ASSOCIATION OF ZOOS & AQUARIUMS




LARGE CANID
(*Canidae*)
CARE MANUAL

CREATED BY THE
AZA Canid Taxon Advisory Group
IN ASSOCIATION WITH THE
AZA Animal Welfare Committee

Large Canid (Canidae) Care Manual

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Introduction

Preamble

AZA accreditation standards, relevant to the topics discussed in this manual, are highlighted in boxes such as this throughout the document (Appendix A).

AZA accreditation standards are continuously being raised or added. Staff from AZA-accredited institutions are required to know and comply with all AZA accreditation standards, including those most recently listed on the AZA website (www.aza.org) which might not be included in this manual.

Taxonomic Classification

Table 1: Taxonomic classification large canids

Classification	Taxonomy
Kingdom	Animalia
Phylum	Chordata
Class	Mammalia
Order	Carnivora
Suborder	Caniformia
Family	Canidae

Genus, Species, and Status

Table 2: Genus, species, and status information for large canids

Genus	Species	Common Name	USA Status	IUCN Status	AZA Status
Canis	*latrans*	Coyote	Species of Concern	Least Concern	
Canis	*rufus*	Red wolf	Endangered	Critically Endangered	Yellow SSP
Canis	*lupus*	Gray wolf	Endangered in part	Least Concern	
Canis	*lupus baileyi*	Mexican gray wolf		Extinct in the Wild	Yellow SSP
Chrysocyon	*brachyurus*	Maned wolf	Endangered	Near Threatened	Yellow SSP
Cuon	*alpinus*	Dhole	Endangered	Endangered	
Lycaon	*pictus*	African wild dog	Endangered	Endangered	Yellow SSP

General Information

The information contained within this Animal Care Manual (ACM) provides a compilation of animal care and management knowledge that has been gained from recognized species experts, including AZA Taxon Advisory Groups (TAGs), Species Survival Plan® Programs (SSPs), biologists, veterinarians, nutritionists, reproduction physiologists, behaviorists and researchers. They are based on the most current science, practices, and technologies used in animal care and management and are valuable resources that enhance animal welfare by providing information about the basic requirements needed and best practices known for caring for *ex situ* large canid populations. This ACM is considered a living document that is updated as new information becomes available and at a minimum of every five years.

Information presented is intended solely for the education and training of zoo and aquarium personnel at AZA-accredited institutions. Recommendations included in the ACM are not exclusive management approaches, diets, medical treatments, or procedures, and may require adaptation to meet the specific needs of individual animals and particular circumstances in each institution. Statements presented throughout the body of the manuals do not represent specific AZA accreditation standards of care unless specifically identified as such in clearly marked sidebar boxes. AZA-accredited institutions which care for large canids must comply with all relevant local, state, and federal wildlife laws and regulations; AZA accreditation standards that are more stringent than these laws and regulations must be met (AZA Accreditation Standard 1.1.1).

AZA Accreditation Standard

(1.1.1) The institution must comply with all relevant local, state, and federal wildlife laws and regulations. It is understood that, in some cases, AZA accreditation standards are more stringent than existing laws and regulations. In these cases the AZA standard must be met.

The ultimate goal of this ACM is to facilitate excellent large canid management and care, which will ensure superior large canid welfare at AZA-accredited institutions. Ultimately, success in our large canid

management and care will allow AZA-accredited institutions to contribute to large canid conservation, and ensure that large canids are in our future for generations to come.

"Large canids" in this document are defined as species in the family Canidae that weigh over 22lb (10kg) as adults, and that are currently and commonly held in AZA member institutions. These include the African wild dog (*Lycaon pictus*), coyote (*Canis latrans*), gray wolf (*Canis lupus*), maned wolf (*Chrysocyon brachyurus*), Mexican gray wolf (*Canis lupus baileyi*), red wolf (*Canis rufus*), and the dhole (*Cuon alpinus*). Recommendations for the domestic dog (*Canis lupus familiaris*), New Guinea singing dog (*Canis lupus hallstromi*), and the dingo (*Canis lupus dingo*), are not included in this manual, although these species are managed by some AZA member institutions.

The Large Canid Care Manual was developed using information from the following AZA Species Survival Plan® (SSP) husbandry manuals:

- Maned Wolf Husbandry Manual, 2007 Edition (Maned Wolf SSP, 2007).
- Mexican Gray Wolf Husbandry Manual: Guidelines for Captive Management, 2009 Edition (Mexican Wolf SSP, 2009).
- Red Wolf Husbandry Manual: Guidelines for Captive Management (Waddell, 1998).

These husbandry manuals provide additional species-specific information on the recommendations made within the Large Canid Care Manual; readers should consult these sources further as appropriate, and when these documents are referenced. A comprehensive list of references and citations can be found in these documents, along with contact information for program managers and advisors. The Large Canid Care Manual also contains information incorporated from published documents, unpublished husbandry documents of the AZA African Wild Dog SSP, the authors' personal experiences with large canid species, and personal communications with Maria Franke (Curator of Mammals, Toronto Zoo) concerning dhole husbandry. Unless stated otherwise, "gray wolf" refers to both gray and Mexican gray wolves.

Physical description: The following table (Table 3) includes basic large canid body size information. Typically, males are larger than females, although there is no sexual dimorphism among maned wolves. There will be some individual-specific variability in body weight and size within each species, and these are general ranges only.

