) can lead to accidental exposure of maned wolves and possible fatal results. This can be avoided by carefully planned pest control programs and subsequent correct applications of pesticides. This is an obvious concern regarding maned wolves and other animal species.

Besides the aesthetic reasons for eliminating pests, a more important reason is eliminating potential diseases found in feral mammals, birds, rodents and insects. The ectoparasites of mammals such as fleas, ticks, and mites, can be transmitted to maned wolves; as well, internal parasites of these same feral animals can be

acquired by maned wolves and cause infection. Feral animals also serve as potential sources of pathogens such as rabies, yersiniosis, leptospirosis, salmonellosis, toxoplasmosis, parvovirus, canine distemper, and others.

Products used on domestic dogs for flea and tick control, such as fipronil (Frontline) and lufenuron (Program), have been used successfully on maned wolves (see Chapter 7).

MANAGEMENT DURING PREGNANCY and PUP REARING-see also Chapter 5

The maned wolf exhibits a typical canid gestation of approximately 65 days. Although in the past males were usually separated from females for birth and pup rearing, it is now recognized that most males will invest a good deal of parental care in pups. Most zoos now leave the pair together for the birth and pup rearing. Alternatively, some zoos opt to separate the male during the birth and first few weeks and then reintroduce him to the dam and pups. In this situation, males are typically housed adjacent to the dam and pups with visual access. Bestelmeyer (2000) examined historical records of births in North America and reported that pups raised by both parents had a higher rate of survival, and females raised by both parents were more likely to successfully raise their own young. Consult Chapter 5 for additional information.

Whelping Areas

Since maned wolves give birth during the winter, adequate heat must be provided in whelping dens to maintain temperatures above 45° F (~7° C). Whelping areas should also be free from drafts. Managers should keep in mind that floor temperatures will affect neonates. The dens and rest/hide boxes used throughout the year may suffice for use during whelping as long as they meet the specifications listed in this chapter section.

Maned wolves will usually move pups, therefore more than one nest box must be provided. Dams seem to prefer a small space with a low ceiling, therefore a typical nest box measures 4' x 4' x 4' (1.2 x 1.2 x 1.2m) high. An A-frame design has been successful. Even smaller spaces may be preferred, although the design should take technical requirements for video monitoring into account. Designs incorporating a partition or L-shaped entry may provide more security to a dam with newborn pups. A small opening covered with freezer strips will help retain heat and enhance the dam's security. Whether nest boxes are contained within a larger structure or constructed as separate individual units depends on climatic conditions at the institution, however, maned wolf litters have been successfully reared in insulated but unheated A-frame boxes when air temperatures routinely dipped below freezing.

In very cold climates, it may be preferable to construct whelping dens inside a larger heated building, eg. 15' x 15' (4.6 x 4.6m) or larger, furnished with 2 or more whelping boxes measuring 4' x 4' (1.2 x 1.2m), so that the dam and pups can be locked in during extreme weather.

Whelping box at CRC

Whelping box at White Oak

Parents with pups should be closely observed to discourage digging under the whelping box or other natural features such as uprooted tree stumps since this is an injury risk to pups and also prevents keepers from monitoring pups.

Most adult maned wolves become defensive of pups and will display very aggressive behavior towards human "intruders". It is recommended that whelping boxes be equipped with a door, e.g. guillotine, that can be operated remotely so that keepers can access feeding areas safely. The ability to separate the dam/sire from pups for routine weighing and inoculations should also be addressed when designing a whelping den/nest box.

Neonatal mortality rates have historically averaged around 50% for the global captive population. Mortality most frequently occurs during the first week of life. It is therefore highly recommended that nest boxes be equipped for remote video monitoring.

Whelping boxes may be bedded with straw, carpet, or cedar chips. Hay is not recommended because of the risk to pups of inhaling small particles.

Changes in Keeper Routine and Public Access

During the first weeks after a birth, some institutions close the exhibit to public viewing in order to provide a quiet and secluded area to new parents. Most managers try to limit care of new litters to a few individuals who are familiar to the parent(s). Consistency in procedures and observations is optimized by limiting the number of individuals dealing with the animals. Other zoos make no changes in routine for new litters.

Disturbances to new families should be kept to an absolute minimum. Barring an imminent health concern, pups should not be handled until the first vaccinations (~6-8 weeks, see Chapter 7). Physical examinations and weigh-ins should be coordinated with regularly scheduled vaccinations.

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Chapter 2 - Addendum I
Two Accounts of Mixed Exhibits – July 2004

1. Sunset Zoo

This is an FYI for those who have or are considering mixing giant anteaters and maned wolves. Sunset Zoo began physical introductions between 1.0, 12 year old giant anteater and 1.1, three year old maned wolves on 1 July. This was after a lengthy introduction period. To date, the arrangement has worked out well. Typically the wolves are curious about the anteater and will approach him cautiously but not aggressively. The anteater seems generally ambivalent towards the wolves and goes about his business as usual (mostly sleeping during the day).

We are still evaluating how the space is being utilized and if there have been any negative affects on the wolves behavior. Typically the anteater goes wherever he pleases and the wolves react. At this point I don't believe the wolves have been negatively impacted by the introduction. They are together approximately 7.5 hours per day.

Thanks to Audubon Zoo staff for providing assistance with the process.

--
Ryan Gulker
General Curator
Sunset Zoo
Manhattan, KS

Ryan Gulker's current address is San Antonio Zoo, 3903 N. St. Mary's Street, San Antonio, TX 78212

2. Fossil Rim Wildlife Center

We have also had success at Fossil Rim with a mixed exhibit of maned wolves and a capybara. We had two male capybaras, which we introduced to a pair of maned wolves through chain link for several weeks. The shared yard space is about an acre with a concrete pool and three concrete houses for shelter. There is no "inside building". The two capybaras were fine with the two adult wolves. Eventually, we lost one capybara and the male wolf.

The capybara was not replaced but the male maned wolf was. The new male wolf chased the capybara around quite a bit at first but then settled down after a few weeks. The pair of wolves reproduced this year and there are now three pups in the yard. The only problem that we have encountered is with the capybara occasionally eating wolf food. Another problem is the capybara fouling the pool water with feces every day, but there are alternate water sources in the yards. The pups sometimes run after the capybara but it doesn't seem to bother him very much. We do not separate so they are all together 24 hours a day.

--
Mary Jo Stearns
Animal Care Specialist
Fossil Rim Wildlife Center
PO Box 2189
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Chapter 2 – Addendum II
Capture Boxes and Restraints

Example of a crowding gate used at Sedgwick County Zoo:

Squeeze cage used at CRC:

CHAPTER THREE
NUTRITIONAL MANAGEMENT

BACKGROUND AND CURRENT RESEARCH EFFORTS

Numerous studies of wild maned wolves have clearly documented the omnivorous habits of this unique canid; the natural diet is evenly split between animal and plant products (Dietz, 1984; Carvalho & Vasconcellos, 1995; Motta-Junior *et al*., 1996; Rodrigues, 2002). In Brazil the fruit *Solanum lycocarpum* is commonly know as *lobeira* ('fruit of the wolf") because it constitutes a large portion of the maned wolf diet. Maned wolves housed in U.S. zoological collections through the 1980s were typically fed the same raw, meat-based products that are offered to exotic felids. The results of a 1985 survey revealed that most U.S. zoos fed commercially available raw, horsemeat-based diets (Nebraska Brand Feline/Canine; Western Plateau Carnivore Diet) to maned wolves. Out of 14 zoos, 11 offered commercial, meat-based diets (frozen horsemeat or beef commercial diets) in addition to a variety of other items. Fruits, vegetables, bread, whole prey and rice were also commonly fed items. Since domestic canids, and probably other facultative carnivores, do not require the high levels of fat and protein characteristic of commercial meat-based diets, the feeding of these products is neither necessary nor appropriate. Diets currently fed to maned wolves in most U.S. zoos now consist of dry dog food, with supplemental fruits, vegetables and prey.

Cystine calculi in the urinary tract pose a significant health threat to captive maned wolves in North America. These stones can predispose maned wolves to urinary tract infection, and urethral stones can lead to obstruction and secondary rupture of the bladder, especially in males. Both captive and free-ranging maned wolves have been shown to excrete cystine and other dibasic amino acids in the urine (Bovee *et al.*, 1981; Mussart & Coppo, 1999).

In domestic dogs, it is now believed that a partial control of cystine stone formation may be accomplished by reduction in methionine and cystine in the diet, by feeding less animal protein, and by promoting more alkaline urine (by the addition of urinary alkalinzers), since cystine is significantly more soluble in an alkaline environment. Urinary pH is typically lower (more acidic) in carnivores fed meat-based diets while the plant-based diets fed to herbivores will result in higher urinary pH. The historical use of high protein, meat-based diets for maned wolves held in U.S. zoos may have exacerbated or promoted cystine stone formation.

In 1998, a maned wolf diet was formulated as a result of collaborative work that included Dr. Rosalina Angel and Dan Hopkins of Mazuri (PMI), Dr. Mary Allen, (then-MWSSP nutrition advisor), and Jennifer Boniface (a graduate student from the University of Maryland). This diet was high in fiber and lower in cystine than commercial dog chows. Mazuri (PMI) manufactured and distributed the maned wolf chow, and the SSP encouraged everyone to use this specially formulated product. The animals were easily converted to this diet and stool quality seemed improved (more formed). However, beginning in 2001 several animals were diagnosed with anemia of unknown origin, had elevated liver enzymes, and seemed unthrifty. A follow-up study conducted by U. of Maryland graduate student Sara Childs, Dr. Mary Allen, and Dr. Roselina Angel (U. MD.) confirmed these reports, and in 2002 the recommendation was made to discontinue the diet, although the Mazuri diet could not be directly linked to the reported health problems (Childs-Sanford, 2005).

Captive maned wolves continue to have cystinuria, "sand" in the prepuce and bladder, and renal and cystic calculi. It is generally agreed that diet contributes to this medical condition and further research is desperately needed to determine nutrient needs of maned wolves. Additional concerns related to the chow diets that are currently fed include chronically loose stools and palatability issues that may result in weight loss. Maintaining a healthy weight can be problematic in some individuals. A survey requesting information about diet, stool quality, and health issues from all MWSSP zoos was initiated in November 2006. Data analysis is

currently underway. Nutrient profiles for current diets will be determined for each institution and correlated if possible with stool consistency and health issues. The MWSSP is searching for funding to continue research into nutritional needs and diets, including additional studies of wild populations and exploring reducing dietary cystine by increasing the ratio of plant:animal protein and maintaining alkaline urine.

RECOMMENDATIONS

Nutrient Content of Diet

Current knowledge supports the feeding of diets:

- of low to moderate protein content, between 20-25% protein, DMB (dry matter basis) to reduce the amount of cystine that the kidneys must excrete
- that promote oral health (dry feeds vs. soft)
- that result in well-formed stool (soybean meal may exacerbate loose stool)
- that result in more alkaline urine (foods with higher carbohydrate, lower animal protein will promote alkaline urine)

Maned wolves in many zoos outside North America are fed small amounts of animal protein in the form of meat, prey or eggs, but the bulk of the diets consist of cooked rice and other grains, fruits and vegetables. Such "home-made" diets require supplementation with vitamins and minerals. Unless the complete diet is evaluated and analyzed it is impossible to know which supplement and how much supplement should be applied to balance such diets. These diets may be preferred over the meat-based diets of 20 years ago, but nutrient balance in home-made diets that consist of multiple food items is difficult to achieve. Unless supplements are applied homogeneously to the total diet, animals have the opportunity to preferentially select some food items over the others. Maned wolves may not receive the entire supplement and thus may consume vitamin or mineral deficient diets. **It is therefore recommended that nutritionally complete commercial products (e.g., dry dog foods, omnivore biscuits) represent at least 60-70% of the dry matter intake for maned wolves.** If this guideline is adhered to, vitamin and mineral supplements are not necessary and additional dietary items such as chopped fruits and vegetables and small amounts of prey may be fed without risk of dilution of the nutrients in the commercial product. **The Maned Wolf SSP is currently assessing diets with a goal of recommending several brands that have proved successful for reproduction and general health.**

Adult maned wolves (body mass 30 kg) should maintain body mass by consuming approximately one pound of dry matter per day. Lactating females and growing pups may consume 1.5 to 2 times that amount. Because low protein dog foods are often low in fat, the addition of vegetable oil or cooked chicken fat will increase the energy density of the diet and may help improve palatability.

Adults: The maned wolf should be able to be maintained on commercial products that are formulated to meet the requirements of the domestic dog. Because domestic dogs fed diets containing soybean meal may produce poorly formed stool, commercial diets with rice or other non-soy plant products are recommended. High performance dog foods, because they are often in excess of 28% protein, and excessive meat or prey should be avoided. It is probably safe to supplement the basal diet with limited amounts of whole prey such as one or two mice daily or 3 small rats per week. Medicating or shifting maned wolves is often facilitated by the use of prey or fruit treats. The use of whole prey may also promote oral health. Oxtails or horse bones with some meat attached are also used to stimulate gums and teeth. The extent to which soft diets promote gingivitis and dental problems seen in some maned wolves has not been determined.

Puppies: A good quality adult maintenance chow is considered nutritionally adequate for growing maned wolves, however, zoos may opt to offer diets formulated for growing puppies to animals under the age of 15 months. Puppies begin eating solids at around 4 weeks of age, and parents begin regurgitating food to pups at this time. A good quality commercial puppy chow can be added to the adult diet beginning around 4 weeks of age. Gradually increase the ratio of puppy:adult chow to approximately 1:1 by the time the pups are ~ 6 months of age,

and then gradually decrease so that the youngsters are weaned over to adult chow by about 1 year of age. As pups begin eating more solids, additional feeding bowls/stations should be provided. Lactating females should not be offered diets with lower than 22% (DMB) crude protein since the nutrient demands on the female during milk production may not be met.

Feeding Schedules/Locations:

If dry dog food or dry omnivore biscuits are fed they may be offered free choice since they will not spoil readily and over-consumption is generally not of concern with maned wolves. The daily ration should be placed in the feeding bowl(s) and completely emptied on a daily basis. Food bowls or containers should be thoroughly washed and rinsed daily. Fresh, potable water should always be available. Food intake should be monitored by weighing food in and food out, if practical.

Other food items such as fresh fruits, vegetables, cooked rice, whole prey, or other perishables should be offered at multiple times during the day to minimize spoilage under high ambient temperatures and promote more naturalistic foraging behavior. More frequent feeding of smaller amounts may also promote more normal gastrointestinal function and create less demand on the GI tract. The extent to which one-meal feeding (daily allotment of food presented in one feed) promotes loose stool has not been objectively studied. All uneaten perishable foods should be removed from the enclosure as soon as is practical to avoid risks associated with bacterial proliferation.

Feeding locations should be protected from the elements. There should be at least one feeding location per maned wolf for those wolves housed together. Feeding stations should be separated by adequate distance and perhaps by visual barriers to discourage dominant animals from taking control of the food and to encourage food consumption by subordinate animals.

Maned wolves with access to outdoor yards are often seen eating insects, small rodents, birds, frogs, and grass. The feeding of 20-30 crickets once or twice per week also stimulates feeding activity and offers some diversity. Grass eating is seen in domestic dogs and does not necessarily indicate GI tract problems or a nutrient deficiency. "Grass stool" produced by maned wolves may be voided as soon as 30 minutes after consumption. Such stool boluses are often coated in foamy mucous and may not contain any other food material, other than grass.

NUTRIENT REQUIREMENTS AND DEFICIENCIES/TOXICITIES

As discussed above, the nutrient requirements of maned wolves are unknown so domestic dog requirements are used as the closest model. There is likely some value in offering diets that do not exceed 28% crude protein since protein (amino acids) in excess of requirement must be excreted. Much of the excess dietary cystine is probably excreted via the kidneys. It is thus prudent to restrict cystine (and methionine) (See Chapter 7).

DIETS THAT HAVE CAUSED PROBLEMS

As noted above, some commercial dry dog foods and omnivore biscuits appear to result in poorly formed stool. The extent to which feeding frequency (more frequent feeds in smaller amounts) may help to alleviate loose stool has not been studied. The addition of prey (rats, mice, etc.) appears to result in firmer stool, however the amount of prey must be monitored closely as these items increase levels of cystine in the diet.

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CHAPTER FOUR
BEHAVIOR AND SOCIAL ORGANIZATION

Studies of captive animals have provided the bulk of information about maned wolf behavior, since observations in the wild are rare and usually of short duration. However, conclusions based on captive studies are supported by the few instances of behavioral observations of wild maned wolves. Institutions receiving an animal should obtain as much information as possible about the individual's past history. This information can be crucial to successful introductions and subsequent reproduction. There is considerable individual variation in temperament, which may be influenced by such factors as early experience (hand vs. mother rearing, group vs. solitary rearing), and prior social grouping. Please refer to the ethogram at the end of this chapter for a description of maned wolf behavior.

SOCIAL GROUPING

Adult maned wolves are usually housed as opposite sex pairs. Animals may be housed together 24 hours a day or may be separated into individual dens at night. Maned wolves can be housed together throughout the year. Little is known about dispersal of young maned wolves in the wild. In captivity, siblings are frequently housed together for the first 2 years. Opposite sex siblings should be separated before they reach 2 years of age in order to prevent reproduction. Same sex pairs or trios of siblings may be housed together for longer periods but should be monitored closely for signs of tension and aggression, particularly in the case of trios. Managers should make every effort to avoid separating one member of a trio from the other 2 individuals. Conditioning animals to enable both routine and non-routine husbandry practices can minimize the need for separating same-sex groups and should be considered an essential management tool.

Parent-reared offspring are usually separated from the dam and sire before the onset of the subsequent breeding season. Compatible pairs may be housed together 24 hours a day throughout the year. Several houses or dens should be made available as pairs may choose to sleep together or separately according to temperament, season, and temperature. Separate feeding stations should also be available at all times to lessen the possibility or severity of aggression. Managers may prefer to separate marginally compatible pairs during the night. Although rare, there have been instances of pairs that are only compatible during the breeding season and that are housed separately the remainder of the year. Bestelmeyer (2000) demonstrated that pup survival is maximized if the sire is left with the family group; therefore, housing the pair together throughout the year is desirable as it may lead to better synchronization of behavior and compatibility. If this is not possible, an attempt should be made to reintroduce the male as soon as possible after the birth of pups.