Table 3: Typical large canid body size ranges

Species	**Body size**	
	Weight	Shoulder height
African wild dog (*Lycaon pictus*)	20-25 kg (44-55 lb)	60-75 cm (24-30 in)
Coyote (*Canis latrans*)	9-22 kg (20-48 lb)	38-51 cm (15-20 in)
Dhole (*Cuon alpinus*)	12-18 kg (26-40 lb)	~50 cm (20 in)
Gray wolf (*Canis lupus*)	25.8-50+ kg (50-110 lb)	66-81 cm (26-32 in)
Maned wolf (*Chrysocyon brachyurus*)	20-30 kg (44-66 lb)	75-91 cm (29.5-36 in)
Mexican gray wolf (*C. lupus baileyi*)	25.8-36.24 kg (50-80 lb)	~67 cm (26 in)
Red wolf (*Canis rufus*)	20-36 kg (44-80 lb)	~66 cm (26 in)

Natural history and behavioral repertoire: Large canids are cursorial animals. Some species can travel long distances (>16 km/10 mi) on a daily basis, or disperse over longer distances (>805 km/500 mi) during seasonal movements. Food and foraging habits range from the omnivorous maned wolf to the seasonally omnivorous coyote, to the predominantly carnivorous red and gray wolves, dhole, and African wild dog; the latter three species prey on ungulates many times their individual size using cooperative hunting strategies. Large canids express a wide variety of social tendencies, from seasonal monogamy in maned wolves to large multigenerational packs of some dholes, African wild dogs, and gray wolves (Sillero-Zubiri et al., 2004). Additional information about maned wolf natural history can be found in Emmons (1998), Silveira (1999), and Bestelmeyer (2000). The coyote may live as single transients, in pairs, or in small generational packs (Gese & Bekoff 2004). There is social flexibility within many species,

related to environmental conditions, prey availability, and prey size. Senses of sight, hearing, and smell are well developed in all species. Olfactory sensitivity in the domestic dog and, presumably, other large canids may be underappreciated in zoo canid management.

Regulating agencies: Federal and state agencies are involved in regulating the care, management, and transport of many species of large canids, and standards exist in addition to the AZA Accreditation Standards (AZA, 2010) and USDA Animal Welfare Regulations (AWR, 2005). All red wolves and Mexican wolves in the USA are held under permit or loan from the U.S. Fish and Wildlife Service (USFWS). In Mexico, all Mexican wolves are on loan from the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT). Both species are part of federal recovery and reestablishment programs.

Table 4: Regulatory Status of Species Covered by the AZA Large Canid Care Manual

Common name	Genus	Species	CITES	USFWS	SEMARNAT
African wild dog	*Lycaon*	*pictus*	not listed	EN	n/a
Coyote	*Canis*	*latrans*	not listed	n/a	n/a
Dhole	*Cuon*	*alpinus*	II	Injurious wildlife, EN	n/a
Gray wolf	*Canis*	*lupus*	II	EN in part	n/a
Maned wolf	*Chrysocyon*	*brachyurus*	II	EN	n/a
Mexican gray wolf	*Canis*	*lupus baileyi*	II	EN	EN
Red wolf	*Canis*	*rufus*	not listed	EN	n/a

Four taxa are listed as Endangered under the U.S. Endangered Species Act (1973), and require an USFWS export or import permit for any international shipment involving a U.S. zoo. For zoos involved with a Mexican wolf transfer to or from Mexico, this requires permits from SEMARNAT. Many countries also require import permits for animals entering the country, which should be obtained by the recipient prior to shipment. Four taxa are listed as Appendix II under CITES which necessitates an export permit from the CITES representative of the country exporting the animal.

Chapter 1. Ambient Environment

1.1 Temperature and Humidity

> **AZA Accreditation Standard**
>
> **(1.5.7)** The animal collection must be protected from weather detrimental to their health.

Animal collections within AZA-accredited institutions must be protected from weather detrimental to their health (AZA Accreditation Standard 1.5.7). Animals not normally exposed to cold weather/water temperatures should be provided heated enclosures/pool water. Likewise, protection from excessive cold weather/water temperatures should be provided to those animals normally living in warmer climates/water temperatures.

Cold weather: Within their enclosures, large canids should have access to a dry den structure with appropriate dry bedding (see Chapter 2, section 2.1 for additional information). In addition to dens, outdoor enclosures should have features that provide windbreaks and rain shelters for all individuals within the social group, whether they choose to shelter together or separately. Where needed to meet the specific needs of individual animals, additional heat can be provided in the form of forced warm air, or electrical heaters such as space heaters, radiant heaters, heat pads or panels, and heat lamps. Standard safety guidelines should be followed in the use of these heating devices for CO_2 production and the potential for fire hazards, and the animals should not be able to gain access to any of the electronic components of the equipment (e.g., wiring).

- Red wolf, gray wolf, dhole, and coyote: These species tolerate cold weather, but should be provided with a dry den structure, with dry bedding such as straw for shelter from rainy or windy conditions. In general, these species do not require supplemental heating in their dens, and do not generally require specific access to an enclosed, climate-controlled environment.
- Maned wolf and African wild dog: These species are more sensitive to cold temperatures. Considerations for minimum temperature should take into account wind, snow, and/or ice in the enclosure, and the age and coat condition of the animal. In climates where the temperature regularly falls below 4.4-7.2°C (40-45°F) , these species should be provided with a den with auxiliary heat, or access to a climate-controlled environment.
- Whelping considerations: Maned wolves, dholes, and often African wild dogs whelp during the winter in North American facilities. Those facilities located in climates where the daily minimum temperature regularly falls below 4.4-7.2°C (40-45°F) during the whelping season for these species should provide a den with adequate supplemental heat. Whelping areas should be kept above 7.2°C (45°F). Red wolf, gray wolf, and coyote whelp in the spring, and generally do not require special heating considerations.

Hot weather: Large canids adapt effectively to high environmental temperatures if they have the opportunity to rest in shaded areas. Sufficient shade should be provided throughout the day within outdoor enclosures to allow all members of a social group to gain access to it, whether resting together with conspecifics or apart from them. Additional approaches that can be used to provide opportunities for the animals to regulate their own temperature in hot weather include the provision of air conditioning, underground dens, mist systems, swamp coolers, and water features in which the animals can cool themselves.

Humidity: Healthy, adult large canids should be able to cope effectively with humidity in the outdoor environments at current AZA member institutions, where average relative humidity ranges from 15-80%. Canids cool themselves primarily through panting, which is more effective when humidity is low, so animals should be monitored in conditions where both temperature and humidity reach extreme highs. Care should be taken that large canids are not physically stressed during hot and humid days in order to prevent possible hyperthermia. An outdoor enclosure with a variety of microclimates will allow individuals to choose the most comfortable local environment within their enclosures. In order to provide optimal humidity and ventilation within enclosed dens or holding areas, hard surfaces should dry quickly and stay dry when they are occupied by canids. The general guideline for optimum humidity in a climate-controlled environment for laboratory mammals is approximately 50% relative humidity, with a critical range of 30-80% (Norwegian School of Veterinary Science Lab Animal Reference Centre, 2007).

Indoor enclosures: It is recommended that large canids not be kept in enclosed, climate-controlled environments for extended periods of time, except for medical or for temporary husbandry management reasons. In these situations, temperatures between 13-29°C (55-85°F) are recommended for an uncompromised adult large canid. It should be noted that pups less than three weeks of age cannot thermoregulate, and additional provisions may be needed to provide adequate ambient heat for new litters (see Chapter 7, sections 7.4 and 7.5 for additional information).

AZA institutions with exhibits which rely on climate control must have critical life-support systems for the animal collection and emergency backup systems available, while all mechanical equipment should be included in a documented preventative maintenance program. Special equipment should be maintained under a maintenance agreement or records should indicate that staff members are trained to conduct specified maintenance (AZA Accreditation Standard 10.2.1).

AZA Accreditation Standard

(10.2.1) Critical life-support systems for the animal collection, including but not limited to plumbing, heating, cooling, aeration, and filtration, must be equipped with a warning mechanism, and emergency backup systems must be available. All mechanical equipment should be under a preventative maintenance program as evidenced through a record-keeping system. Special equipment should be maintained under a maintenance agreement, or a training record should show that staff members are trained for specified maintenance of special equipment.

1.2 Light

Careful consideration should be given to the spectral, intensity, and duration of light needs for all animals in the care of AZA-accredited zoos and aquariums.

Although it is difficult to assess all of the effects of photoperiod on large canids, some species, such as gray wolves, red wolves, coyotes, and maned wolves, have seasonal reproductive cycles and seasonal coat shedding cycles that are related to the photoperiod (Kreeger, 2003). For coyotes, red wolves, gray wolves, and maned wolves, the timing of the seasonal estrous cycle is highly influenced by photoperiod (see Chapter 7, section 7.1 for additional information). Therefore, it is generally recommended that large canids be kept under natural light with a natural diurnal cycle; this is best accomplished by housing them in outdoor enclosures.

The artificial lighting within indoor holding areas used for veterinary or temporary husbandry purposes should mimic the natural light cycle of the local holding institution, and lights can be placed on timers to simulate appropriate light cycles. Skylights can also be used to utilize natural light within indoor areas. All of the effects of photoperiod and reversed seasonality in the keeping of large canids from the southern hemisphere that are kept in the northern hemisphere may not be completely understood and might be an area of future research.

1.3 Water and Air Quality

AZA-accredited institutions must have a regular program of monitoring water quality for collections of aquatic animals and a written record must document long-term water quality results and chemical additions (AZA Accreditation Standard 1.5.9). Monitoring selected water quality parameters provides confirmation of the correct operation of filtration and disinfection of the water supply available for the collection. Additionally, high quality water enhances animal health programs instituted for aquatic collections.

AZA Accreditation Standard

(1.5.9) The institution must have a regular program of monitoring water quality for collections of fish, pinnipeds, cetaceans, and other aquatic animals. A written record must be maintained to document long-term water quality results and chemical additions.

As the primary enclosures provided to large canids are typically outdoor areas with constant fresh air, normal air quality considerations are generally not applicable. Veterinary or temporary husbandry holding buildings should have at least 15-20 fresh air changes per hour, and sufficient ventilation to allow wet surfaces to dry quickly (Norwegian School of Veterinary Science Lab Animal Reference Centre, 2007). However, care should be taken to avoid drafts within indoor enclosures, especially in whelping areas (see Chapter 7, section 7.4 for additional information).