Maned wolves have been successfully housed and pups reared in enclosures adjacent to other canid species (e.g., Mexican gray and red wolves, Sullivan 2002). Visual barriers are not necessary but physical barriers are required, e.g. double fencing.

INTRODUCTIONS

Introductions of male-female pairs are rarely unsuccessful. Timing the introduction near the beginning of the breeding season (i.e. late summer or very early fall in North America) may facilitate the process. Brief fighting may occur until one individual establishes dominance. Any continued severe aggression should not be allowed, and animals should be watched closely for signs of wounds and other injuries. **Attempts to introduce unrelated same-sex animals over 1 year of age have not been successful and are not currently recommended**.

A set protocol for introductions of maned wolves should be in place before the process is begun. Visual and olfactory contact through a wire-mesh barrier for a period of time prior to physical access is critical. The length of time this limited access should occur is dependent upon the wolves' behaviors, but 1-2 weeks is usually long enough

for the individuals to become familiar with each other. Each individual maned wolf should have access to the new enclosure (exhibit or holding area) before introductions take place. This allows the new individual(s) time to become familiar with the enclosure. If both maned wolves are new to the enclosure, sufficient time should be allowed for both animals to learn the enclosure prior to introduction.

Physical introductions should take place in an area that facilitates quick and safe separation of the pair if undue aggression occurs. Behaviors such as lunging, growling, gaping and chasing can be expected during the initial introduction period. Wrestling and biting is also seen and may occur until dominance is established. Following the initial introductions some zoos continue to separate pairs at night for a period of time.

CHANGES IN BEHAVIOR DURING THE BREEDING SEASON

In North America maned wolves breed primarily from late September through January, with a mean estrus date of November 16 (Rodden *et al.*, 1996). Females cycle once each year. Increased frequency of synchronized activities such as reclining and moving together, male following female (or vice versa), and friendly behaviors such as male sniffing and licking female's anogenital area are indicators of the onset of estrus. Keepers should observe new pairs outside the breeding season in order to establish baseline levels of interactions; increasing the frequency of observations as the breeding season approaches. It is recommended that all potential breeding pairs be observed during the season, beginning in early September and continuing through the breeding season (possibly mid-January). Copulations may not be observed, so it is crucial that affiliative behaviors be noted to aid in predicting a parturition date. Rodden *et al.* (1996) determined that in the absence of observed copulation, the amount of time a pair spent in close proximity provided strong evidence of breeding activity. An ethogram and data collection form is included as Addendum I at the end of this chapter.

Pairs with young should be isolated as much as possible from other maned wolves. It is recommended that family groups be separated by a solid barrier from maned wolves housed in an adjacent exhibit, e.g. buffered fencing with a corridor between enclosures or cinderblock/gunite in exhibits). In cases where a breeding pair has been separated for management purposes, housing the sire adjacent to dam and pups is recommended. Sires have been successfully reintroduced to the dam and pups at several institutions, primarily when pups are between 2-10 weeks old. Managers are urged to review the Zoo Biology paper regarding reintroductions of male maned wolves to females with pups (Bestelmeyer, 1999).

For additional information about reproductive behavior, please refer to Chapter 5-Reproduction.

COMMUNICATION

Maned wolf communication involves visual, auditory, and olfactory cues. Since adult maned wolves occupy large home ranges and interact infrequently, one would anticipate the development of mechanisms for long-range communication. It would also be expected that close-up communication would involve a few distinctive displays leaving little room for misunderstanding. Indeed, visual displays such as the friendly tail-up approach and play invitation bow, and the agonistic piloerect pace, gape, and submissive crouch convey a clear message to the recipient.

Olfactory information is communicated via urine and feces, which are deposited at ground level and on elevated objects throughout the exhibit, particularly along boundaries. Frequency of urine marking increases prior to and during the breeding season, with both partners showing an interest in each other's urine marks (Brady & Ditton, 1979). Presumably information about reproductive status is ascertained from sniffing and tasting the partner's urine. In close-up interactions, both sexes frequently sniff and lick the partner's anogenital area. Feces are often deposited in specific areas, usually around the perimeter of the exhibit. Since almost all the hormonal metabolites are excreted in feces, information about sex and reproductive status is likely transmitted via this route as well (Wasser *et al.*, 1995).

Brady (1981) described the vocal repertoire of the maned wolf. The most commonly heard vocalizations in

captive maned wolves are the submissive whine, the repetitive (or "pulsed") whine, growls of varying intensity, and the roar-bark. All convey information about the mood of the individual. Submissive whines and growls are heard during aggressive interactions between adults and are also used by pups in interactions with each other and with parents. The repetitive whine is used in two contexts: in a sexual context by a male toward a female in estrus, and in a feeding context by dam prior to nursing young pups or by either parent to solicit food begging from older pups. The roar-bark can be heard over long distances and may function as a spacing mechanism between same-sex adults (Kleiman, 1972). Additionally, the roar-bark may occasionally be used in a sexual context, typically directed by a male toward a female in estrus when the pair is visually separated.

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Chapter 4 – Addendum I
Maned Wolf Ethogram and Data Collection form

Behavioral States

Lying down: mid-section of body in contact with the ground
Sit: hind end in contact with the ground
Stand: stationary upright position
Walk: locomotion without in-air phase
Trot: locomotion with in-air phase where hind legs do not extend forward past the midline of the body
Run: locomotion with in-air phase where hind legs extend forward past the midline of the body
Pace: walk back and forth over the same, small area
Eat: consume solid food
Drink: consume water or other liquid

Behavioral Events

Solitary Behaviors
Self-groom: lick own body
Sniff: investigate object with nose; nose <10 cm from object
Scratch: scratch own body with hind leg
Stalk: ears erect and forward, body tense and either standing or moving slowly with attention focused forward
Dig: scratch ground with one or both front paws to make a depression
Cache: bury food in hole or cover food with substrate

Elimination/Marking
Leg lift: urination with hind leg lifted off the ground
Squat: urination using a squatting posture, sometimes one hind leg may be lifted slightly off the ground
Urinate over: urinate in the same spot another wolf urinated or defecated within five minutes
Taste urine: lick urine of another wolf, usually accompanied by flehmen
Flehmen: teeth chatter; lips often pulled back
Straddle: stand over and rub genitals on bush, grass, or other object
Face rub: rub face or neck on a surface
Body rub: rub side of body on a surface
Defecate: self-explanatory

Affiliative (Friendly)
Sniff conspecific: investigate another wolf anywhere except the anogenital region
Groom conspecific: lick body of another wolf
Sniff/lick anogenital region: sniff or lick another wolf's anogenital region
Tail out: tail lifted or held horizontally along line of back
Tail up: tail lifted above level of back
Play invite: stamp or bow on forelegs with ears up while facing other animal, or use foreleg to paw at shoulder of another animal
Play chase: chase another animal, usually with ears forward and without piloerection
Open mouth: head and ears up, alert, mouth open with tongue out
Wrestle: stand together on hind legs, front legs on other's shoulders, usually silent and with open mouth
Present: female stands or walks with anogenital region oriented toward male's face, back often slightly arched, tail deflected up or to the side
Pulse whine: rapidly repeated soft high-pitched vocalization
Attempt mount: male attempts to mount female

Mount: male mounts female and exhibits pelvic thrusts
Tie: occurs after the mount and lasts a minimum of 60 seconds

Agonistic

Charge/lunge: advance towards other wolf, piloerect, stiff forelegs, ears back
Growl: growl at conspecific
Gape: open mouth, ears back, oriented toward other wolf; often accompanies charge
Agonistic chase: chase another animal, usually with ears back and piloerect
Piloerect pace: walking with stiff forelegs, head down, piloerect, ears usually back, often moving parallel to other animal
Submissive crouch: body in crouch or semi-crouch and oriented sideways to other animal, head rolled sideways while looking at other animal and often whining
Submissive whine: long, high-pitched vocalization, usually accompanying the submissive crouch
Bite: snapping jaws shut
Bark: short, loud, hoarse vocalization; not necessarily an agonistic behavior

Parental Behaviors

Regurgitate: disgorge partly digested food to mate or pups
Regurgitation solicitation: animal crouches low to the ground, approaches another wolf with ears flattened and tail wagging, and nudges the body or mouth area with the muzzle
Muzzle bite: bite the muzzle of another animal, usually in response to a regurgitation solicitation
Nurse: suckle; female may be lying down or standing
Groom: lick body of pup or mate
Repetitive whine: bouts of short, loud high-pitched vocalizations, usually emitted by an adult wolf with a closed or slightly open mouth; often followed by nursing or regurgitation
Carry pup: carry pup with mouth around pup's neck or midsection
Attempt carry: open mouth around pup's neck or midsection; sometimes the pup is dragged but it is never lifted off the ground
Gape: open mouth, ears back, teeth bared; often occurs during weaning

MANED WOLF ETHOGRAM **DATE:**

♂ **ID:** ♀ **ID:** **Start Time:**

Observer: **Weather:** **NOTES:**

Time Minute	Active-A Inactive-I Not Visible-NV				**Initiate** Approach-A Follow-F Bark-tick marks		**Scent Mark** Urinate-U Defecate-D Straddle Weed-SW Face/Neck Rub-FR Flehman-F		**Friendly** Sniff Body-S Pulse Wine-PW Tail Out/Up-TO Play Initiation-PI Wrestle-W		**Sexual** Sniff/Lick AG- Sag ♀ Presents-P ♂ Atmp Mount-AM ♂ Mounts-M Tie - T		**Agonistic** Gape/Growl-G Lunge/Charge-L Chase-C Piloerect Pace-PP Submissive Whine /Crouch-SW	
	Solitary		Social											
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
1														
2														
3														
4														
5														
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CHAPTER FIVE
REPRODUCTION

Ever since the arrival of the first maned wolves at zoos in US zoos in 1975, the species has never reproduced with consistency in captivity. For example, from 1996 through 2003, only 43% of the 108 recommended breeding pairs produced offspring and ~ 35% of pregnant females lost their pups within a few days of giving birth. Analysis of birth and death records from the International Maned Wolf Studbook from 1980 through 1998 confirmed that the main cause of pup mortality was parental incompetence (66%). Therefore, reproductive research has been one of the main focuses for this species. During the past 10 years, we have made significant progress in improving our knowledge in maned wolf reproduction.

CHARACTERISTICS OF THE BREEDING CYCLE

Maned wolves are monestrous; females cycle once a year. The onset of the breeding season appears to be a response to a decreasing light cycle. In the northern hemisphere breeding typically occurs from late September through early January; almost 50% of pups are born between December and January, while the majority of births in South American occur from May – July.

The mean estrus date is November 16, based on back-dating 65 days from mean birth date of January 20 for 37 litters born in North America from 1988 through 1994 (Rodden *et al.*, 1996). Data from the International Studbook suggest that first copulations can occur in the age range of 1-2 years in both sexes, although first successful breeding typically occurs at around 34 months of age. Pregnancy can result from the first breeding. The average age of males and females at first reproduction is around 5 to 6 years. Reproductive senescence is typically reached by age 12 in both sexes.

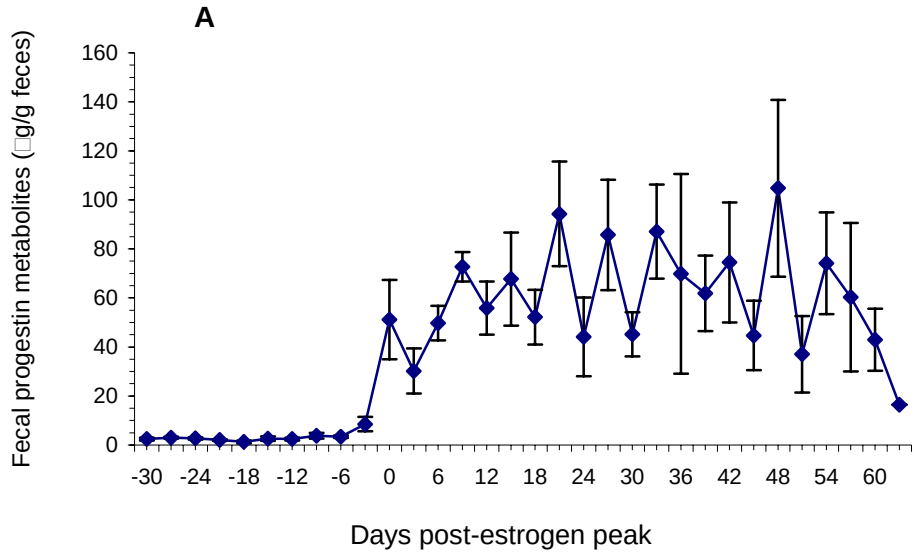
Pro-estrus: Pro-estrus lasts from 3 to 18 days and is characterized by vaginal swelling and secretion and increasing mutual interest between male and female, indicated by solicitous tail flagging, play invitations, sniffing, anogenital investigations and attempt mounts (Velloso *et al*., 1998) that further increase in frequency throughout estrus.

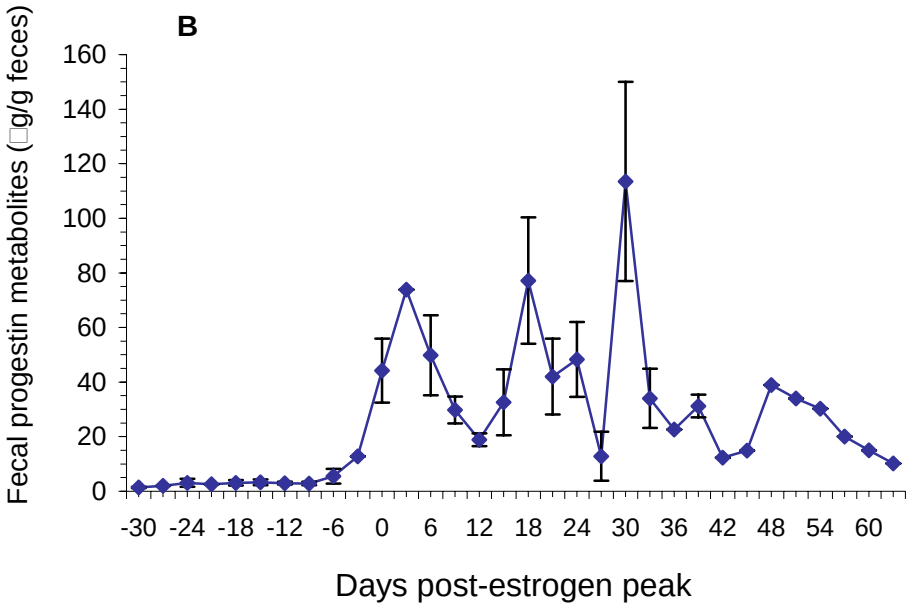
Estrus: Estrous can last up to 10 days. Visible signs of estrus in the maned wolf are vaginal swelling (pink in color) and discharge (discharge appears pink or bloody before, clear during, and thick yellow at the end of estrus). Male sniffing and licking the female's anogenital area, and a female averting the base of the tail to one side, exposing the vulva to the male ("presenting") are clear indicators of approaching estrus. Copulation with pelvic thrusts and attempted copulations occur frequently during estrus. Maned wolves exhibit a copulatory tie lasting from less than 1 minute to several minutes. Rodden *et al.* (1996) reported that the proportion of time spent in close association was the single most powerful behavioral measure for distinguishing breeding from non-breeding pairs. In the absence of observed copulations, valuable information can be gleaned from simply noting changes in the amount of time a pair spends in close proximity. Sexual behavior (e.g. female "presenting" and copulation) is very rarely observed outside of the estrus period.

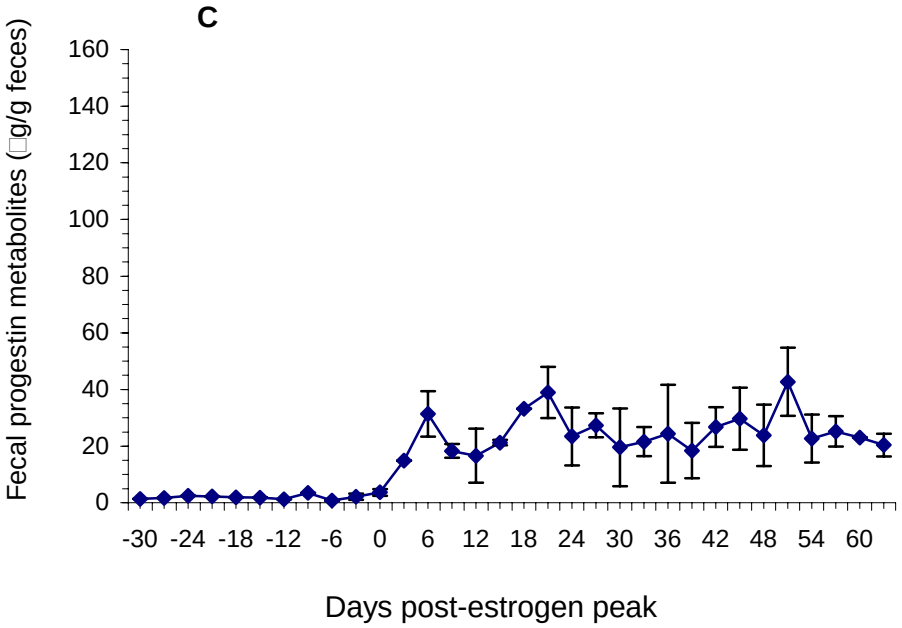
Pregnancy: Gestation lasts 58-72 days, with a mean of 65 days (Dietz, 1985). Detecting pregnancy has been a key problem in captive breeding efforts. Changes in a female's appearance during gestation are usually minimal. Numerous instances of pseudopregnancy have been noted by maned wolf managers. In these cases, copulatory behavior was observed, and females often exhibited the slight abdominal swelling associated with pregnancy. Some even produced milk, but no birth occurred (Rodden, unpublished data). Comparison of behaviors between pregnant and pseudopregnant females revealed that pregnant females exhibited higher rates of agonistic behavior during the pro-estrous and estrous periods and lower frequencies of approach behavior during estrous period than do females who became pseudopregnant (Rodden *et al.*, 1996).