Water sources and quality: Large canids will regularly drink from or splash in water features within their enclosures, and a source of clean drinking water should always be available in the form of a water bowl or clean pool. Large canids do not appear to be competitive over access to water if it is freely provided. Small, shallow pools are recommended for large canid enclosures in zoos and aquariums where summer temperatures are extreme, as animals will use the pool to regulate their temperatures. The safety

precautions described in Chapter 2, section 2.2 for the use of wet moats as containment barriers should be applied to all water features. Pools should also be drained to an appropriate level when young pups are present in the enclosure (see Chapter 7, section 7.4).

As natural water features are difficult to monitor for water quality and depth, they are generally discouraged. Water features, such as moats, streams and pools, may be kept clean by using regular dump-and-fill, constant change, or filtration and sterilization methods. When water bowls or drinkers are provided, they should be emptied and refilled daily and disinfected weekly. Some large canids will defecate or urinate in standing water, water bowls, or pools, so water quality should be carefully monitored throughout the day. All cleaning aids, disinfectants, and chemical agents used to clean water bowls and water features should be safe, nontoxic, and biodegradable. Veterinarians at each institution should develop disinfection protocols for water features, and identify safe and effective cleaning and disinfecting products.

1.4 Sound and Vibration

Consideration should be given to controlling sounds and vibrations that can be heard by animals in the care of AZA-accredited zoos and aquariums. Large enclosures and multiple hiding places within enclosures provide large canids with some opportunities to escape or cope with any unexpected loud noises. Features such as plantings and hedgerows inside or outside of an enclosure may also help muffle undesirable noises. However, large expanses of artificial rock wall (e.g., gunite) may magnify undesirable noise, and should be avoided in the design of large canid enclosures. The acoustic environment of a concrete-walled indoor space may be very noisy and disturbing to large canids.

Excessive sound stimuli can result in behavioral and physiological responses in animals (Coppola et al., 2006), and in extreme cases can be a contributing factor to immunosuppression, intestinal problems, and other veterinary health disorders relating to glucocorticoid responses (Spreng, 2002). Sales et al. (1997) and Coppola et al. (2006) discuss sound stimuli in relation to domestic dog welfare in kennel/shelter environments, and this research may be useful in the development of more specific sound and vibration recommendations for large canids. A more complete understanding of the sensitivity of large canids to sounds and vibrations and the effects on their well-being is an area in need of research.

Chapter 2. Habitat Design and Containment

2.1 Space and Complexity

Careful consideration should be given to exhibit design so that all areas meet the physical, social, behavioral and psychological needs of the species. Animals should be displayed, whenever possible, in exhibits replicating their wild habitat and in numbers sufficient to meet their social and behavioral needs (AZA Accreditation Standard 1.5.2).

AZA Accreditation Standard

(1.5.2) Animals should be displayed, whenever possible, in exhibits replicating their wild habitat and in numbers sufficient to meet their social and behavioral needs. Display of single specimens should be avoided unless biologically correct for the species involved.

Enclosure size: Large canids should be provided with large, complex outdoor spaces. Enclosure shape, topography, substrate, plantings, and proximity to the public should all be considered in the design process. A large and varied enclosure will provide greater opportunity for canids to express their full range of natural, species-appropriate behaviors, and eliminate the associated stress and unnatural behaviors associated with housing in low quality environments.

General housing guidelines for large canids are listed in Table 5 below. The size of a primary enclosure for the long-term holding of a large canid should be at least 465 m^2 (5,000 $ft^{2)}$; the more solitary maned wolf should be provided with larger areas: >930 m^2 (10,000 ft^2). Canids kept in undersized areas may show diminished well-being as evidenced by pacing, aggression, nervousness, poor reproduction, and poor care of offspring. An appropriately sized enclosure is dependent upon the size of the social group, the reproductive state of the individuals in a social grouping, and the enclosure complexity. Enclosures of the sizes listed below have resulted in healthy, long-lived canids that are able to express species-appropriate behaviors, and these enclosure size recommendations (together with enclosure complexity and variability considerations discussed below) are considered adequate by the AZA Canid TAG to prevent abnormal or maladaptive behaviors in these species.

Table 5: General housing recommendations for large canids

Social group	Primary enclosure	Holding area
Same sex group of 2 animals or non-reproductive pair	- 465 m^2 (5,000 ft^2) - Maned wolf: 930 m^2 (10,000 ft^2) - Add 93 m^2 (1,000 ft^2) for each addition member of a compatible same sex or non-reproductive grouping	2 holding/shift pens of 19 m^2 (200 ft^2) each
Single generation breeding enclosure	- 930 m^2 (10,000 ft^2)	3 holding/shift pens of 19 m^2 (200 ft^2) each
Multi-generational breeding enclosure	- 930 m^2 (10,000 $ft^{2)}$ or larger - Plus a secondary enclosure of 465 m^2 (5,000 ft^2)	3 holding/shift pens of 19 m^2 (200 ft^2) each
Potential grouping for reintroduction to the wild	- 1,860 m^2 (20,000 ft^2) - Plus a secondary enclosure of 465 m^2 (5,000 ft^2)	3 holding/shift pens of 19 m^2 (200 ft^2) each

Providing minimum safe distances for individuals to move within social groups, as well as around humans entering enclosures with the animals, should be carefully considered for these social but competitively aggressive predatory species. In non-competitive circumstances, large canids in a natural social group will rest and move within several feet of one another. However, the inter-individual distances maintained by large canids are dependent upon the individual social relationships within the group, which can change over time. The shape of enclosures is in important factor. For example, a 465 m^2 (5,000 ft^2) enclosure with dimensions 20 m x 23 m (70 ft x 72 ft) is better than a 3 m x 155 m (10 ft x 500 ft) enclosure. Multiple visual barriers within enclosures will decrease the stress associated with proximity to an aggressive competitor. However, all facilities housing large canids should have sufficient holding space to accommodate irreconcilable aggression between conspecifics if this occurs.

Enclosure design: Large canids are primarily cursorial animals, and should be provided with flat, smooth ("run-able") ground so that they can walk, trot, or run. Earthen berms or long slopes are desirable topographical features. However, enclosures should not be barren. Many plants, furniture of varying sizes, and a variety of walk-able surfaces are highly recommended. Enclosures should be furnished with deadfall, logs, or boulders, and should be planted with trees and shrubs to provide shelter, shade, and escape from conspecifics. Natural or artificial shelters should be provided in both on- and off-exhibit areas to allow animals privacy and escape from inclement weather or insects. Examples of appropriate shelters include: hollow logs, rock overhangs, underground dens, deadfall, logs, boulders, trees, bushes, holding buildings, and artificial den boxes made of wood or PVC (see Chapter 7, section 7.4 for additional information on dens). Many canids use elevated areas (e.g., berms, tops of den boxes, elevated platforms) for resting, which can promote better visibility for both canids and zoo visitors. Exhibit furniture should not be situated close to the primary containment barrier where animals could use it for climbing, jumping, and escape.

Large canids should be housed on natural substrates such as grass, dirt, sand, or forest litter. Natural substrates allow and encourage species-appropriate behaviors such as walking, trotting, running, caching food items, and digging shallow depressions for resting. The combination of substrate, topography, and furnishings will help to increase the quality of the exhibit from the animals' perspective. Large canids will occasionally dig their own dens, and may be encouraged to do so by providing the proper substrate in the enclosure. However, natural-made dens can make the task of inspecting, removing, treating, or monitoring adults and pups difficult and dangerous. There is also the potential for natural-made dens to collapse. The length, depth, and location of the den and soil type should be considered when deciding whether to fill in the den or allow its continued use by the animals (see Chapter 7, section 7.4 for artificial alternatives). The following substrates and natural enclosure materials have been provided to promote species-appropriate behaviors in large canids:

- Plantings and grasses
- Substrate piles/dirt and brush piles
- Tall grass patches for "hiding"
- Fallen trees
- Fallen leaves
- Pine cones, acorns and other mast
- Straw
- Dry grasses
- Sand
- Bark mulch
- Pine needles
- Pine shavings

The use of a variety of natural plantings (e.g., shrubs, trees, tall grasses) within outdoor enclosures will increase the natural variability of the environment, especially where grasses and shrubs are allowed to grow to maturity. Large canids will scent mark on plants and objects within their enclosures (see Appendix F for large canid ethograms). Water features, such as pools and streams, also provide a constant source of change within the exhibit.

Enclosures should be large enough and contain sufficient visual barriers to provide the occupants with a degree of privacy and ability to avoid the public, staff, and each other when desired. When large canids do not have sufficient visual privacy or feeling of security, they are more likely to show stereotypic abnormal behavior, including pacing, spinning or twirling, excessive self-grooming (licking or chewing), and a range of other abnormal or inappropriate behaviors and conditions such as increased agonistic interactions, diarrhea, hair loss, decreased appetite, weight loss, reproductive failure, or maternal neglect. Exhibits should have public viewing access limited to no more than 50% of the circumference. Animals should be provided with options for choosing to hide or to spot "danger" coming from a distance. Large canids will seek out opportunities for long views of their surroundings, and if too confined may exhibit the stress-indicating behaviors noted above.

Care should be taken when designing enclosures to avoid tight corners (<90°). Large canids tend to climb and jump in corners, and can also trap subordinates in these areas. A circular perimeter pen design may reduce stereotypic pacing and scattered running responses during capture procedures. All enclosures should be constructed in an area that drains well to prevent the collection of water in the yard, especially in the vicinity of the dens. Exhibits should also be designed to allow access to large equipment for landscaping and construction, so that exhibit furniture (e.g., large rocks, deadfall, etc.) can be easily added, moved, or removed from the enclosures.

Enclosure cleaning: Good sanitation, including daily removal of feces, old bones, and uneaten food from enclosures, reduces the incidence of intestinal parasites and disease in canids. Prompt removal also

eliminates the attraction of insects, such as biting flies, or other pests to the enclosure area. However, the benefits of daily cleaning should be evaluated in each facility and weighed against the stress it may cause the animals.

Hard-surface holding areas should be cleaned daily and disinfected weekly unless directed otherwise by a veterinarian. Removal of fecal material from natural substrate should occur daily. Food containers should generally be cleaned and disinfected daily. For facilities with multiple canid enclosures, animal care staff should utilize proper footwear and disinfecting procedures (e.g., footbaths filled with disinfectant) prior to entering any pen or holding area, in order to reduce the incidence and spread of intestinal parasites and disease. All cleaning aids, disinfectants, and chemical agents should be safe, nontoxic, and biodegradable.