Endocrine hormone profiles: Measures of reproductive steroids excreted in feces have been reported by Wasser *et al*. (1995), Gross *et al.* (1991), and Velloso *et al*. (1998) using radio immunoassay, and by Songsasen *et al*. (2006) using enzyme immunoassay. The studies indicate that fecal steroid measures can be used to assess the reproductive status of female maned wolves. Cycling females show an estrogen surge followed by a sustained rise in progesterone levels. Copulation begins 2-6 days after the estrogen peak, and last up to 9 days after the peak (Songsasen *et al*., 2006). As in domestic dogs, pseudopregnant maned wolves excreted overall lower concentrations of progestins than did their pregnant counterparts (Velloso *et al*., 1998; Songsasen *et al.*, 2006). However, it remains to be determined if the monitoring of longitudinal profiles would be useful as a diagnostic tool for pregnancy in this species. Preliminary data show that females experiencing neonatal loss have lower progesterone during the second half of gestation than those that successfully raise their young. It is possible that the lower progestin excretion in maned wolves that lose pups may be related to an adrenal/stress response, perhaps due to sub-optimal management (Figure 1). Comparing fecal corticoid metabolites between females that successfully raised young and those that lost their pups show significant higher cortisol in the latter group, especially during the periovulatory period (Figure 2). Finally, there is preliminary evidence that male maned wolves may play a role in estrus induction of the females. This evidence may be relevant to zoos that separate pairs during breeding season and place them back together early on during the non-breeding season. In 2004, a pair that had been separated for the duration of the normal breeding season copulated shortly after being reintroduced and gave birth in June, more than 2 months after the typical end of the birth season.

Figure 1: Mean (± SEM) Longitudinal profiles of fecal progesterone metabolites for female maned wolves that (A) conceived and successfully raised pup (n =5), (B) conceived but failed to raise pups (n = 2), (C) became pseudopregnant (n = 2) and (D) were unpaired (n = 3). Each data point is a pool of 3-day mean.




B
Fecal progestin metabolites (□g/g feces)
160
140
120
100
80
60
40
20
0
-30 -24 -18 -12 -6 0 6 12 18 24 30 36 42 48 54 60
Days post-estrogen peak


C
Fecal progestin metabolites (□g/g feces)
160
140
120
100
80
60
40
20
0
-30 -24 -18 -12 -6 0 6 12 18 24 30 36 42 48 54 60
Days post-estrogen peak

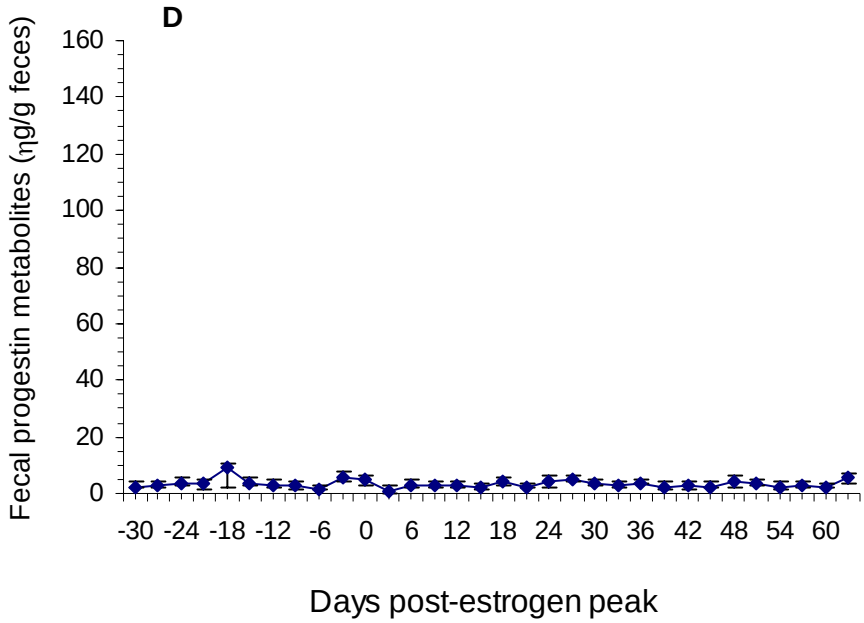
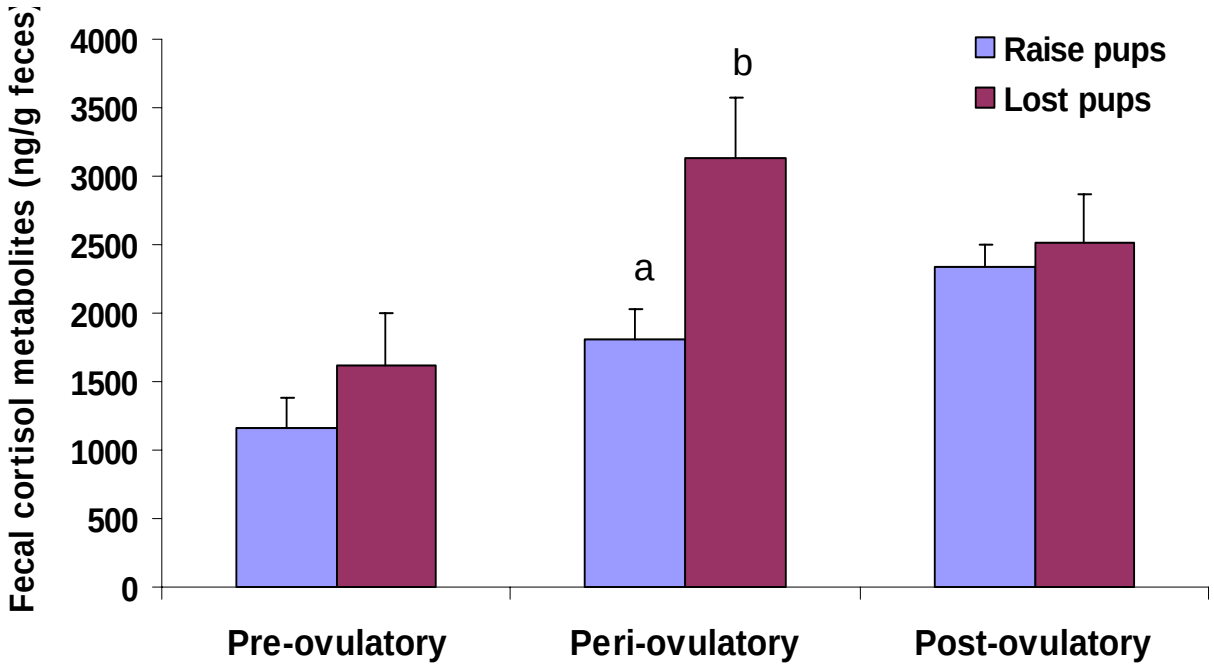


Figure 2: Mean (± standard error of mean) fecal corticoid metabolites in female maned wolves that successful raised (n=5) or lost (n=2) offspring shortly after birth. [a,b] Different letters indicate significant differences ($P < 0.05$).



MANAGING BREEDING PAIRS & FAMILIES

A **Birth Survey** is included as **Addendum I** at the end of this chapter. While the MWSSP does not require mangers to complete the survey, it is a very helpful tool for collecting information that may prove extremely valuable to your institution for future litters.

Housing: In their native habitat, pairs of maned wolves can occupy home ranges measuring 25-30 km² or larger (Rodrigues, 2002; Dietz, 1984). Institutions intending to house more than one adult pair of breeding animals are strongly encouraged to separate pairs by as much physical distance as possible. If adult pairs must be housed in adjacent exhibits, a solid barrier is required. Offspring may be housed adjacent to parents for, although the potential effects on the parents' subsequent breeding/pup rearing are not clearly known at this time. Breeding pairs may be housed in proximity to other canids and other taxonomic groups (Sullivan, 2002). Visual barriers are not absolutely necessary, but physical barriers are required, e.g. double fencing. Because there can be a good deal of individual variation in response to neighboring species, caution should always be exercised when introducing new "neighbors".

Since maned wolves give birth during the North American winter, adequate heat must be provided in whelping areas to maintain temperatures above 45° F (~7° C). Whelping areas should also be free from drafts. Managers should keep in mind that floor temperatures will affect neonates. The dens and rest/hide boxes used throughout the year may suffice for use during whelping, as long as they meet the specifications listed in this chapter section (see also Chapter 2). Institutions anticipating births are encouraged to contact Management Committee members for advice regarding whelping den requirements.

Maned wolves will usually move pups, therefore more than one nest box must be provided. Dams seem to prefer a small space with a low ceiling, therefore a typical nest box measures 4' x 4' x 4' high. An A-frame design has been successful. Even smaller spaces may be preferred, although the design should take technical requirements for video monitoring into account. Designs incorporating a partition or L-shaped entry may provide more security to a dam with newborn pups. A small opening covered with freezer strips will help retain heat and enhance the dam's security. Whether nest boxes are contained within a larger structure or constructed as separate individual units depends on climatic conditions at the institution, however, maned wolf litters have been successfully reared in insulated but unheated A-frame boxes when air temperatures routinely dipped below freezing. If given the opportunity, pregnant females may attempt to dig a den for whelping, usually under an existing object in the landscape such as a large shrub or exposed tree roots. In very cold climates, it may be preferable to construct whelping dens inside a larger heated building, e.g. 15' x 15' or larger, furnished with 2 or more whelping boxes measuring 4' x 4', so that the dam and pups can be locked in during extreme weather.

Nest boxes may contain bedding of straw, carpet, or cedar chips. Hay is not recommended because of the risk to pups of inhaling small particles. Floor covering in the whelping area should provide good traction for pups, therefore "wood wool" type products are not recommended for bedding material. Neonatal mortality rates have historically averaged around 50% for the global captive population. Mortality most frequently occurs during the first week of life. It is therefore highly recommended that nest boxes be equipped for remote video monitoring.

Most adult maned wolves become defensive of pups and will display very aggressive behavior towards keeper staff. The whelping box and exhibit area should be designed to allow keeper staff to safely access feeding areas. The ability to separate the dam/sire from pups for routine weighing and inoculations should also be addressed when designing a whelping den.

Left: Whelping box at CRC
Right: Whelping box at White Oak

Changes in Keeper Routine and Public Access:

During the first weeks after a birth, some institutions close the exhibit to public viewing in order to provide a quiet and secluded area to new parents. Most managers try to limit care of new litters to a few individuals who are familiar to the parent(s). Consistency in procedures and observations is optimized by limiting the number of individuals dealing with the animals.

Disturbances to new families should be kept to an absolute minimum. Barring an imminent health concern, pups should not be handled until the first vaccinations (~6-8 weeks, see Chapter 7). Physical examinations and weigh-ins should be coordinated with regularly scheduled vaccinations.

Introductions: A protocol for introducing new pairs should be in place before initiating the process. Visual and olfactory contact through a wire-mesh barrier for a period of time prior to physical access is critical. The length of time this limited access should occur is dependent upon the wolves' behaviors, but 1-2 weeks is usually long enough for the individuals to become familiar with each other. Each individual maned wolf should have access to the new enclosure (exhibit or holding area) before introductions take place. This allows the new individual(s) time to become familiar with the enclosure. If both maned wolves are new to the enclosure, sufficient time should be allowed for both animals to learn the enclosure prior to introduction.

Physical introductions should take place in an area that facilitates quick and safe separation of the pair if undue aggression occurs. Behaviors such as lunging, growling, gaping and chasing can be expected during the initial introduction period. Wrestling and biting is also seen and may occur until dominance is established. Following the initial introductions some zoos continue to separate pairs at night for a period of time.

Introductions of male-female pairs are rarely unsuccessful. Brief fighting may occur until one individual establishes dominance. Any continued severe aggression should not be allowed, and animals should be watched closely for signs of wounds and other injuries. There have been a few cases of pairs that were incompatible outside the breeding season, however the majority of pairs are housed together year round with no problems.

The annual MWSSP Population Plan may, for a variety of reasons, request that established pairs be separated and one or both members re-paired with new mates. This can be accomplished without negatively impacting breeding success. If an animal is to be moved to a new institution for breeding, it is advisable to move the individual several months before the beginning of the breeding season. This will ensure adequate time for the wolf to pass quarantine and adjust to new areas and keepers before introductions begin. If an existing pair is separated for re-pairing with other individuals at the same institution, care should be taken to separate the existing

pair for at least several weeks and by as much physical distance as possible before attempting to introduce a new mate.

Gestation and Birth: The gestation period for maned wolves ranges from 58-72 days; the mean is 65 days (Dietz, 1985). Pregnancy can be very difficult to determine because there are usually only minor changes in physical appearance. In addition, there have been numerous instances of pseudopregnancy in which mating is observed, the female's abdomen appears to get larger and she produces colostrum, but there is no birth. Ultrasound has been used successfully 30 days post-copulation, while radiographs are only diagnostic when done during the final 3 weeks of pregnancy when bone formation is occurring. There have been no reports of problems associated with the use of ultrasound or radiographs, although these techniques are not commonly used. More recently Relaxin assays used at least 30 days post-breeding have successfully predicted pregnancy, however the test requires obtaining a blood sample. A study is currently underway to establish a non-invasive method (urine sample) to determine pregnancy in maned wolves. Conditioning a female to allow ultrasound or a blood draw without sedation and to provide a urine sample on command is an extremely valuable management tool.

Behavior observations can be used to assess whether the pair has bred (in the absence of observed copulations) and also help determine whether a breeding pair will actually produce pups. Rodden *et al.* (1996) includes instructions for analyzing behavior data recorded on the data sheet found in Chapter 4 Addendum I. In the absence of observed copulations, the proportion of time spent in social interactions is the most powerful behavioral measurement for distinguish breeding individuals from non-breeding ones. In addition, breeding females exhibit higher levels of scent marking both before and after estrous periods than do non-breeding females. For reasons that are not entirely clear, successful breeding pairs can be distinguished from unsuccessful breeders on the basis of higher rates of agonistic behavior before and during estrus (Rodden *et al.*., 1996). As whelping approaches, elevated levels of aggression have been noted between pair members (Encke *et al.*, 1970; Uka, 1986), and by females toward keepers (Brady & Ditton, 1979).

In pregnant females, indicators of impending birth include: restlessness 2-3 days prior to birth, aggression to male mate and sometimes aggression towards keepers. Physical changes can include abdominal swelling in the last 2-3 weeks, nipple development, milk in teats 2 weeks pre-delivery and hair loss around nipple area.

Family Groups: It is strongly recommended that pairs be housed together year round, including throughout gestation, birth, and pup rearing. The historical records of captive maned wolves in North America indicate that pups raised by both parents have a higher rate of survival, and that female pups raised by both parents are more likely to successfully raise their own young (Bestelmeyer, 2000).

During the first 2-3 weeks after birth, females spend a majority of their time with the pups, leaving only to feed and defecate. They typically keep the male away from pups during the first few weeks; experienced sires will rarely attempt to enter a whelping den with the dam and young pups. Pups begin to venture out of the den when they are 4-6 weeks old, and at this time the sire begins to take an active interest in the pups, regurgitating to them and engaging in play bouts. The weaning process also begins at this time, continuing until pups are fully weaned at around 5-6 months of age. See **Chapter 6-Pup Development & Hand Rearing Protocol** for additional information about pup growth and development.

Although the MWSSP recommends leaving pairs together for the birth, some managers may opt to separate the sire for management reasons or out of pair incompatibility concerns. In such cases, the MWSSP urges managers to house the sire adjacent to dam and pups, preferably with visual access, and to reintroduce the sire to the family as soon as possible. Successful reintroductions have occurred when pups were 6-12 weeks old. Initiating the introduction through a fence barrier is recommended. Look for affiliative behaviors such as Tail Up or Pulse Whine (see Ethogram at end of Chapter 4). Sires may try to push whole prey items through the fence to pups, or regurgitate to pups.

Most zoos separate pups from parents when they are approaching 10 months of age, to prevent interference with the subsequent breeding season. Pups may be housed adjacent to parents during subsequent

breeding. There is some recent evidence from wild populations that juvenile females may remain in the home territory and possibly assist with rearing the next litter (Louise Emmons, pers. comm.). Same-sex littermates may continue to be housed together; male-female sibs should be separated when they are 2 years old to prevent inbreeding.

Diet Changes **(See also Chapter 3-Nutrition):** Pregnant females typically do not require changes in the diet; a high quality dog chow should constitute the bulk of the diet. As excess weight is rare in maned wolves, a pregnant animal should be offered plenty of chow, and the amount should be increased should she demonstrate an increase in appetite.

The appetite may decrease abruptly 1-2 days prior to birth. Females usually resume eating 1-3 days post partum. Special attention should be paid to maternal nutrition during lactation, particularly females with larger litters. Lactating females should be fed chow containing a minimum of 22% (DMB) crude protein. Lactating females and growing pups may consume up to 1.5-2 lbs. chow each per day.

Parents begin regurgitating to pups when they are about 4-6 weeks old, and pups begin eating solids at this age. A good quality adult maintenance dog chow is considered nutritionally adequate for growing maned wolves, however, zoos may opt to offer diets formulated for growing puppies to animals under the age of 15 months. A good quality commercial puppy chow can be added to the adult diet beginning around 4 weeks of age. Gradually increase the proportion of puppy chow to a maximum of 50% by the time the pups are ~ 6 months of age, and then gradually decrease so that the youngsters are weaned over to adult chow by 1 year of age. As pups begin eating more solids, additional feeding bowls and stations should be provided. Pups can be offered small whole feed items, e.g. small mice, beginning at about 6 weeks of age. The size of whole feed items can be increased as the pups grow.

ASSISTED REPRODUCTION

The canid family presents a challenge in terms of developing techniques for artificial reproduction (a Canid TAG priority). In females, the ovaries are surrounded by a bursa that must be surgically removed in order to observe follicular development using the laparascopic techniques developed for other carnivores, e.g. felids. In general, estrous induction is not as reliable in canids as in other species, however, in recent years several protocols have been developed for use in domestic dogs. One protocol (deslorelin) has been used to successfully induce estrous in gray wolves (Asa *et al.*, 2006) and in Mexican grey wolves (Siminski, in prep). Ovulation can be predicted by measuring plasma progesterone and examining vaginal smears, although these techniques may not be practical in wild animals.