Urine or feces marking: In the large canids, olfactory information among conspecifics is communicated via urine and feces that are deposited at ground level and on elevated objects throughout the exhibit, particularly along boundaries. For all species, the frequency of urine marking increases prior to and during the breeding season, with both sexes showing an interest in each other's urine marks. Animals can gain information about the reproductive status of a conspecific by sniffing and tasting the partner's urine.

Urine scent marks are not generally removed in well-planted and well-furnished, large, outdoor enclosures. Feces are often deposited in specific areas, usually around the perimeter of the exhibit. In some species, such as maned wolves, territories in the wild are marked by site-specific defecation spots (e.g., termite mounds, ant mounds, shrubs, trees, grass) (Dietz, l984). Since almost all the hormonal metabolites are excreted in feces, information about sex and reproductive status may be transmitted via this route as well (Velloso et al., 1998; Hodges, 1996). In outdoor dirt-floored enclosures, the daily removal of feces does not completely eliminate the scent information available to canids.

Red wolf and Mexican wolf: When red wolves and Mexican wolves are managed within zoos and aquariums for potential future release to the wild, enclosures are generally cleaned by keepers entering the enclosure with the animals. Training wolves to shift is considered too great of a 'habituation-to-humans' hazard for wolves that may be prone to conflicts with humans when released. For keeper safety in these circumstances, it is a strongly recommended management practice to have more than one person accessing an enclosure at a time. When moving about inside an enclosure for feeding or cleaning, keepers should remain together within the enclosure, and should move in a circular route. This allows the animals to avoid the keepers, and maximizes the spacing between keepers and animals. Cutting through the center of an enclosure or failing to remain together may inadvertently separate animals, causing confusion and increased levels of stress, as well as compromising keeper safety. Repeatedly using the same circular pattern should reduce the stress level experienced by the animals, as they will learn to recognize the feeding and cleaning routine (see Chapter 8, section 8.3 for additional information).

The same careful consideration regarding exhibit size and complexity and its relationship to the animal's overall well-being must be given to the design and size all enclosures, including those used in exhibits, holding areas, hospital, and quarantine/isolation (AZA Accreditation Standard 10.3.3).

AZA Accreditation Standard

(10.3.3) All animal enclosures (exhibits, holding areas, hospital, and quarantine/isolation) must be of a size and complexity sufficient to provide for the animal's physical, social, and psychological well-being; and exhibit enclosures must include provisions for the behavioral enrichment of the animals.

Holding areas: An ideal holding facility consists of a primary outdoor enclosure with an adjacent secondary enclosure and three shift pens (one indoors) adjacent to the primary enclosure. The AZA Maned Wolf SSP recommends up to one holding pen per animal for this species (AZA Maned Wolf SSP, 2007). Holding pens should be easily accessible from the main enclosure, and animals should be made familiar with them through feeding and/or continuous access. Shift areas should be accessible to animals at times other than capture or potentially stressful husbandry procedures so that the animals do not become reluctant to use them. All holding buildings should be well lit and ventilated, easily sanitized, and have remotely operated doors for shifting animals. Off-exhibit holding or shifting areas, particularly when used for restraint or capture, should be fully covered and high enough (2.1 m/7 ft) to allow for the flight/jump/climb response of an animal. Maned wolves and African wild dogs should have a temperature-controlled space in cold weather (see Chapter 1, section 1.1). Holding areas constructed of hard materials (e.g., concrete) should be covered with suitable soft substrates (described above) and bedding

materials (e.g., hay or straw) to prevent damage to foot pads and provide opportunities for animals to perform a wide range of species-appropriate behaviors.

2.2 Safety and Containment

Animals housed in free-ranging environments should be carefully selected, monitored and treated humanely so that the safety of these animals and persons viewing them is ensured (AZA Accreditation Standard 11.3.3).

AZA Accreditation Standard

(11.3.3) Special attention must be given to free-ranging animals so that no undue threat is posed to the animal collection, free-ranging animals, or the visiting public. Animals maintained where they will be in contact with the visiting public must be carefully selected, monitored, and treated humanely at all times.

Pests and predators: The containment features described in this section should deter entry into the enclosures by feral dogs and cats, skunks, raccoons, bobcats, coyotes, pumas, and foxes. These animals could pose a health risk to large canids from both an injury and a disease/parasite perspective. Feral animals can serve as potential sources of pathogens such as rabies, yersiniosis, leptospirosis, salmonellosis, toxoplasmosis, parvovirus, and canine distemper (AZA Maned Wolf SSP, 2007). When pest control programs are implemented within an institution, extreme caution should be exercised to ensure that animals do not consume rodents that have ingested rodenticides, and at no time should large canids have primary access to any rodenticide compound used. Rodent pests should be handled through a well-planned, veterinary-supervised, continuous pest control program.

Animal exhibits and holding areas in all AZA-accredited institutions must be secured to prevent unintentional animal egress (AZA Accreditation Standard 11.3.1). Exhibits in which the visiting public may have contact with animals must have a guardrail/barrier that separates the two (AZA Accreditation Standard 11.3.6).

AZA Accreditation Standard

(11.3.1) All animal exhibits and holding areas must be secured to prevent unintentional animal egress.

AZA Accreditation Standard

(11.3.6) Guardrails/barriers must be constructed in all areas where the visiting public could have contact with other than handleable animals.

Exhibit design should be considered carefully to ensure that all areas are secure, and particular attention should be given to shift doors, gates, keeper access doors, locking mechanisms, and exhibit barrier dimensions and construction. The containment of large canids in an enclosure should have a dig barrier, a perimeter wall or a moat, and a climbing barrier if walls can be climbed. A double-door enclosure access system leading to all areas containing large canids is highly desirable, and a secondary perimeter fence is strongly recommended to surround all holdings of large canids.

Dig barriers: The entire enclosure perimeter should have an underground component to prevent animals from digging out. Large canids that are seeking to escape an enclosure by digging will generally begin to dig at the perimeter fence or wall. Care should be taken to monitor all digging, wherever it occurs within an enclosure. To prevent digging out at the perimeter barrier, a 90 cm (3 ft) wide section of chain-link fencing can be installed by lacing it with smooth wire or “hog rings” to the base of the vertical fencing. This digging barrier should come to the fence at approximately 90° to the vertical fence, and should be buried 15-30 cm (6-12 in) below ground level. A concrete footing 15-20 cm (6-8 in) wide and 90-120 cm (3-4 in) deep should be poured at all gates to prevent digging at these areas, and any other areas where a digging barrier cannot be installed.

Mesh walls and climbing barriers: Large canids have the ability to climb all types of mesh fencing. When this type of barrier is used, a climbing barrier should ensure complete containment. Perimeter mesh walls should be of sufficient strength, gauge, opening size, and tautness to prevent the animals from tearing, snipping, or stretching the mesh, or from becoming entangled in it. Nine gauge or heavier steel wire in 5 cm (2 in) square chain-link fabric suspended on 6.1 cm (2.4 in) metal line pipes and 7.4 cm (2.9 in) terminal posts that are set in concrete satisfies all the requirements for large canid mesh wall containment.

Vinyl-coated wire is not recommended because canids can easily chew off and may swallow pieces of the coating. Plastic or nylon mesh will not permanently contain most large canids. Cable mesh can be used, but should be sufficiently taut to prevent an animal (of any age) that attempts to escape from getting its foot entangled, and preventing animals pushing their muzzles through far enough through the

cables to allow them to use their carnassial teeth to snip through it. Frequent fence inspection is strongly recommended, as one facility experienced a failure with 9-gauge chain-link fabric, and grey wolves are capable of snipping 9-gauge cable wire with their carnassials. All mesh fencing should be checked for gaps or stretching at bar attachment and extra precautions should be taken where there is any compromised fencing that would allow a canid to get its carnassial teeth close enough to the mesh to bite it.

As canids are skillful climbers and good jumpers, it is recommended that the vertical height of a mesh wall be at least 2.5 m (8.2 ft) including the addition of a 1 m (39 in) overhanging climb barrier extending into the enclosure at a 35-45° upward angle. Maned wolves can be contained with a 2.2 m (7.2 ft) vertical wall together with an overhanging climbing barrier. Overhanging mesh wire climbing barriers need not be as heavy a gauge as the primary mesh barriers, as there will not be many (or any) opportunities for large canids to chew on or snip through the overhanging barrier. Horizontal wire strands or hot wire are not generally recommended for use as climbing barriers, as they will not contain a highly motivated large canid attempting to climb out. Hot wire can be used as a feature to discourage a large canid from using an area within an enclosure, but it should not be used as a primary containment barrier.

Mesh used as a common containment barrier between conspecifics or another species should have an opening size (e.g., ≤2.5 cm x 2.5 cm/1 in x 1 in) that prevents any possibility of individual animals at any age from getting body parts (e.g., paw, tail, etc.) through the containment barrier into the adjacent enclosure. For additional information on interactions between conspecific groups in adjacent enclosures see Chapter 4, section 4.2. Holding enclosures for large canids should also be constructed of mesh this size.

Solid walls: Solid walls used as primary containment barriers for maned wolves should not be less than 2.2 m (7.2 ft), or 2.5 m (8.2 ft) for other large canid species. Solid walls 3.5 m (11.5 ft) tall are sufficient to contain large canids, as long as the walls cannot be climbed. Solid walls that are less than this height, and any areas where solid walls of any height can be potentially climbed, should have a climbing barrier as described above. Hybrid walls with the lower part solid and the upper part mesh with a climbing barrier are commonly used for large canids in zoos and aquariums. For zoos and aquariums in climates where large amounts of snow are possible during the winter, care should be taken to ensure that snow banks do not provide a means for animals to escape their enclosures.

Moats: Dry moats are effective at containing large canids if the vertical containment wall meets the vertical parameters stated above for solid walls, and the horizontal jumping distance is at least 5.5 m (18 ft) for most large canid species, or 3.7 m (12.1 ft) for maned wolves. Within the enclosure, the moat should be sloped (~30° from horizontal) facing into the center for at least part of the distance, in order to allow animals the ability to get out of the moat and back into the enclosure. If a dry moat is used when pups are present, all sides facing into the enclosure should be sloped. For zoos and aquariums in climates where large amounts of snow are possible during the winter, care should be taken to ensure that snow banks do not provide a means for animals to escape their enclosures.