During the 1990 and 1991 breeding seasons, a number of males in the MWSSP were electroejaculated in order to obtain baseline information about semen quality. The results of the 2 studies were distributed to all participating institutions in 1991 and 1992 (D. Wildt *et al.*, unpublished report; D. Schmitt, unpublished report). A total of 12 males were electroejaculated. Semen was collected once from 10 animals, and 4 times evenly spaced over a 2-month period from the other 2 males. In general, the quality of the ejaculates obtained was lower than that observed in other canids in terms of total sperm counts and percent of structurally abnormal sperm cells, although most of the animals evaluated during the survey were spermatogenic.

Table 1 shows seminal characteristics of samples obtained from 9 of 10 wolves collected by Wildt *et al*. in 1990. Males of proven fertility (n = 6) had higher ($P < 0.05$) proportions of structurally normal sperm than unproven counterparts (n = 3). With one exception (seminal volume), there also was a strong tendency for other characteristics to be higher in wolves of proven than in unproven individuals but the difference was not significant ($P > 0.05$), likely due to small sample size.

Table 1. Seminal characteristics (mean ± SEM) of samples obtained from nine maned wolves using electroejaculation.

Seminal Characteristics	All Males (n = 9)	Proven Males (n = 6)	Non-Proven Males (n = 3)
Volume (ml)	2.0 ± 0.6	1.4 ± 0.3	3.1 ± 1.2
Sperm cell concentration ($X10^6$/ml)	29.5 ± 9.3	36.9 ± 13.2	17.1 ± 10.4
Total sperm count ($X10^6$/ejaculate)	44.4 ± 11.4	50.9 ± 17.6	33.5 ± 9.4
Sperm motility (%)	65.0 ± 6.1	71.0 ± 3.3	55.0 ± 18.2
Total motile sperm ($X10^6$/ejaculate)	29.1 ± 7.7	36.9 ± 12.4	16.0 ± 5.6
Structurally normal sperm (%)	50.1 ± 8.1	62.8 ± 10.1[a]	22.3 ± 8.7[b]

a,b, different letters between proven and non-proven male columns indicate differences ($P < 0.05$)

Recently, Songsasen *et al*. (unpublished data) collected from four wolves at 4 to 8 week intervals during 2002/2003 and 2003/2004 breeding seasons. A single semen collection was made from two of these wolves during the non-breeding season (July 2003). There was a definitive temporal change in sperm concentration throughout the breeding season, with a rise beginning in November and a peak in December followed by an immediate diminution. Other factors, including seminal volume and sperm motility/structural integrity were not affected (data not shown). No sperm or semen was produced by males during the non-breeding season. It appears that semen production in some males is positively correlated with changes in testosterone concentration through out the year (Songsasen *et al.*., unpublished data). It is worth noting that semen collection using electroejaculation technique does not affect fertility of male wolves. Two males participating in this study were subjected to electroejaculation 2 weeks prior to successful breeding; a total of 7 pups were born from these two males.

Low semen quality and urine contamination of the ejaculate are the limiting factors in the development of assisted reproductive technique such as semen cryopreservation and artificial insemination in this species.

CONTRACEPTION

See Chapter 7 – Medical Management

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Chapter 5 - Addendum I
Maned Wolf SSP Birth Survey

Date_____
Institution_____
Survey filled out by_____

Circle dam or pair where appropriate.

Date litter was born_____/_____/_____ sex ratio _____._____._____
month day year

Sire studbook#_____
Previous litters: #of pair reared___, #of dam reared___, #of hand-reared___, #eaten ____.

Dam studbook#_____
Previous litters: #of pair reared___, #of dam reared___, #of hand-reared___, #eaten____.

Most recent *vaccinations and dates:* (include brand names)
Sire:
Dam:

When was this pair first introduced?_____
Is this pair:
_____kept together all year - 24 hrs./day
_____kept together all year - separated at night
_____together during breeding season only - *dates* _____
_____together during estrus only
_____other - *describe*

"Home" enclosure measurements _____ On exhibit? Y/N

List all carnivores that are adjacent to the maned wolf exhibit, and if they have visual contact.

List all additional carnivores, including other maned wolves, within close proximity (give approx. distance), and indicate if they have visual contact.

Are the wolves in a mixed-species exhibit? If yes, list the species.

Was the sire separated from the dam for whelping? Y/N *date* _____

If the sire was separated from the dam for whelping, give *date* when he was re-introduced to participate in the pup rearing _____.

If he was not re-introduced was he allowed fence contact? Y/N *date* _____

Was the dam / pair corralled in a smaller area within the familiar enclosure for whelping? Y/N *date* _____

Was the dam / pair moved to an unfamiliar enclosure for whelping?
Y/N *date* _____

After whelping, give *date* when dam/pair were given access to a larger enclosure for pup rearing. _____

Provide *whelping pen* measurements _____ On exhibit? Y/N

***Describe* the whelping pen & pup rearing pen enclosures: (i.e., grass, trees, shelters, terrain etc.)**

How many whelping boxes were provided? #_____ length _____, width _____, height _____

After whelping, were they given access to other houses or dens boxes? Y/N *date* _____

What material was the whelping box floor made of?

Did the pups have adequate traction? Y/N

Was bedding provided? Y/N *describe*

Whelping box heat source? Y/N *describe*

Whelping box light source? Y/N *describe*

Was the whelping box temperature and / or humidity monitored? Y/N *describe*

Pregnancy confirmed by: *(check all that apply and give dates)*
_____observed copulation
_____weight gain
_____X ray
_____ultrasound
_____fecal steroid / assays
_____the birth itself
_____other - *describe*

***Describe & date* any changes in dam during pregnancy:**
appetite:
activity level:
behavior towards keepers:
behavior towards pen mate:
other:

***Describe & date* any changes in dam during lactation:**
appetite:
activity level:
behavior towards keepers:
behavior toward pen mate:
other:

***Describe & date* any changes in Sire during dam's lactation:**
appetite:
activity level:
behavior towards keepers:

behavior towards pen mate:
other:

Describe **current diet:**

diet items:
diet amounts:
feeding frequency:
supplements:
other:

Describe **diet changes and *dates* provided:**
during pregnancy: during lactation:
diet items:
diet amounts:
feeding frequency:
supplements:
other:

Was keeper routine changed prior to and following whelping? If so, note changes & when they were made.

Indications that birth was imminent: (check all that apply)
_____dam off food? *when?*
_____circling nest box
_____digging in nest box
_____in & out of the box
_____pacing
_____direct attention to hindquarters - AG licking
_____contractions
_____increased interest by male - *describe*
_____other - *describe*

How was birth observed?
_____not observed
_____direct
_____through peephole
_____through one-way glass
_____via video monitor
_____other - *describe*

Was the birth video taped? Y/N

Was there audio coverage? Y/N *describe method*

What *time* was straining first observed _____ contractions first observed? _____

What *time* was first pup born?

***Note time each pup* was born:**
1_____
2_____
3_____
4_____

***Date & time* sire was first allowed in the whelping box _____.**
Number of pups, if any, eaten by dam _____ sire _____ unknown _____

At what *time* was the dam first observed cleaning a pup? _____

At what *time* was the sire first observed cleaning a pup? _____

At what *time* was nursing first observed? _____

Give *date & time* the dam first leaves the whelping box without a pup _____ with a pup _____.
Give *date & time* the sire first leaves the whelping box with a pup _____.

***Describe & date* how often the dam / sire moved the entire family to another location in the enclosure. (for the first six weeks)**

After giving birth when was dam first offered food? *date* _____

When did dam first eat after giving birth? *date* _____

Were pups pulled for hand-rearing? Y/N *date* _____

What indicators were used to determine that hand-rearing was necessary?
_____restless behavior? *describe*
_____neglect
_____disappearance of a pup
_____traumatic injury to a pup
_____death of a pup
_____SSP recommendation
_____Zoo policy
_____other - *describe*

Reason for first human physical contact with pups: *(check all that apply & give date)*
_____sexing
_____weight
_____vaccinations
_____pulling for hand-rearing
_____other - *describe*

Give *date* when dam was first observed regurgitating to pups: _____

Give *date* when sire was first observed regurgitating to pups: _____

What would you like to change for next year's breeding season?

Other comments?

CHAPTER SIX
PUP DEVELOPMENT AND HAND-REARING PROTOCOL

GROWTH and DEVELOPMENT

The developmental information presented below is based on observations of both mother- reared (Brady & Ditton, 1979; Pithart *et al.*, 1986) and hand-reared maned wolf litters.

Eyes: Inner corners begin cracking open around Day 7-8; often accompanied by mild mucous discharge. Eyes fully open by Day 11. Pups may not see very well until 3-4 weeks of age.

Ears: Begin to open around Day 7; fully open by Day 16. Ears fully erect around Day 33.

Pelage: Dull black at birth, with white tail tip and a few white hairs under chin. Changes in fur color begin at the head and move posteriorly. The fur on the head begins to lighten around Day 7. By Day 25-30 the head and torso are grayish in color, with red fur beginning to emerge. The mane and legs remain black. The tip of the tail, under the chin and inside the ears is white. Adult coloration develops by 10 weeks, although youngsters usually remain a pale shade of red until they are adults.

Teeth: The deciduous teeth begin to erupt around Day 10, starting with the upper canines and incisors, closely followed by the lower canines and incisors. The first lower premolar appears around Day 16, followed by the first upper premolar and the 2nd lower premolar. By Day 28 the first lower molar begins to erupt. At five weeks, in addition to the incisors and canines, pups have 2 upper and 2 lower premolars (on each side) plus 1 upper and 1 lower molar. The deciduous teeth begin to shed at around 13-14 weeks of age. The incisors are lost first; permanent incisors begin to erupt at 17 weeks. Between 21-23 weeks, the canines and premolars start shedding. Adult premolars and canines begin to erupt around 24 weeks; in most cases the 4th premolar precedes the adult canines. The 2nd set of upper and lower molars also begins to appear around 24 weeks. Adult dentition is completed by 7-8 months of age displaying the typical canid pattern of 3 incisors, 1 canine, 4 premolars, 2 molars on each side of the upper jaw, and 3 incisors, 1 canine, 4 premolars, 3 molars on each side of the lower jaw.

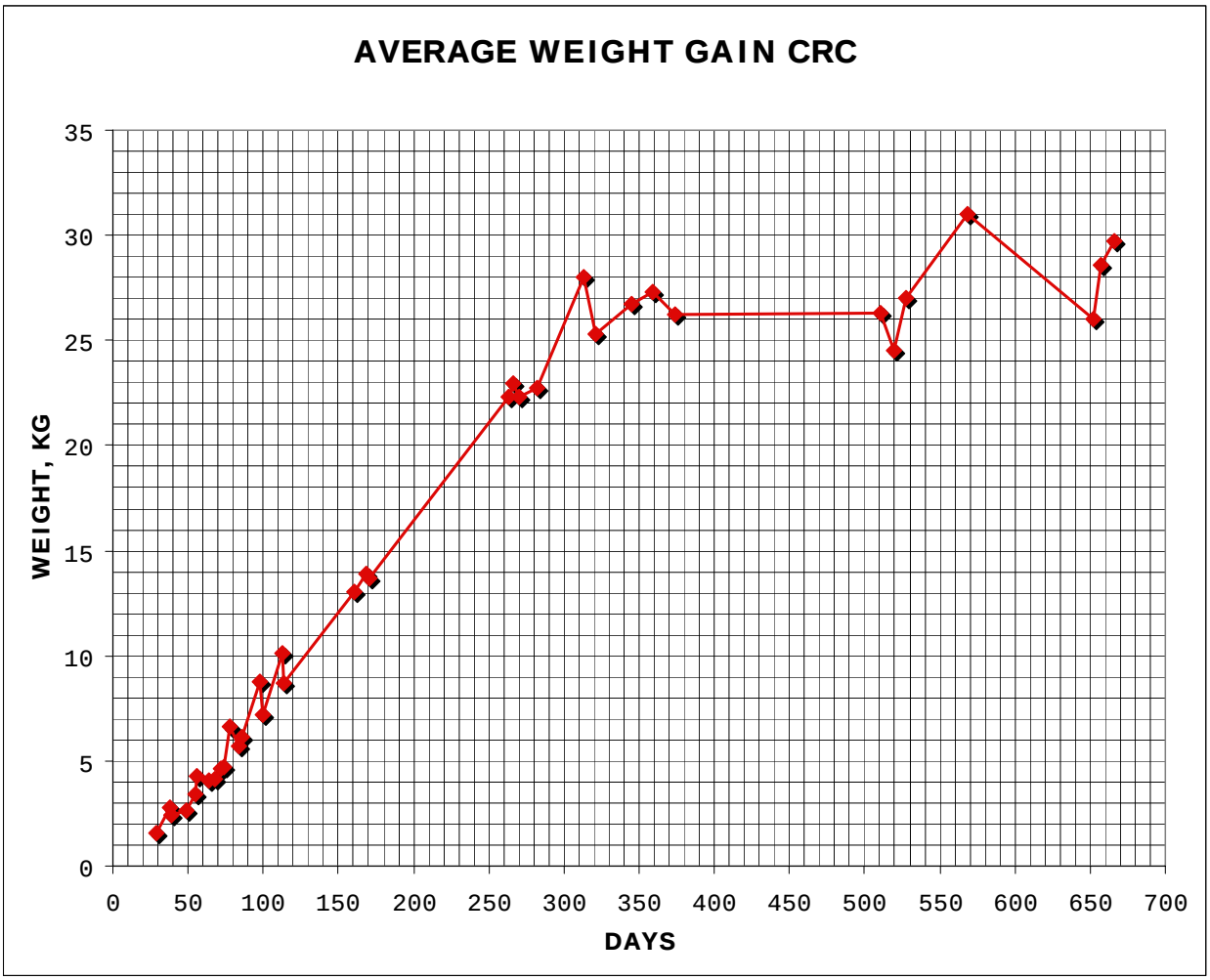
Motor Development: Pups can roll and crawl at birth. They begin to stand briefly around Day 10, and by 21-23 days pups can walk quite well. Coordination improves steadily from that point on, and by 27-28 days the pups can climb and pounce. Initially their gait is that of a walking "trot" (legs move in diagonal pairs) with the front legs between the rear when running; as their coloration matures their gait shifts to the adult pace (legs move in lateral pairs). Pups begin to regularly urinate and defecate without stimulation after the first month.

Behavior: The first play bites appear around Day 18-20. Play behavior increases as coordination improves, and includes pouncing, scruff bites, wrestling, and other typical infant canid behaviors. The period from 5-8 weeks of age is often characterized by very rough physical play, sometimes resulting in minor cuts and scratches. The intensity of play fighting decreases after 7-8 weeks of age, although pups continue to interact frequently. In hand-reared pups, a fear response (growls, attempted biting, submissive crouch) to strange humans begins between Day 27-35.

Weaning: Pups nurse every 2-3 hours for the first month. After the first 4 weeks, the frequency of nursing bouts gradually declines until pups are fully weaned at around 4 months of age (Brady & Ditton, 1979). Parents begin regurgitating food when the pups are around 4 weeks old. Both sire and dam regurgitate to pups up to 10 months of age, although Rasmussen and Tilson (1984) reported that pups aged 24-42 weeks

solicited food from the sire 3 times more frequently than from the dam. The MWSSP recommends that pups be separated from parents by 10 months of age, prior to the subsequent breeding season.

Figure 1 presents average weights for 6.5 pups (4 litters) parent-reared at NZP-CRC, 2002-2007.



HAND-REARING

BACKGROUND

When maned wolves were added to the AZA's Species Survival Plan program in 1985, hand rearing was not an immediate priority. Our knowledge at that time was based on a few published accounts describing hand-rearing procedures at institutions in North America and abroad (Acosta, 1972; Encke *et al*., 1970; Hora *et al.*, 1975; Rodden & Blakely, 1987). However, for reasons that are still unclear, the 1986-1988 breeding seasons proved disastrous with regard to both reproduction and neonatal mortality. By 1989, the age structure of the captive population of maned wolves in North America resembled an inverted pyramid, with the majority of animals approaching post-reproductive age and only a few replacements coming up through younger age classes. Our concern for the genetic and demographic implications resulting from a lack of young animals prompted a decision in 1989 to hand-rear every litter born during the next few breeding seasons in order to assure recruitment of young animals into the SSP population.

The program was extremely successful. From 1989-1994, 50 (25.25) pups in 23 litters were successfully hand-reared at 14 institutions. A hand-rearing protocol was developed by John Lewis, Director, John Ball Zoo in 1989. Several members of the MWSSP assisted in preparing updates and revisions, including John Collette (Dickerson Park), Mike Blakely (Kansas City), John Lukas (White Oak Conservation Center) and Melissa Rodden (NZP-CRC). In 1993 a video and accompanying booklet describing hand-rearing techniques and growth and development of a litter of 2.1 pups born in February 1991 was prepared by Mark Rosenthal and staff at Lincoln Park Zoo, Chicago with funding from the Institute of Museum Services. In addition to the Lincoln Park litter, the booklet also summarizes growth and feeding information for maned wolves hand-reared at six MWSSP participating institutions. The video and booklet, entitled *Maned Wolf Diaries: Growth and Development of Hand-Reared Pups* (Lincoln Park Zoological Society, 1993), was distributed to all MWSSP institutions in November 1993. Additional copies are available; contact the Species Coordinator. With Lincoln Park Zoo's permission, portions of this chapter are excerpted from *Maned Wolf Diaries*, and readers are strongly urged to consult the video and booklet to supplement the information presented here.

CURRENT HAND-REARING POLICY

The recruitment of young animals into the MWSSP resulting from the success of the hand-rearing program 1989-1994 allowed us to shift our focus away from hand-rearing toward management changes to promote successful parent-rearing of young. Only 9 puppies have been hand reared since 1995; hand-rearing recommendations have been limited to genetically valuable pairs near the end of their reproductive life span, with no or a poor history of reproduction. Additionally, hand rearing is usually recommended only if the survival of the litter is clearly in danger; parents should be given every opportunity to raise pups. The focus now is on providing breeding pairs with conditions conducive to successful parent rearing. Since January 2005, 35 litters totaling 81 pups have been successfully parent-reared at 12 institutions in the MWSSP.

Decisions about hand-rearing are made by the Management Committee on an annual basis and distributed to all institutions. Any questions about hand-rearing policy should be directed to the Species Coordinator. The Species Coordinator should be kept well-informed of all breeding or suspected breeding dates, birth dates and pup death dates. Every effort will be made to place singleton hand-reared pups with another hand-reared litter to ensure proper socialization. Both the Red wolf and Mexican grey wolf SSP programs have successfully cross-fostered pups from one litter to a parent-reared litter of similar age in recent years. Upon the recommendation of the MWSSP Management Committee, cross-fostering may be suggested as an alternative to hand-rearing should a parent-reared litter of appropriate age be available. Decisions about hand-rearing or cross-fostering will continue to be made on a case by case basis by the Management Committee.

Hand-Rearing Protocol

Monitoring: It is essential that the birth and first 7-10 days of litters recommended for hand-rearing be closely monitored for signs of trouble. In order to minimize disturbance to new parents, zoos are strongly urged to install video monitoring equipment well in advance of the birth.

Removing pups: If at all possible, do not remove newborn pups from the dam until the entire litter has been born. There is typically a 1-3 hour interval between births, although up to 8 hours is not unusual. One instance of 24+ hours from the birth of a stillborn pup to the subsequent live birth of a sibling was recorded in 1993 at Oklahoma City Zoo. It is extremely important that priority litters be closely monitored for at least the first 7 days, since trouble most commonly develops during this time. Below is a list of typical behaviors that indicate a problem may be developing. Managers must use their own judgment and knowledge of their animals to determine when the survival of a litter is seriously jeopardized.

1. Dam becomes restless; up & down frequently, in & out of den repeatedly.
2. Dam begins to persistently carry pups in & out.
3. Dam begins to lick and mouth pups frequently and persistently.
4. Dam lies apart from pups and makes no effort to pull them close to her body.
5. Pups should appear strong and vigorous. If they appear lethargic and make no attempt to move close to dam, it indicates they are getting cold and weak. Nursing activity should be observed every 2 to 3 hours (Brady & Ditton, 1979).

Immediate care: A hot water bottle or other heat source is recommended during transport from the den area to the nursery. Once at the nursery, weigh and sex each pup, take rectal temperature, and examine for injuries. Use natural markings or specific fur clip patterns to individually identify each pup. Veterinary staff should be on hand to ascertain the medical status of each individual and determine the appropriate immediate action. This may include warming the pup, administering 5% dextrose orally, electrolyte replacement, beginning a course of antibiotics, etc. Do not give oral fluids or dextrose to a pup that is cold. Warmed parenteral fluids are preferred in this case.

Passive immunity: In the domestic dog, a small amount of maternal antibodies are transferred through the placenta during fetal development. However, studies have demonstrated that this immunity is short-lived, and that domestic dogs receive the bulk of passive immunity through colostrum (Bouchard *et al.*, 1992). However, in a study of domestic dog puppies, Bouchard *et al.* found that "intestinal absorption of immunoglobulins is minimal after 12 hours and thus, another route of administration should be used." Subcutaneous injection of 16 ml of serum at birth provided the greatest increase in the hand-reared pups' serum immunoglobulins, although concentrations were lower than in the control group (pups left with dam).

The MWSSP recommends that institutions holding potential breeding pairs aseptically collect a small supply of adult maned wolf serum opportunistically prior to the birth season. Serum may be frozen for up to 6 months. A good reference for this technique in cats is Levy *et al.*, 2001. To maximize effectiveness, it is recommended that serum be administered both orally and by SQ injection: 1cc SQ when pups are pulled, followed by 5 0.5cc oral doses spaced evenly over the next 24 hours (2.5cc total). Give another 1cc serum SQ 24 hours after the last oral dose.

***Formula*:** Liquid Esbilac, domestic dog replacement formula manufactured by Pet-Ag, Inc., is the recommended formula for hand rearing maned wolves.

It is also recommended that the enzyme Lactaid (McNeil Consumer Products Co.), which aids in the digestion of milk sugars, be added to the formula according to the manufacturer's directions (~4 drops per quart added 24 hours in advance of feeding).

Formula should be diluted with sterile water or an electrolyte replacement for the first several days, e.g. Pedialyte (Ross Products Division, Abbott Laboratories). Liquid Esbilac should also be diluted by at least 50% on the first day and the concentration gradually increased up to full strength over the next 5-7 days. If powdered formula is used, it is recommended that it be prepared by weight rather than volume. Dilute to an 8-10% concentration on the first day, e.g. to make 100g of formula, mix 10g powder with 90g. sterile water. Increase the concentration by 2% per day up to the manufacturer's recommended full strength concentration of 20%. At the first sign of gastrointestinal upset, substitute or dilute formula with an electrolyte replacement until symptoms disappear.

Feeding Schedule and Amounts

First feeding: It is recommended that the first bottle offered to pups contain 5% dextrose or Pedialyte in order to safely determine whether pups can nurse and swallow effectively, thus preventing potentially fatal problems resulting from inhalation of formula. If pups nurse well, begin offering formula at the next feeding. If, however, pups do not respond or nurse correctly, tube feeding may be indicated (consult a veterinarian). Attach a size 8 French feeding tube to a 12cc syringe containing formula. Estimate the length of tube needed by measuring it from the tip of the pup's nose to the level of the last rib. Lubricate the tube with a small amount of K-Y jelly. Hold pup upright with one hand and gently guide the tube down the throat with the other hand until the predetermined distance is reached. Once tube is in place, depress the plunger slowly and steadily. If tube feeding is indicated, it is best to use no more than half the calculated volume for the first few tubings. Avoid injecting air into the stomach.

Formula feeding: It is recommended that pups be weighed at the same time each day, and the weight used to calculate the amount of formula to be offered over the next 24 hours. Offer each pup 20-25% of its body weight spaced evenly over the 24 hour period. Avoid sudden jumps in amounts fed from one feeding to the next. Feedings every 3 hours are recommended for the first 5-7 days; the number of feedings/day can then be gradually reduced. For example, 3 week old pups should be fed about 5 times per day. Table 1 presents feeding schedules and amounts fed for one representative hand reared litter. Please refer to Lincoln Park Zoo's *Maned Wolf Diaries* for additional feeding information.

Several zoos have successfully used human preemie nipples for very young pups. Once the pups are about 10 days old, the nipple holes can be enlarged or the preemie nipple replaced with a regular human infant nipple. Young pups need to be stimulated to urinate and defecate. Keepers can emulate mother's tongue by gently rubbing the anogenital area with moistened cotton immediately before and after each feeding. Pups begin to occasionally urinate and defecate on their own as early as 10 days, although manual stimulation should be continued through the first month.

Solids should be introduced relatively early, at about 20 days of age, to prevent cataracts and enhance coat development (Vainisi *et al.*, 1981). Small amounts of ground puppy chow, eg. Purina ProPlan (Ralston Purina), Science Diet Growth (Hill's Pet Nutrition, Inc.), commercial brand beef baby food, and pureed calf liver have all been successfully added to bottled formula.

***Weaning*:** The MWSSP strongly recommends pups be weaned to a commercial brand of puppy chow; chicken based brands are preferred to soy based because they are more easily digested. Wean pups gradually beginning around 4 weeks of age. It has been noted that pups will frequently begin to resist taking a bottle when they are between 3 and 4 weeks old. Although there is considerable variation in the procedures used successfully to wean pups, generally speaking nursery staff begins by offering pups a small bowl of ground or whole puppy chow soaked in water or diluted formula once or twice a day. Many pups adjust to the change more easily if the gruel mixture is presented on keeper's fingers. Most pups are observed lapping water at 30-40 days of age; discontinue formula by the time pups are 5-6 weeks old. Offer pups free choice dry puppy chow along with chow soaked in water. Weight and hydration should be monitored daily to ensure that pups are ingesting adequate amounts of solids and liquids. Pups usually begin to prefer the dry chow by 7-8 weeks of age.

Housing: Care should be taken at all times to isolate young pups from exposure to canid disease agents, including keepers' or nursery staffs' pet dogs. It is recommended that newborn pups be housed in an incubator maintained at 85°F (29.4°C) and 50% humidity. Higher temperatures and/or humidity may result in fur loss. Cotton sheets or towels provide comfortable bedding while reducing the potential for snagging claws or ingesting material. Pups may be removed from incubator by 3 weeks of age; during the final week, begin reducing the incubator temperature to room temperature.

Once removed from the incubator, house pups in an area large enough to allow adequate physical activity. Non-abrasive, non-slip, easily-cleaned surfaces are recommended. Provide one or more shelters, e.g. small boxes or carry-all crates, and a cloth-covered hot water bottle for comfort. Heating pads are not recommended because of the potential electrical hazard.

Immunizations: All maned wolf pups should be vaccinated against canine distemper, parvovirus and rabies (in rabies endemic areas).

Canine Distemper: AAZV's Distemper Vaccine Subcommittee recommends using Merial's PUREVAX® Ferret Distemper recombinant canary pox vector vaccine for all exotic carnivore species that are susceptible to the canine distemper virus.

Schedule: Pups should be vaccinated every three (3) weeks, beginning at six (6) to eight (8) weeks of age and ending at 16 weeks. Vaccinate again at six (6) months, and check the titer at this time. Annual boosters are recommended. Vaccine should be given IM, not SC, for increased effectiveness. Titers are generally lower with the recombinant vaccines; titers below 100 do not necessarily indicate lack of protection. We are trying to gather information on appropriate titers; please send any information to the Maned Wolf SSP Veterinary Advisor. Cornell Veterinary Medical/Diagnostic Laboratory, Upper Tower Rd. Ithaca, NY 14853 is the recommended laboratory for both distemper and parvo titers. Ph: (607) 253-3900. Website: http://diaglab.vet.cornell.edu.

Parvovirus: Since 1992, three zoos have reported cases of parvo-like syndrome in pups post-vaccination with Fort Dodge's modified-live KF-11 (see Backues, 1994), therefore the **MWSSP DOES NOT recommend the use of modified-live products on pups less than six (6) months of age or any individual with a low titer (<1:80)**. As of 2006, killed canine parvovirus vaccine is no longer being manufactured. Thus, the current recommendation is to initially vaccinate maned wolf pups with a killed feline parvo vaccine, such as Fel-O-Vax® PCT. Initial results using Fel-O-Vax® PCT are reported in Addendum I at the end of Chapter 7.

Schedule: Pups should be vaccinated with a killed product, e.g. Fel-O-Vax® PCT by Ft Dodge, every two (2) to three (3) weeks beginning at six (6) weeks of age and ending at 16 weeks (follow manufacturer guidelines). Do a follow up titer and send results to veterinary advisor.

- If titer is acceptable (> 1:80), begin using a modified-live product, such as Duramune® Max PV by Ft. Dodge, at six (6) months of age and continue using MLV product every six (6) months.
- If titer is unacceptable (<1:80), continue using killed product at 6 months of age, and check titer again. Switch to MLV product at one (1) year of age (if titer is >1:80) and continue immunizing adults annually with MLV.

It is important to remember that killed vaccine does not provide good protection. Every effort should be made to minimize pups' potential for exposure to infectious disease.

Rabies: Maned wolves exhibited in rabies-endemic areas should be vaccinated with a killed product.

Schedule: Pups should be immunized at 12 weeks, one (1) year and annually thereafter.

Please refer to Chapter 7 - MEDICAL MANAGEMENT, for additional information about immunizations

Reporting Recommendations: Daily logs should be maintained regarding feeding schedules, amounts offered and consumed, feeding methods (e.g. bottle, bowl), and weight gain. Complete records of all medical procedures should be kept. Clear and concise records of physical and behavioral development should be maintained.

1. General Information
   - Pups' ISIS #s, sex, birthdate, parents' studbook numbers.
   - Date pulled and reason for pulling.

2. Feeding Schedule
   - Formula used; dilution factor; additives, eg. Lactaid.
   - Average daily food consumption summarized by weeks.
   - Number of feedings per day summarized by weeks.
   - Type of nipple used, and any changes.

3. Weaning Process
   - When solids introduced.
   - Method by which solids were introduced.
   - What solids and in what order were they introduced.
   - When were bottles discontinued.
   - When was formula discontinued.

4. Development
   - Weekly weights.
   - Age eyes and ears opened.
   - Age and description of changes in pelage.
   - Pattern of tooth eruption.
   - Motor development: when standing, walking, running etc.
   - Behavioral changes: e.g. age when fear of strangers noted; development of play behavior; appearance of uninhibited fighting between littermates.
   - Vocalizations: age when whine, growl, bark first noted.

5. Housing
   - Use of incubator (temp, rel. humidity)
   - Age when removed from incubator.
   - Description of housing after incubator: flooring, heat source, "nest box".
   - Age when put on exhibit.

6. Medical
   - Description of any medical treatments required.
   - Dates and brand names of vaccinations.

Table 1. Summary of growth and feeding for a litter of 1.1 maned wolf pups hand-reared at NZP-CRC.

Stbk #1351 & #1352 born 29.Dec.91 to Sire #476 and Dam #368 were pulled approximately 6 hours after birth due to maternal trauma.
Formula = Esbilac powder mixed with boiled water. Formula was prepared by weight, not volume, beginning with an 8% solution (e.g. 8g powder in 92g water). The concentration was increased by 2% each day up to 20%. Stool quality was monitored closely. All feeding equipment was sterilized until pups were removed from the incubator at 21 days.
Date presented below are MEANS for the 2 pups:

Age (Days)	Weight (kg)	No. Feed per day	Formula Conc.(%)	Tot. Form. EATEN(cc)	Solid Food INTAKE
1	.3395	8	8	34	
7	.3725	8	20	84	
14	.6885	7	20	147	Start calf liver homogenate 3X/dy, beginning with .75cc/dy increasing by .5cc/day.
18					Begin to resist bottle. Switch from Preemie to Evenflo nipple.
21	1.075	6	20	209	3cc Liver; Begin intro GRUEL = ground Science Growth mixed w/ formula.
28	1.575	4	20	266	8cc Liver; ~2.5tsp Gruel + ~ pinkie mouse.
35	2.150	3	<20	355	8cc Liver; ~4.5tsp Gruel + 2 pinkie mice.
39					STOP Bottles.
42	2.475	3	<10	135	6cc Liver;~14 Tbsp Gruel + 1 pinkie. ** Gruel: substitute water for formula.
44					STOP Formula; STOP Liver
49	2.940	2		0	172g Gruel + 1 "fuzzy" mouse.
51					Switch to DRY Science Diet Growth Chow
56	3.899	2			144g chow + 1 mouse

Table 2. Maned wolf growth rates for 5 hand-reared litters.

WEIGHTS in KG

Age (wks)	Litter #1	Litter #2	Litter #3	Litter #4	Litter #5
<1	.3917 (Day 2)	.3397 (Day 2)	.4565 (Day 1)	.353 (Day 1)	.393 (Day 1)
1	.5300	.3540	.660	.571	.528
2	.8467	.4823	1.15	.862	.690
3	1.29	.6753	1.725	1.279	1.016
4	1.87	1.03	2.225	1.845	1.582
5	2.36	1.417	2.55	2.90 (Day 39)	2.50
6	2.91	1.80	3.10	3.40 (Day 45)	---
7	3.57	2.40	3.425	---	3.6 (Day 53)
8	4.30	3.05	3.90	4.80 (Day 59)	---
9	4.90	3.73	4.40	5.50 (Day 67)	5.0 (Day 67)
10	5.76	4.57	5.28	---	---
11	6.27	4.85	5.80	6.50	5.7 (Day 75)
12	7.12	5.48	6.25	---	---

Litter #1: 3.0 born 17.Dec.1990 at Woodland Park Zoo.
Litter #2: 2.1 born 19.Feb.1991 at Lincoln Park Zoo
Litter #3: 0.2 born 31.Dec.1992 at NZP-CRC
Litter #4: 1.0 born 25.Dec.2005 at Buffalo Zoo
Litter #5: 1.0 born 24.Dec.2006 at Buffalo Zoo

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CHAPTER SEVEN
MEDICAL MANAGEMENT

PRESHIPMENT

Complete medical history and diet information should be received and reviewed by receiving institution's veterinary staff prior to shipment. Preshipment examination should include a complete physical examination, CBC/chemistry panel, fecal examination, urinalysis, heartworm test if in heartworm endemic area, thoracic and abdominal radiographs. In addition, the animal should be current on all recommended vaccinations (see below). The wolf should be permanently identified with a tattoo of the studbook number on the inner thigh (left for female, right for male) or via transponder chip inserted to the left of the midline in the shoulder area. Since the compatibility between microchip systems is still evolving, a specific vendor is not currently recommended. However, microchip ID # and vendor information should be reported to the studbook keeper and entered into the animal's institutional records.

QUARANTINE

Quarantine should be a minimum of 30 days on an all-in-all-out basis. It is recommended that release from quarantine is contingent on at least two negative fecal examinations one week apart. Any deficits in the preshipment recommendations should be corrected during quarantine. Vaccination history should be carefully reviewed and re-vaccination considered if history is questionable.

PREVENTIVE MEDICINE

The following guidelines are specifically for maned wolves and are in addition to the comprehensive preventive medicine program for all collection animals.

Parasite Monitoring

Fecal exams (flotation and direct) should be done at least semi-annually. Internal parasites are only occasionally seen. These include ascarids, trichuris, cestodes, and strongyles. Standard anthelmintics at canine dosages have been successfully used. A re-check fecal examination should be done two weeks post treatment.

Ectoparasites seen include ear mites, fleas, flies, and occasionally ticks. Again, standard canine dosages of domestic animal products and environmental treatment have been successfully used for ectoparasite control.

All maned wolves in heartworm endemic areas should be tested annually and placed on prophylaxis using ivermectin or milbemycin at standard canine dosages.

Vaccinations

Maned wolves are vaccinated annually against canine distemper, parvovirus and rabies (in rabies endemic areas). Vaccines are preferably administered by hand to adults during the annual physical exam.

Modified-live vaccines (MLV) are typically preferred over killed vaccines because they produce a bigger and longer-lasting immune response. However, there have been several reports of vaccine-induced illness in maned wolves (Backues, 1994; Thomas-Baker, 1985). Thus, it is important to use a killed or recombinant canary pox vector vaccine on naïve/young animals until their immune response is acceptable (as indicated by adequate

titers). Also, vaccinating with only one antigen at a time may help reduce the chance of an adverse vaccine reaction (e.g., administer rabies/distemper/parvo vaccines separately).

We are gathering information on appropriate vaccinal titers in maned wolves (See **Addendums IV and V** for more information). The recommended laboratory for **distemper** and **parvo** titers is Cornell Veterinary Medical/Diagnostic Laboratory, Upper Tower Rd. Ithaca, NY 14853, phone number (607) 253-3900, website http://diaglab.vet.cornell.edu. Kansas State Veterinary Diagnostic Laboratory runs **rabies** titers, address: 1800 Denison Avenue, Manhattan, KS 66506, 866-512-5650. Website: http://www.vet.ksu.edu/depts/dmp/service/rabies/index.htm. Please send titer results to the Maned Wolf SSP Veterinary Advisor.

Canine Distemper AAZV's Distemper Vaccine Subcommittee recommends using Merial's PUREVAX® Ferret Distemper recombinant canary pox vector vaccine for all exotic carnivore species that are susceptible to the canine distemper virus. "Because of its high demonstrated safety record and inability to induce canine distemper as with earlier attenuated vaccines, any possible risks are considered minimal. However, as with any off-label use of a biological product, the vaccine should be given with the discretion of the user. Users are also encouraged to obtain pre-and post-vaccinal titers where possible" and to report any new observations with this product to the maned wolf veterinary advisor on any issues.

Schedule: Pups should be vaccinated every three (3) weeks, beginning at six (6) to eight (8) weeks of age and ending at 16 weeks. Vaccinate again at six (6) months, and check the titer at this time. Annual boosters are recommended. Vaccine should be given IM, not SC, for increased effectiveness. Titers are generally lower with the recombinant vaccines; titers below 100 do not necessarily indicate lack of protection.

Parvovirus It is recommended that adult maned wolves with an acceptable titer be vaccinated annually with a modified-live product such as Duramune® Max PV by Fort Dodge. Semi-annual vaccination should be considered in high-risk areas. Since 1992, three zoos have reported cases of parvo-like syndrome in pups post-vaccination with Fort Dodge's modified-live KF-11 (see Backues, 1994). Therefore the **MWSSP DOES NOT recommend the use of modified-live products on pups less than six (6) months of age or any individual with low titers (<1:80).** As of 2006, killed canine parvovirus vaccine is no longer being manufactured. Thus, the current recommendation is to initially vaccinate maned wolf pups with a killed feline parvo vaccine, such as Fel-O-Vax® PCT. Initial results using Fel-O-Vax® PCT are reported in **Addendums I and V** at the end of this chapter.

Schedule: Pups should be vaccinated with a killed product (e.g. Fel-O-Vax® PCT by Ft Dodge) every two (2) to three (3) weeks beginning at six (6) weeks of age and ending at 16 weeks (follow manufacturer guidelines). Do a follow up titer and send results to veterinary advisor.

- If titer is acceptable (> 1:80), begin using a modified-live product (e.g. Duramune® Max PV by Ft. Dodge) at six (6) months of age and continue using MLV product yearly.

- If titer is unacceptable (<1:80), continue using killed product at 6 months of age, and check titer again. Switch to MLV product at one (1) year of age (if titer is >1:80) and continue immunizing adults annually with MLV.

Every effort should be made to minimize pups' potential for exposure to infectious disease.

Rabies Maned wolves exhibited in rabies-endemic areas should be vaccinated with a killed product.

Schedule: Pups should be immunized at 12 weeks, one (1) year and annually thereafter. Many states require that domestic canines be vaccinated once every 3 years. However, since "adequate" titers to prevent rabies infection in maned wolves are not known, annual boosters are recommended until more information is available about titer response to killed rabies vaccine in maned wolves. Serology may help determine how often an individual needs a rabies vaccine booster (Kansas State University). For more information on Rabies vaccination visit: http://www.avma.org/issues/policy/rabies_control.asp.

Other One case of a fatal **adenovirus** outbreak in a litter of pups was reported in 1994. Efficacy and side effects of the available adeno vaccines (modified-live) are unknown so no recommendations can be made at this time.

In areas where diseases such as **leptospirosis, Lyme disease or coronavirus** are common, managers may want to consider immunizations; however, the efficacy and safety of the available products are unknown at this time.

ANESTHESIA

Two commonly used anesthesia regimens are a ketamine/xylazine combination (approx. 6-8 mg/kg ketamine and 1.1 mg/kg xylazine) or telazol (approx. 3-6 mg/kg). Telazol gives a smooth induction and a relaxed state of anesthesia, although recoveries may be prolonged. Xylazine is reversible, but it should be avoided in compromised animals.

Another protocol that has been used with favorable results is Medetomidine (0.03 mg/kg) + Butorphanol (0.2mg/kg) + Midazolam (0.1mg/kg); reverse with atipamezole (five times the medetomidine dose) and naltrexone (5 times the butorphanol dose) +/- flumazenil. The advantage of this protocol is that it is completely reversible and provides for a quick recovery. It is a matter of personal preference which combination is used.

Supplemental anesthesia with isoflurane or sevoflurane is preferred. Routine monitoring during anesthesia and recovery is essential.

ANNUAL PHYSICAL EXAMINATION

It is recommended that animals undergo an annual physical examination. Blood is collected for CBC/chemistries and serum banked. Since anemia has been reported, a reticulocyte count may be indicated. ISIS 2002 reference values are given in Tables 1 and 2. Thoracic and abdominal radiographs are taken. Teeth are examined, scaled and polished as needed. The animal is weighed; average adult male weight is 31 kg, average adult female weight is 30 kg. Maned wolves can be trained to stand on a scale for more frequent weighing. Urine is collected and analyzed, including pH and microscopic exam for cystine crystals. Abdominal ultrasonography is another method that can be utilized to screen for kidney and bladder stones.

A urine sample should be collected for cystinuria screening. Place the sample in a cryovial or other sterile, non-breakable container. Label the sample with the animal's studbook number and mail sample with an accompanying sheet of information including date, species, Stbk #, gender, birth date, and return address to Paula Henthorn at:

Dr. Paula Henthorn
University of Pennsylvania School of Veterinary Medicine
Section of Medical Genetics
3900 Delancey Street
Philadelphia, PA 19104-6010
Tel: (215) 898-9601
Fax: (215) 573-2162
e-mail: henthorn@vet.upenn.edu

Thyroid panel database for maned wolves:

Dr. Scott Jacques at Texas Veterinary Medical Diagnostic Lab (TVMDL 979-845-3414) is helping collect data on thyroid levels in maned wolves. When submitting serum (1-2 ml), please include the following information: "these samples are for the maned wolf thyroid database as per Dr. Hammond, MWSSP Veterinary Advisor." Please forward results to Dr. Hammond.

As samples are collected, please note if the animal was sick (or showing signs of hypothyroidism, etc) or healthy during the sample collection. If known, please note the reproductive history, i.e. paired but no breeding or pregnancy success vs. successful litter.

CONTRACEPTION

The Maned Wolf SSP recommends following the guidelines for large canids developed by the AZA's Wildlife Contraception Center, http://www.stlzoo.org/animals/scienceresearch/contraceptioncenter/ . The guidelines have been included in the revised *AZA Standardized Animal Care Guidelines for Large Canids* (in prep), excerpted here:

The development of safe, reversible methods for contracepting over-represented animals is a research priority for the AZA's Canid TAG. The progestin-based melengestrol acetate (MGA) implant, previously the most widely used contraceptive in zoos, has been associated with uterine and mammary pathology in large felids (Munson 1993), and these side effects are also likely to occur in other carnivores. Instead, the AZA Wildlife Contraception Center recommends GnRH agonists [e.g., Suprelorin® (deslorelin) implants, leuprolide acetate injectable implants, or Lupron®] as safer alternatives. However, dosages and duration of efficacy have not been well established for all species. The GnRH agonists can be used in either females or males, and side effects are generally those associated with gonadectomy, especially weight gain, which should be managed through diet.

Following is general information on contraceptive options for canids. More details and ordering information can be found at http://www.stlzoo.org/contraception.

Ovariohysterectomy: Ovariohysterectomy of females is the safest method for long-term control of reproduction for large canids that are eligible for permanent sterilization. Permission for permanent sterilization must be granted by the program managers and/or the Maned Wolf SSP Coordinator.

Vasectomy: Vasectomy of males will not prevent potential adverse effects to females that can result from prolonged, cyclic exposure to the endogenous estradiol and progesterone associated with the pseudo-pregnancy that follows all spontaneous ovulations in canids. This approach is **not** recommended.

Gonadotropin releasing hormone (GnRH) agonists [Suprelorin® (deslorelin) implants, leuprolide acetate injectable implants, or Lupron®]: GnRH agonists achieve contraception by reversibly suppressing the reproductive endocrine system, preventing production of pituitary (FSH and LH) and gonadal hormones (estradiol and progesterone in females and testosterone in males). The observed effects are similar to those following gonadectomy but are reversible. They first stimulate the reproductive system, which can result in estrus and ovulation in females or temporary enhancement of testosterone and semen production in males. Then, down-regulation follows the initial stimulation. The stimulatory phase can be prevented in females by daily Ovaban administration for one week before and one week after implant placement.

GnRH agonists should not be used during pregnancy, since they may cause spontaneous abortion or prevent mammary development necessary for lactation. They may prevent initiation of lactation by inhibiting progesterone secretion, but effects on established lactation are less likely. New data from domestic cats have shown no effect on subsequent reproduction when treatment began before puberty.

Monitoring the efficacy of GnRH agonists in females can be done by noting the suppression of estrous behavior or by measuring gonadal steroids in feces. Suprelorin® has been tested primarily in domestic dogs and cats, whereas leuprolide acetate and Lupron® have been used primarily in humans, but they should be as effective as Suprelorin®, since the GnRH molecule is identical in all mammalian species.

Although GnRH agonists can also be an effective contraceptive in males, monitoring effectiveness is more challenging because it requires regular semen collections to ensure the absence of sperm. If this contraceptive is used in males, disappearance of sperm from the ejaculate following down-regulation of testosterone may take an additional 6 weeks, as with vasectomy. It should be easier to suppress the onset of spermatogenesis in seasonally breeding species, but that process begins at least 2 months before the first typical appearance of sperm. Thus, treatment should be initiated at least 2 months before the anticipated onset of breeding.

Progestins: Melengestrol acetate (MGA) implants were previously the most commonly used method. Other synthetic progestins include Depo-Provera® (medroxyprogesterone acetate) injections and Ovaban® (megestrol acetate) pills. Although MGA has proven effective in canids, possible side effects include uterine and mammary disease, in addition to weight gain and symptoms of diabetes mellitus. Other progestins are also very likely to cause these same side effects, although data are not available for them all. Because estradiol seems to synergize with progestins to exacerbate deleterious effects on uterine and mammary tissue, progestin treatment should never be initiated during proestrus, a time when endogenous estradiol is elevated. In the gray wolf, proestrus (based on blood in vaginal smears) begins an average of 6 weeks before estrus. This means that for some individual females, estradiol may be elevated as much as 2 months or more prior to what is considered the beginning of the breeding season. The ideal time to begin progestin administration is during deep anestrus.

If progestins must be used, they should be administered for no more than 2 years and then discontinued to allow for a pregnancy. Discontinuing progestin contraception and allowing a non-pregnant cycle does not substitute for a pregnancy. In fact, non-fertile cycles are more likely to exacerbate deleterious effects, since both estradiol and progesterone are elevated during estrus, and ovulation is followed by hormonal pseudo-pregnancy with high progesterone. Use of progestins for more than a total of 4 years is not recommended. MGA implants last at least 2 years, and clearance of the hormone from the system occurs rapidly after implant removal. Progestins are considered safe to use during lactation.

Androgen: Mibolerone is a synthetic androgen in pill form that is approved for female dogs. However, it may stimulate aggressive behavior, so is **not** recommended.

Vaccines: The porcine zona pellucida (PZP) vaccine may cause permanent sterility in canids after only one treatment, due to a cellular response causing depletion of oocytes. This approach is **not** recommended.

Physical separation may be the safest form of contraception provided there is space at the facility. Any contraception, whether permanent or temporary should be reported to the SSP Coordinator and the veterinary advisor.

FIELD SURVEY RECOMMENDATIONS

If the opportunity arises to examine wild maned wolves, the following is a suggested protocol for sample collection provided by Dr. Sharon Deem:

Anesthesia data
PE including morphometrics
Ectoparasites for identification
Feces for endoparasites
Cystocentesis for U/A, immediate microscopic examination of sediment for cystine crystals and
    Dioctophyma renali ova
Whole blood for CBC and parasite examination
Serum for chemistry profile and thyroid panel (see above)
RBCs for genetics and PCR (cells that are centrifuged to the bottom after spinning down the blood)
Serum banked for nutritional profile
Serology for canid panel;
    Dirofilaria immitis

Toxoplasmosis
Rabies
Canine adenovirus
Canine herpesvirus
Canine distemper
Canine parvovirus
Canine brucella
Coronavirus
Leptospirosis (17 serovars)

Please share any data obtained with the Maned Wolf SSP Veterinary Advisor.

REPORTED DISEASES

Cystine calculi, both in the kidney and bladder are commonly reported. Maned wolves may have a genetic predisposition to developing cystinuria as evidenced by the presence of cystine in the urine of wild maned wolves. Dietary factors may also contribute to the disease (research is ongoing). Many institutions have treated with Thiola (tiopronin), U/D diet and urine alkalinization (potassium citrate [Urocit-K] 62-125 mg/kg SID-BID) with varying success. Urethral obstruction due to cystine calculi does occur, particularly in males. This may present as an animal straining (like constipation) and is an emergency situation. Surgery may be indicated. Untreated urethral obstruction can lead to bladder rupture and death.

All maned wolves should be regularly screened for the presence of cystinuria.

Dermatitis has been reported at several institutions. These included *Staph. aureus* pyoderma, and more commonly, acute moist dermatitis. Some of the dermatitis seen seems to have an allergic basis, possibly related to exhibits containing tall, damp grass. This type of dermatitis usually responds to judicious use of corticosteroids and/or antihistamines. Atopy has been implicated in several maned wolves with dermatitis. Changing the diet to a novel protein source seems to have helped alleviate the problem. An interdigital fungal dermatitis that is non-responsive to antibiotics and steroids has been reported. This condition has responded well to systemic anti-fungals such as ketoconazole or itraconazole.

Virus: Several occurrences of **canine parvovirus** have been reported in maned wolves (see Fletcher *et al.*, 1979; Mann *et al.*, 1980; Montali & Kelly, 1989). Vaccine-related parvoviral-type syndrome was reported by Backues (1994).

Vaccine-induced **canine distemper** was reported by Thomas-Baker (1984) in one litter of captive-born pups.

One case of **adenovirus** occurred in 1994 in a litter of 1.2 5-month-old parent-reared pups. The two female pups died as a result of the infection; the male recovered.

Traumatic injuries reported include footpad lacerations, long-bone fracture caused by darting and other fractures with unknown history.

Idiopathic degenerative neuropathy and/or myopathy have been seen in two related individuals.

Proliferative gingivitis with associated tooth loss in all age groups has been reported.

Damage to or destruction of the right kidney due to the giant kidney worm (*Dioctophyma renale*) has been seen in individuals imported from the wild. It is seen in individuals fed raw fish, the intermediate host of the parasite. However this disease has never been reported in maned wolves captive born in North America.

Neoplasia: Ovarian tumors, especially dysgerminomas, are found quite frequently in adult female maned wolves (Munson & Montali, 1991) and Sertoli cell tumors have been seen in males. ***Fibrosarcomas*** have been seen in multiple older animals.

Hypothyroidism has been successfully treated in one female maned wolf. The main clinical sign was an unthrifty hair coat. Poor reproductive performance may be another sequela. The SSP is currently working on baseline thyroid values for this species (see **Addendum VI**).

Adverse drug reaction: One suspected case of bone marrow suppression thought to be induced by the antibiotic **trimethoprim/sulfadiazine (TMS)** occurred in 1992. A report prepared by Mitchell Bush, DVM was distributed to all MWSSP participants in February 1993, (see **Addendum II** at end of chapter).

In 2004, Frank Ridgley, DVM (Buffalo Zoo) reported adverse reactions from the use of metronidazole (see **Addendum III**).

NECROPSY REPORT

The death of any maned wolf should be reported to the MWSSP Coordinator immediately. A complete necropsy should be performed following the **MWSSP Necropsy Protocol (Chapter 8)**. Completed necropsy reports should be sent to the Veterinary and Pathology Advisors who will then forward copies to the SSP coordinator.

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TABLE 1. ISIS 2002 Clinical Pathology Reference Values for Maned Wolves < 2 years old.

Reference Ranges for Physiological Data Values

Test	Units	Mean	St. Dev.	Minimum Value	Maximum Value	Sample Size[a]	Animals[b]
WHITE BLOOD CELL COUNT	*10^3/µl	12.56	3.998	4.700	24.60	193	61
RED BLOOD CELL COUNT	*10^6/µl	4.87	0.76	3.35	7.36	169	52
HEMOGLOBIN	g/dl	12.3	2.4	6.6	19.3	172	53
HEMATOCRIT	%	37.2	6.6	22.1	58.5	202	61
MCV	fL	76.0	7.2	60.0	92.3	167	52
MCH	pg/cell	25.4	3.0	12.9	32.5	165	50
MCHC	g/dl	33.5	3.2	18.9	46.8	172	53
PLATELET COUNT	*10^3/µl	252	106	95	690	29	17
NUCLEATED RED BLOOD CELLS	/100 WBC	2	1	1	7	21	14
RETICULOCYTES	%	1.4	0.6	0.5	2.0	5	2
SEGMENTED NEUTROPHILS	*10^3/µl	8.767	3.335	1.450	23.10	178	54
LYMPHOCYTES	*10^3/µl	2.909	1.296	0.778	8.260	181	56
MONOCYTES	*10^3/µl	0.454	0.408	0.053	3.937	162	53
EOSINOPHILS	*10^3/µl	0.645	0.466	0.055	3.092	176	56
BASOPHILS	*10^3/µl	0.106	0.108	0.000	0.381	33	20
NEUTROPHILIC BANDS	*10^3/µl	0.220	0.555	0.000	3.540	45	23
ERYTHROCYTE SEDIMENTATION RATE	mm/Hr	0	0	0	0	5	2
CALCIUM	mg/dl	10.1	0.7	8.3	12.5	187	58
PHOSPHORUS	mg/dl	8.8	2.2	3.7	12.7	140	48
SODIUM	mEq/L	144	4	132	156	141	50
POTASSIUM	mEq/L	5.0	0.6	3.8	6.9	143	52
CHLORIDE	mEq/L	111	3	103	119	125	43
BICARBONATE	mEq/L	20.0	2.8	18.0	22.0	2	2
CARBON DIOXIDE	mEq/L	19.1	3.5	15.0	26.0	13	8
OSMOLARITY	mOsmol/L	298	20	275	322	4	3
IRON	µg/dl	130	52	47	278	24	4
BLOOD UREA NITROGEN	mg/dl	17	6	5	44	186	60
CREATININE	mg/dl	0.8	0.3	0.2	1.6	115	51
URIC ACID	mg/dl	0.2	0.2	0.0	0.6	34	23

TOTAL BILIRUBIN	mg/dl	0.3	0.1	0.1	0.8	120	53
DIRECT BILIRUBIN	mg/dl	0.0	0.1	0.0	0.3	40	10
INDIRECT BILIRUBIN	mg/dl	0.2	0.1	0.1	0.4	28	7
GLUCOSE	mg/dl	125	21	75	190	189	58
CHOLESTEROL	mg/dl	321	81	142	497	156	46
TRIGLYCERIDE	mg/dl	66	43	16	215	130	36
CREATINE PHOSPHOKINASE	IU/L	562	327	44	1630	71	21
LACTATE DEHYDROGENASE	IU/L	153	119	42	567	72	32
ALKALINE PHOSPHATASE	IU/L	297	231	10	2046	189	59
ALANINE AMINOTRANSFERASE	IU/L	49	26	12	180	177	55
ASPARTATE AMINOTRANSFERASE	IU/L	42	11	16	96	186	60
GAMMA GLUTAMYLTRANSFERASE	IU/L	5	2	0	12	71	28
AMYLASE	U/L	314	214	118	1949	117	36
LIPASE	U/L	129	0	129	129	1	1
TOTAL PROTEIN (COLORIMETRY)	g/dl	5.6	0.7	4.0	7.7	185	57
GLOBULIN (COLORIMETRY)	g/dl	2.6	0.4	1.8	3.9	116	45
ALBUMIN (COLORIMETRY)	g/dl	3.0	0.5	2.0	4.5	116	45
FIBRINOGEN	mg/dl	96	105	0	300	46	15
Body Temperature:	°F	100.8	1.6	96.8	104.0	56	35

[a] Number of samples used to calculate the reference range.
[b] Number of different individuals contributing to the reference values.

TABLE 2. ISIS 2002 Clinical Pathology Reference Values for Maned Wolves > 2 years old.

Reference Ranges for Physiological Data Values

Test	Units	Mean	St. Dev.	Minimum Value	Maximum Value	Sample Size[a]	Animals[b]
WHITE BLOOD CELL COUNT	*10^3/µl	9.133	3.371	3.700	25.90	362	92
RED BLOOD CELL COUNT	*10^6/µl	5.76	0.88	3.37	9.73	301	82
HEMOGLOBIN	g/dl	14.3	1.8	9.0	19.3	279	83
HEMATOCRIT	%	42.9	5.6	25.6	68.5	377	97
MCV	fL	74.8	7.4	42.1	109.0	299	82
MCH	pg/cell	25.4	2.3	16.7	34.4	272	79
MCHC	g/dl	33.4	2.1	21.9	45.6	277	83
PLATELET COUNT	*10^3/µl	205	74	78	435	71	36
NUCLEATED RED BLOOD CELLS	/100 WBC	1	1	0	3	26	19
RETICULOCYTES	%	1.3	1.0	0.0	2.7	10	7
SEGMENTED NEUTROPHILS	*10^3/µl	6.475	2.822	0.083	19.90	336	86
LYMPHOCYTES	*10^3/µl	1.751	0.789	0.005	4.500	342	89
MONOCYTES	*10^3/µl	0.278	0.252	0.000	2.049	300	87
EOSINOPHILS	*10^3/µl	0.609	0.447	0.000	3.120	308	83
BASOPHILS	*10^3/µl	0.114	0.132	0.000	0.582	45	33
NEUTROPHILIC BANDS	*10^3/µl	0.520	0.931	0.000	6.210	89	43
ERYTHROCYTE SEDIMENTATION RATE	mm/Hr	1	1	0	4	8	5
CALCIUM	mg/dl	9.4	0.6	7.4	11.2	327	88
PHOSPHORUS	mg/dl	4.3	0.8	2.4	7.3	283	80
SODIUM	mEq/L	145	3	135	156	307	82
POTASSIUM	mEq/L	4.6	0.4	2.7	5.9	308	85
CHLORIDE	mEq/L	115	3	102	130	290	76
BICARBONATE	mEq/L	19.2	4.0	14.0	24.0	8	6
CARBON DIOXIDE	mEq/L	18.6	2.6	13.0	25.0	64	34
OSMOLARITY	mOsmol/L	297	6	291	305	5	4
IRON	µg/dl	107	36	43	181	16	10
MAGNESIUM	mg/dl	3.80	4.06	1.30	12.50	7	5
BLOOD UREA NITROGEN	mg/dl	26	8	10	58	329	90
CREATININE	mg/dl	1.5	0.3	0.8	2.6	273	86

URIC ACID	mg/dl	0.3	0.3	0.0	1.2	94	40
TOTAL BILIRUBIN	mg/dl	0.3	0.2	0.0	0.9	281	84
DIRECT BILIRUBIN	mg/dl	0.1	0.1	0.0	0.5	51	24
INDIRECT BILIRUBIN	mg/dl	0.2	0.2	0.0	0.6	43	23
GLUCOSE	mg/dl	107	25	47	207	328	92
CHOLESTEROL	mg/dl	273	74	0	499	304	79
TRIGLYCERIDE	mg/dl	27	13	0	102	183	51
CREATINE PHOSPHOKINASE	IU/L	149	96	33	608	116	52
LACTATE DEHYDROGENASE	IU/L	157	147	24	880	186	57
ALKALINE PHOSPHATASE	IU/L	41	43	5	340	321	87
ALANINE AMINOTRANSFERASE	IU/L	95	83	0	605	277	85
ASPARTATE AMINOTRANSFERASE	IU/L	44	31	5	291	315	85
GAMMA GLUTAMYLTRANSFERASE	IU/L	4	3	0	11	160	51
AMYLASE	U/L	490	379	74	2218	160	55
LIPASE	U/L	174	78	12	367	31	17
TOTAL PROTEIN (COLORIMETRY)	g/dl	6.6	0.6	5.3	8.5	283	85
GLOBULIN (COLORIMETRY)	g/dl	3.5	0.5	2.4	5.0	235	74
ALBUMIN (COLORIMETRY)	g/dl	3.1	0.3	2.1	4.0	234	73
FIBRINOGEN	mg/dl	97	118	0	400	32	18
PROGESTERONE	ng/dl	1.680	0.000	1.680	1.680	1	1
TOTAL TRIIODOTHYRONINE	ng/ml	46.9	65.3	0.7	93.0	2	2
TOTAL THYROXINE	µg/dl	1.7	0.5	1.0	2.1	4	4
Body Temperature:	°F	99.7	1.4	96.8	104.0	315	70

[a] Number of samples used to calculate the reference range.
[b] Number of different individuals contributing to the reference values.

Chapter 7 – Addendum I
Preliminary Report on use of Killed Feline Panleukopenia vaccine in Maned Wolves for protection against Canine Parvovirus

Prepared by Mitchell Bush, DVM, NZP-CRC
December 2006
Unpublished Report

A Killed Canine Parvovirus vaccine is no longer commercially available. The use of modified-live Canine Parvovirus vaccines has resulted in several adverse reactions in unvaccinated young Maned wolves.

In an attempt to protect 2 Maned wolf pups this year (2006) we reverted back to a procedure used years ago using a killed feline Panleukopenia vaccine to protect canids against Canine Parvovirus.

Two pups at CRC were titered (both 1:20) and then vaccinated with Fel-O-Vax® PCT by Fort Dodge (1ml) at the age of 6, 8, 10 and 12 weeks. Titers at 12 weeks were 1:320 and 1:160. The pups received an additional Fel-O-Vax® vaccine at 14 weeks while awaiting the titer results. With these titers the pups were vaccinated with a modified-live parvo vaccine (Duramune® Max PV by Fort Dodge) when they were 6 months of age. No problems with this protocol were noted.

A similar study using Fel-O-Vax® in maned wolf pups was conducted by Dr Scott Citino at White Oak Conservation Center with slightly different results. His 3 pups were vaccinated at about 9, 12, 15 and 18 wks of age and only one developed an acceptable titer. The 3 were then vaccinated with Duramune® Max PV by Fort Dodge at 21 wk and all developed adequate titers with no ill effects.

The use of Fel-O-Vax® may be useful in protecting naive maned wolf pups against Canine Parvovirus, but it is recommended to monitor titers. It is **"Possible"** that some protection against modified-live vaccine related problems is afforded by prior vaccination with Fel-O-Vax® before the use of Duramune® Max PV by Fort Dodge.

Chapter 7 - Addendum II
Suspected case of bone marrow suppression thought to be induced by the antibiotic trimethoprim/sulfadiazine (TMS)

Reported by Mitchell Bush, DVM, National Zoological Park

This is to alert veterinarians who care for maned wolves of a suspected case of bone marrow suppression in a 3 year old male thought to be induced by the antibiotic trimethoprim/sulfadiazine (TMS). The past history included dysuria with fine calculi in the urinary bladder seen on radiographs. The straining due to cystitis was controlled by oral TMS in October 1991 and again in December 1991. The wolf was also receiving Thiola, in an attempt to reduce cystine stone formation, until April 1992. In October 1992 the wolf again presented with stranguria and was again placed on TMS following a diagnostic evaluation that was unremarkable except for a moist dermatitis of the front foot. Two weeks later the wolf developed acute anorexia and depression. The hemogram showed a WBC of 500 and a HCT of 40. There were also decreased platelets on the blood smear. Despite extensive supportive care the wolf died the next day. At necropsy there was an extensive cellulitis of the front leg that had the dermatitis and bone marrow smears showed a hypoplasia of the myeloid elements.

Bone marrow suppression is reported in dogs and cats due to TMS, but it more commonly involves the erythroid elements: however, it can also affect the myeloid cells. In domestic species, discontinuation of the drug usually reverses the bone marrow suppression. Specific treatment is the administration of Leucovorin, which is an antidote to drugs like TMS that act as folic acid antagonists.

TMS is still an excellent drug for use in maned wolves, but the clinician should be aware of this potential problem since this species may be more sensitive. We have used TMS for many years in maned wolves with good therapeutic results and no previously recognized problems.

Chapter 7 – Addendum III
Possible reaction to Metronidazole

Reported by Frank Ridgley, DVM, Buffalo Zoo

This message is to pass on some observations of metronidazole use in maned wolves at the Buffalo Zoo. There have been two cases that were suspected to have been the result of toxicity of the drug. I will report it in the form of a timeline. I am reporting this because I could not find any reference to this happening in the husbandry manual or in literature searches. A historical episode happened in 1997, before my time here at Buffalo, and is strictly from clinical notes and technician memory. A recent event happened in January, 2003 with similar signs to the episode in 1997.

96M171 "Olive Oil" 0.1

9-17-97 Metronidazole 400 mg PO BID for 10 days, No weight recorded 9-26-97 Metronidazole 1000 mg SID PO (37mg/kg) for 10 days 10-2-97 Metronidazole 1625mg SID PO (60 mg/kg) for 5 days 10-6-97 continued to 10-17 10-11-97 head tremor noted 10-15-97 Ataxia, head tremor 10-16-97 Physical exam under anesthesia and stopped medications 10-17-97 Still exhibiting neurologic signs and uncoordinated 10-22-97 85% better 10-25-97 Appears normal 9-19-98 Metronidazole 500mg BID PO for 10 days (30mg/kg), no signs of toxicity 8-29-01 Metronidazole 250mg BID PO for 5 days, no signs of toxicity

M02046 "Scotty" 1.0

4-3-03 Metronidazole 230.5 mg PO SID for 10 days
9-27-03 Weight 23.6 kg
11-13-03 Metronidazole 250 mg PO SID for 10 days
12-8-03 Metronidazole 250 mg PO BID for 15 days
1-3-04 Metronidazole 250 mg PO BID for 28 days
1-16-04 Neurologic signs were noticed by keeper in PM. Wolf would not rise and seemed like he could not visually locate us in the room. He would not rise from lateral recumbency when touched with a pole but kept his head raised. I was suspicious that the early signs were missed by the keepers because of severe cold weather at the time and limited access outside for the wolves to watch behaviors. Pupils were dilated and rear legs showed less motor activity than the front. Metronidazole was stopped (13 days into treatment). This wolf had been on metronidazole after chronic loose stool and it had only been responsive to metronidazole. Repeated stool cultures showed no significant abnormalities, fecal floats and direct exams were always negative, non-responsive to diet changes or anti-inflammatory medications. This wolf had his first documented bout of cystinuria earlier in the fall and was currently on potassium citrate. 1-17-04 His clinical signs did not change overnight and an early AM exam was performed. Physical exam was largely unremarkable. Urinalysis was WNL. Serum chemistry showed a very mild hypokalemia and hypocalcaemia, and a mild hyperproteinemia and hyperbilirubinemia. CBC showed a leucopenia characterized by a lymphopenia and monocytopenia. Weight: 23.6 kg 1-18-04 Evidence of moving around his holding by scattered food and water but no consumption measured and still appears to exhibit the same neurologic signs. 1-19-04 Much more responsive today and vocalizing. Attempts to rise when stirred and eating mice readily. Still appears very ataxic in hindlimbs. 1-20-04 Continued improvement. Standing today very well and eating readily. More normal acting behavior with growling, focused glare and ear pinning. 1-22-04 Appears totally normal in yard. Serum titers for distemper or rabies did not show a rise. Stool returned to normal after starting on L-glutamine supplementation.

Hope this helps to prevent this from happening in the population again. I certainly thought that I was staying conservative on the dose as compared to what I am used to seeing used in the private sector and I was conservative based on the first wolf's doses. I am not sure if maned wolves are very sensitive to metronidazole or there was some other factor that amplified the toxic effects.

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Chapter 7 - Addendum IV
Maned Wolf Pup Vaccination Schedule

Recommended Vaccine Schedule for maned wolf pups June 07

Age	Type of vax	Titer check
7wks	Purevax CDV + Fel-O-Vax PCT	yes
10wks	Purevax CDV + Fel-O-Vax PCT	
13wks	Purevax CDV + Fel-O-Vax PCT	
16wks	Rabies + Purevax CDV + **Fel-O-Vax PCT OR Duramune** (see below)	yes
19wks	Purevax CDV + Fel-O-Vax PCT if prev low	yes, if prev low
24wks	Rabies + Purevax CDV + **Fel-O-Vax PCT OR Duramune** (see below)	yes, if prev low

Recommended vaccines

Parvo: Fort Dodge's feline killed Fel-O-Vax PCT (for pups and naïve animals)
Fort Dodge's canine MLV Duramune Max Pv (if prev vax with good titers)

Canine Distemper: Merial's purevax Recombinant ferret distemper vaccine (canary pox-vector)

Comments:

1. If inadequate parvo titers (<1:80) at 16wks, vax with Fel-O-Vax PCT q3 wks
2. If adequate titers are evident at 16wks, then vax with MLV Duramune parvo vax
3. Booster at 6 months and then yearly
4. Modified-live vaccines (MLV) are typically preferred over killed vaccines because they produce a bigger and longer-lasting immune response. However, there have been several reports of vaccine-induced illness in maned wolves (Backues, 1994; Thomas-Baker, 1985). Thus, it is important to use a killed or recombinant canary pox vector vaccine on naïve/young animals until their immune response is acceptable (as indicated by adequate titers). Also, vaccinating with only one antigen at a time may help reduce the chance of an adverse vaccine reaction (ie, administer rabies/distemper/parvo vaccines separately). This is more important when multiple MLV vaccines are used.

Please send all vaccine inquiries and titer results to the maned wolf SSP vet advisor:

Dr. Elizabeth Hammond,
vet@lioncountrysafari.com

Chapter 7 – Addendum V
Vaccination and Titer Information

White Oak maned wolf pup parvo vaccination and titer schedule
2006

ID	SB	DOB	Age at vax	Date of vax	Type of vax	Parvo titer (IgG)
Y53014	2844	1-Dec-05	9wks	9-Feb-06	Fel-o-Vax	
			12wks	2-Mar-06	Fel-o-Vax	
			15wks	23-Mar-06	Fel-o-Vax	1:100
			18+wks	17-Apr-06	Fel-o-Vax	0
			21wks	9-May-06	Duramune	1:25
			35wks	22-Aug-06		1:1600
Y53015	2845	1-Dec-05	9wks	9-Feb-06	Fel-o-Vax	
			12wks	2-Mar-06	Fel-o-Vax	
			15wks	23-Mar-06	Fel-o-Vax	0
			18+wks	17-Apr-06	Fel-o-Vax	0
			21wks	9-May-06	Duramune	1:1600
			35wks	22-Aug-06		1:3200
Y53016	2846	1-Dec-05	9wks	9-Feb-06	Fel-o-Vax	
			12wks	2-Mar-06	Fel-o-Vax	
			15wks	23-Mar-06	Fel-o-Vax	0
			18+wks	17-Apr-06	Fel-o-Vax	0
			21wks	9-May-06	Duramune	0
			35wks	22-Aug-06		1:1600

Notes:

1. Pups were vaccinated concurrently with Merial Purevax ferret distemper vaccine and Ft Dodge Fel-O-vax PCT IV

2. If no parvo titer at 16wks, then repeat above, if good parvo titer, start MLV Durammune

3. Continue vaccination with Fel-O-Vax q 3wks until titer evident, then start MLV

4. Booster at 6 months and then yearly

Louisville Zoo maned wolf pup vaccination and titer information 2007

ID	SB	DOB	Age	date of vax	CDV titer	Parvo titer
102744	T0706	7-Jan-07	6wks	22-Feb-07	1:12	1:10
			9wks	15-Mar-07		
			12wks	5-Apr-07		
			15wks	27-Apr-07	1:384	1:320
102745	T0707	7-Jan-07	6wks	22-Feb-07	NA	NA
			9wks	15-Mar-07		
			12wks	5-Apr-07		
			15wks	27-Apr-07	1:512	1:320

Pups were vaccinated concurrently with Merial Purevax ferret distemper vaccine
and Ft Dodge Fel-O-vax PCT

Fossil Rim maned wolf pup vaccination and titer information 2007

ID	SB	DOB	age	Date of vax	CDV titer	Parvo titer	
7035	T0710	22-Feb-07	6wks	6-Apr-07			
			9wks	27-Apr-07			
			12wks	18-May-07			
			16wks	14-Jun	1:128	1:80	Rabies vax
			19wks	*5-Jul	1:256	1:320	
7036	T0711	22-Feb-07	6wks	6-Apr-07			
			9wks	27-Apr-07			
			12wks	18-May-07			
			16wks	14-Jun	1:128	1:160	Rabies vax
			19wks	*5-Jul	1:256	1:320	

Pups were vaccinated concurrently with Merial Purevax ferret distemper vaccine
and Ft Dodge Fel-O-vax PCT
*EXCEPT 5 Jul pups given Ft Dodge
Duramune Max PV

Chapter 7 – Addendum VI
Thyroid Levels

Name or Animal ID	Thyroid Levels			
	T3(ng/ml)	T4(ug/dl)	Free T4 (pmol/dl)	TSH (ng/ml)
Canine Average	2.32	1.45	2.25	0.25
Maned Wolf Average	0.72	0.75	1.41	0.13
Pebbles	0.8	0.5	1.017	0.1
Pebbles(medicated*)	0.79	1.3	1.673	0.66
Louise	0.68	0.5	1.364	0.07
Waylon	0.74	0.96	2.162	0.26
Thelma	0.39	0.5	0.94	0.08
Guido	1.13	QNS	QNS	QNS
Vinnie	1.03	0.5	0.708	0.03
Katy	0.71	0.5	0.309	0.07
Caesar	0.65	0.5	0.18	0.03
Emma	0.61	1.4	4.106	0.09
BamBam	0.73	0.57	1.364	0.09
Stripe	0.74	0.59	1.9951	0.06
Hannah	0.38	1.1	3.6041	0.03
17825	1.01	0.5	1.094	0.03
20057	0.31	0.77	0	0.14
Cheyenne	0.7	0.87	1.216	0.2
Elwood	0.87	0.76	0.463	0.09
Jake	0.96	0.94	0.476	0.04

*levothyroxine supplementation

CHAPTER EIGHT
MANED WOLF NECROPSY PROTOCOL

A necropsy examination is one of our most important, occasionally our only, and often our last opportunity to collect valuable diagnostic and archival samples after an animal dies. Listed below is a sample necropsy report and list of recommended tissues for routine collection during a necropsy examination. Please collect two sets of tissues for formalin fixation; submit one set to your pathologist and archive one permanently at your institution. Also, please request that your referral pathology lab or pathology department permanently archive the paraffin blocks and glass slides from these cases. If they are unable to perform this service, please send the blocks and slides to the Maned Wolf SSP Pathology Advisor (DO NOT forward formalin fixed tissues without first contacting the Pathology Advisor). While it may not always be possible to collect all of the recommended samples from each animal, the more consistently these goals can be achieved and reports generated the greater the chance that we will accurately identify diseases and disease trends in our collections. Please send copies of your completed gross and histologic necropsy reports (using your standard forms or the attached form with inclusion of results from ancillary diagnostics) to the SSP Maned Wolf Veterinary Advisor and Pathology Advisor listed below. The submitted information will be included in a year-end summary report for captive collections of Maned Wolves in the United States.

Veterinary Advisor:
Elizabeth Hammond, DVM
Lion County Safari
2003 Lion County Safari Rd
Loxahatchee, FL 33470
Phone: (561) 793-1084 x 320
Fax: (561) 662-0288
Email: vet@lioncountysafari.com

Pathology Advisor:
D McAloose
Wildlife Conservation Society
2300 Southern Blvd
Bronx, NY 10460
Phone: (718) 220-7105
Fax: (718) 220-7126
Email: dmcaloose@wcs.org

Special tissue request:
There are 2 projects that make use of female reproductive tracts. Please follow directions in #1 and #2 below to provide the appropriate tissues to Drs. Munson (1 ovary + uterus) and Songsasen (1 ovary and reproductive tract, including vagina and cervix from female; testis from male).

1. Dr. Linda Munson has requested reproductive tracts (uterus, 1 ovary, mammary gland) from female maned wolves regardless of their contraceptive history. **Addendum I** contains instructions and a contraceptive pathology survey that should accompany the sample. Please forward the appropriate tissues/tissue sections and survey to:

Dr. Linda Munson
University of California School of Vet. Medicine, Davis
Dept VMPMI, 4206 Vet Med 3A
1 Shields Ave
Davis, CA 95616
lmunson@ucdavis.edu (916) 754-7567.

2. Dr. Nucharin Songsasen has requested the reproductive tracts (1 ovary, vagina and cervix) of females and testis of males. For females: remove 1 ovary, vagina and cervix; for males: remove testes. Store in plastic bag with ~10ml of 0.9% saline solution. Ship in ice packs. Ship overnight via FedEx to:

Dr. Nucharin Songsasen
NZP Conservation & Research Center
1500 Remount Road
Front Royal, VA 22630
songsasenn@si.edu (540) 622-8415

3. Cystinuria has been reported in maned wolves. Please collect and have the composition of any stones or crystals that are found evaluated. Please include this information in final necropsy reports.
A urine sample should be collected on filter paper for cystinuria screening. Label the sample with the animal's studbook number and mail sample with an accompanying sheet of information including date, species, Stbk #, sex, birth date, and your return address to Paula Henthorn at:

Dr. Paula Henthorn
University of Pennsylvania
School of Veterinary Medicine
Section of Medical Genetics
3900 Delancey Street
Philadelphia, PA 19104-6010
henthorn@vet.upenn.edu (215) 898-9601
Fax: (215) 573-2162

General Necropsy Information:

1) **Tissue collection**
   a) Collect normal and abnormal tissue samples from all major organ systems
   b) Tissue samples should be no thicker than 0.5 cm
   c) Tissue: 10% neutral buffered formalin (ratio) = 1:10
      i) Multiple tissue samples can be placed in a single bucket, but maintain ratio
   d) Tissues can be saved or shipped in smaller volumes of formalin once adequately fixed
2) **Specific tissue collection and fixation recommendations**
   a) Lymph nodes: Label as to location (e.g. mesenteric, mandibular) when grossly abnormal or in cases of suspect hematopoietic or lymphoid disease
   b) Heart: Collect 3 longitudinal sections (minimum) such that atrium, ventricle and valve from right and left freewalls (include papillary muscle) and interventricular septum (include right AV valve and aortic outflow tract) are collected
   c) Gastrointestinal tract: Open entire tract along its long axis. Collect 2-3 sections (3-4 cm long) from each part of tract. Label as to location when grossly abnormal or in cases of suspect gastrointestinal tract disease.
   d) Urinary tract: Please collect and submit any stones or crystals found in the urinary tract (kidneys, ureters, bladder, urethra) for urolith analysis.
   e) Endocrine organs: Collect organs from both the left and right sides. Longitudinally section adrenal gland to assess cortical and medullary tissue.
   f) Brain: Fix whole if possible. Place in separate bucket to improve fixation if possible.
      i) If only half brain can be collected, section brain longitudinally along midline
   g) Eye: Fix whole (not punctured and do not inject with formalin)
   h) Neonates and fetuses:
      i) Collect umbilical stump and surrounding tissue.
      ii) Evaluate for malformations (e.g. cleft palate, cardiovascular abnormalities).
      iii) Evaluate lungs (e.g. float in formalin if breathing occurred; sink if not inflated (e.g. stillborn), diseased (e.g. pnemonia)).
      iv) Collect fetal membranes if available.
      v) Measure crown-rump length
      vi) Assess hydration status
      vii) Verify sex
      viii) Evaluate for evidence of nursing (presence of milk fluid and/or curd in stomach and presence of "milk stool" (yellow-white semisolid material in colon) with absence of meconium (greenish/brown pastey material)
3) **Diagnostic samples**
   a) Infectious disease

i) Store at –70C (to maintain DNA, proteins, RNA)
ii) Submit samples (e.g. culturettes, fresh tissue, feces) to internal or external labs as necessary for ancillary diagnostic testing

b) Toxicology
i) Store tissues or samples at –20C
ii) In cases of suspected toxicity, contact a toxicologist for recommendations relative to tissue collection, preservation, test type and shipping instructions
iii) For generic toxicologic sample collection, collect two sets (1 in aluminum foil, 1 in plastic or glass) 5 grams each, of the following tissues
(1) Stomach contents
(2) Adipose tissue
(3) Kidney
(4) Liver
(5) Eye

4) Frozen Tissue

a) Archival
i) Freeze 3-5 cm blocks of tissue from major organs (e.g. heart, lung, liver, kidney, spleen, intestines, brain, spleen) in small plastic bags
(1) Store at –70C or in liquid nitrogen
(a) Store at conventional freezer temperatures if ultralow freezer space not available

Gross Necropsy Report – General information

Species:	Sex (M/F/U):
Common Name:	Date of birth/age:
Institution/Owner:	Date of death:
Accession/ID No:	Type of death (Euth/Natural):
Studbook No.:	Prosector:
Other ID No:	Necropsy Date:
House Name:	Necropsy Location:
Enclosure ID/indoor/outdoor:	Body weight:
Captive Born or Wild Caught:	Pathologist:
Parent or hand-reared:	Pathologist Phone Number:
Dam:	
Sire:	
Environmental/weather conditions at time of death:	
Movements or relocations (date, from and to):	
Diet:	
Contraceptive history (contracepted: Y/N; type; date):	
Proven breeder (Y/N; number of offspring produced; birth history (live, stillborn, abortion, etc.)	

Clinical history and past treatments (add additional sheets for clinical history or ancillary diagnostics (e.g. serum chemistries, radiography) as necessary):

Tissue examination and collection checklist

TISSUE	GA	FF	Histo	Photo	-20 -70	TISSUE	GA	FF	Histo	Photo	-20 -70
GENERAL-external						**ABDOMEN**					
Oral cavity & teeth						Diaphragm					
Tonsils						Stomach					
Skin and nails						Small intestines					
Subcutis						Large intestines					
Skeletal muscle						Pancreas					
Peripheral nerves						Spleen					
Mammary gland						Liver & gall bladder					
Umbilicus						Lymph nodes					
BONES & JOINTS						Aorta & vessels					
Bone marrow (femur)						Kidneys					
Bones						Ureters					
Hips						Urinary bladder					
Knees						Urethra					
Tarsi						Adrenal glands					
Shoulders						Ovaries					
Carpi						Oviduct/Uterus					
Atlantooccipital						Vagina/vulva					
CAVITIES						Testes					
Thoracic cavity						Access sex gland					
Abdominal cavity						Penis/prepuce					
PLUCK						HEAD					
Tongue						Eyes					
Thyroids/parathyroids						Ears & bullae					
Esophagus						Skull/nasal cavity					
Trachea & Lungs						Brain/Meninges					
Heart/Pericardial sac						Pituitary gland					
Aorta & other vessels						SPINE					
Thymus/lymph nodes						Vertebral column					
						Spinal cord					

GA = Gross appearance: NGL=no gross lesions; AB=abnormal; NE=not examined; NF=not found; NP=not present
FF = Tissue fixed in formalin: + = tissue collected and fixed
Histo = Tissue submitted for histology: + = tissues submitted
AD = Ancillary diagnostics: + = ancillary diagnostic performed (please include results w/report)
PHOTO = Photograph: + = PHOTO taken
-20/-70 = Frozen tissue temperature: please list storage temp as –20, -70 or other temp if applicable

Gross Descriptions:
Please describe all abnormal gross findings, including dimensions (cm x cm x cm).

General condition (include assessment of body and post-mortem condition, skin, SQ fat stores, body orifices):

Musculoskeletal system (axial, appendicular, tympanic bullae, spinal column, joints, muscle):

Body cavities (thoracic, abdominal, pericardial, also assess fat stores in these locations):

Hemolymphatic systems (tonsil, lymph nodes, spleen, thymus, bone marrow):

Respiratory system (nasal passages, pharynx, larynx, trachea, bronchi, lungs):

Cardiovascular system (heart, valves, chambers, pericardial sac, vessels):

Digestive system (tongue, oral cavity, teeth, esophagus, stomach, small and large intestines, anus, liver, gall bladder, pancreas):

Urinary system (kidneys, ureters, urinary bladder, urethra):

Reproductive system (testes/ovaries, uterus, cervix, penis/vagina, accessory sex organs, mammary glands, placenta, fetus)**:**

Endocrine system (adrenal glands, thyroids, parathyroids, pituitary gland):

Nervous (brain, spinal cord, peripheral nerves) **and special senses** (eyes, ears):

Gross Diagnoses (please add additional diagnoses if necessary):

1.

2.

3.

4.

5.

Gross Comments:

Results of ancillary post-mortem diagnostics (e.g. cytology, fluid analysis, urinalysis, bacteriology, toxicology, virology, parasitology):

1.

2.

3.

4.

5.

Please attach/forward histology report to pathology advisor if available.

Chapter 8 - Addendum I
Dr. Linda Munson's Instructions & Survey

CAG Contraceptive Pathology Survey Adapted for Maned Wolf SSP

MEMORANDUM

TO: MANED WOLF SSP VETERINARIANS AND MAMMAL CURATORS

FROM: Dr. Linda Munson
Pathologist Advisor for AZA Contraceptive Advisory Group
University of California- Davis
Dept. VM-PMI
4206 Vet Med 3A
1 Shields Avenue
Davis, California 95616
Phone: *530-754-7963, FAX: 530-752-3349, Email:* lmunson@ucdavis.edu

DATE: May 23, 2007

SUBJECT: Update on Contraceptive Health Surveillance Center for Maned Wolf SSP

The AZA Contraceptive Advisory Group is continuing the pathology surveillance of reproductive tracts from captive and free-ranging female animals to determine if there are adverse reactions to contraceptives. For the most important current AZA CAG concerns, we need the reproductive tracts from all female mammals *regardless of their contraceptive history.*

SAMPLES TO SUBMIT: *Females:* Intact, formalin-fixed reproductive tracts (uterus, 1 ovary and mammary gland if possible) obtained from necropsy or ovariohysterectomy of any female mammal.

FIXATION: Remove uterus and 1 ovary for processing (process and ship 2nd ovary to Dr. Nucharin Songsasen following the instructions on page 1 of MWSSP Necropsy protocol). Make a *small* incision into one horn (bicornuate uteri). Immerse tissues in buffered formalin for at least 72 hours (ratio of tissue to formalin = 1:10).

SHIPPING: Wrap fixed tracts in formalin-soaked paper towels, enclose in a leak-proof plastic container and ship by ground US mail (Federal Express is not necessary) to the above address.

REQUIRED INFORMATION TO INCLUDE: It is important that you also provide the information requested on the enclosed form. We will not be able to include your case without this information. This valuable data allows us to assess the effects that age and parity, as well as duration and dose of the contraceptive treatment can have on the lesions.
When the data are collated, we will send a brief report on the reproductive tract to you and will be certain to acknowledge your institution's contribution in any publication resulting from this survey.

Thank you in advance for contributing to this survey. Your involvement will contribute to our understanding of the effects of contraceptives on reproductive health in zoo mammals.

Revised *May 23, 2007*

AZA CONTRACEPTIVE PATHOLOGY SURVEY
If no information is available please indicate so in the spaces provided

Submitting veterinarian _____

Institution: _____

Address: _____ Phone #: _____

_____ Contact email: _____

Species: _____ ISIS: _____ Sex: M F

SB#: _____ Zoo ID#: _____ House Name: _____

Birth date: _____ Date when tract was obtained: _____

Was it necropsy or surgery? _____ Weight (kg): _____

According to the animal's record:

Has this animal been cycling? Y N

Has this animal been bred? Y N Dates of pregnancies? _____

Has this animal spent its entire reproductive life at your zoo? Y N

If no, sites of previous residence: _____

Previous ID #s (if known): _____

FOR MELENGESTROL (MGA) IMPLANTS: ___ Never been contracepted

Has this animal shown signs of estrus while implanted? Y N

Implant #	Implant Weight	Animal Weight	Date Inserted	Date Removed
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____

OTHER CONTRACEPTIVE *If more space is needed please use the back*

1) Type: _____ Route: _____

Dose: _____ Body weight (kg):_____

Treatment dates: _____

2) Type: _____ Route: _____

Dose: _____ Body weight (kg):_____

Treatment dates: _____

OTHER PROBLEMS THAT MAY AFFECT REPRODUCTION? (use the back if needed)

Please send tissues and form to:
Dr. Linda Munson, Univ. of California, Dept VM-PMI, 4206 Vet Med 3A, 1 Shields Ave, Davis, CA 95616
Ph (530) 754-7963 lmunson@ucdavis.edu **Revised Sep/04**

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