Wet moats containing water should be of the same horizontal and vertical dimension as the dry moat, with a climb barrier on the exterior side of the wet moat. Most large canids are good swimmers. Wet moats should be of the same depth as dry moats so that they provide effective containment even if accidentally drained. Wet moats should also have a ledge on all edges facing into the enclosure where there is not a sloped bottom, so that animals can get out of the water easily. If pups are present, a wet moat should not be used. If the wet moat is drained with pups present, the side of the moat facing into the enclosure should be sloping. For zoos and aquariums in climates where temperatures drop below freezing during the winter, care should be taken to ensure that frozen water within wet moats does not provide a means for animals to escape their enclosures.

Other types of containment: Metal bars can be used as primary containment. If used, bars should be spaced closer than 5 cm (2 in) apart to prevent the limbs or heads of pups from becoming trapped. This spacing also minimizes the likelihood of injuries that may result from animals biting on the bars. Metal bars should not be used to separate large canids from conspecifics or other carnivorous mammals. Metal bars will not prevent a paw or a tail from slipping into an adjacent enclosure resulting in injury. The use of metal bars for new large canid enclosure construction is not recommended.

Transparent barriers, such as glass, Plexiglas, and Lexan, offer a pleasant unobstructed view of canids for visitors. Care should be taken when introducing new canids to enclosures with viewing

windows, as animals may initially perceive these transparent barriers as open space and accidentally run into them. Temporarily striping the barrier with tape may help. Hot wire can be used as a feature to discourage a canid from using a particular area within an enclosure, but never as a primary barrier.

Small holding or shift pens: For dirt floor holding pens of <46.5 m^2 (500 ft^2), galvanized chain-link mesh should be buried under the entire pen as an anti-dig barrier. Concrete floors will also prevent digging, and are useful for temporary veterinary enclosures, but may cause raw footpads in nervous canids. Concrete floors should only be used in enclosures housing large canids for short-term periods. It is advisable to have a solid or mesh roof completely covering small holding and shift pens of this size for complete containment, especially if animals are to be captured and restrained within the enclosure.

Doors: Doors used in large canid enclosures can be solid or mesh, and made of materials similar to those described above for primary exhibit containment. Slide doors or guillotine gates that animal caretakers can operate without entering the exhibit are useful for large canid management. These gates should have an opening of at least 91 cm (36 in) high and 61 cm (24 in) wide, and should be securable in the closed position; a motivated large canid can lift an unsecured guillotine gate. Swing gates can be used for animal shifting, but are less efficient and may require entry into the exhibit to open or secure. Swing gates are best reserved for staff access. For the safety of the animals and staff, any gaps between the doors and the containment walls should be less than 5 cm (2 in). It is recommended that animal caretakers entering a canid enclosure should pass through two levels of containment (e.g., primary and secondary). This design feature should be considered in the facility design.

Secondary perimeter fencing: Large canid facilities should be contained within the institution's USDA standard perimeter fence (AWR 2005). This fence should be 2.5 m (8 ft) high with gates that can be closed if an animal escapes its enclosure. The perimeter fence provides staff with an opportunity to capture an escapee before it leaves the institution.

Safety: Maintenance checks as well as fence and perimeter inspections should be part of a keeper's daily routine. Fence lines should be inspected daily to detect any need for repairs, sharp protrusions, and to fill in substantial holes to prevent possible escape or injury from digging. Fence integrity is imperative to the safety of all the animals in an enclosure. Large canid enclosures should be also checked daily to ensure that canids cannot injure themselves on any exhibit furniture or enrichment initiative provided to the animals.

All emergency safety procedures must be clearly written, provided to appropriate staff and volunteers, and readily available for reference in the event of an actual emergency (AZA Accreditation Standard 11.2.3).

AZA Accreditation Standard

(11.2.3) All emergency procedures must be written and provided to staff and, where appropriate, to volunteers. Appropriate emergency procedures must be readily available for reference in the event of an actual emergency. These procedures should deal with four basic types of emergencies: fire, weather/environment; injury to staff or a visitor; animal escape.

Natural disasters: Natural disasters such as forest fire, hurricane and flood can sometimes be anticipated. Protocols should be developed that address moving animals at any time of the year, and should include crate and transportation availability, as well as an agreement with other zoos and aquariums in the local/extended area in regards to housing displaced canids on a temporary basis. It is advisable to plan a response in advance with the cooperation and guidance of local emergency agencies.

Staff training for emergencies must be undertaken and records of such training maintained. Security personnel must be trained to handle all emergencies in full accordance with the policies and procedures of the institution and in some cases, may be in charge of the respective emergency (AZA Accreditation Standard 11.6.2).

AZA Accreditation Standard

(11.6.2) Security personnel, whether staff of the institution, or a provided and/or contracted service, must be trained to handle all emergencies in full accordance with the policies and procedures of the institution. In some cases, it is recognized that Security personnel may be in charge of the respective emergency (i.e., shooting teams).

Emergency drills should be conducted at least once annually for each basic type of emergency to ensure that all staff is aware of emergency procedures, and to identify potential problematic areas that may require adjustment. These drills should be recorded and evaluated to ensure that procedures are being

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(11.2.4) The institution must have a communication system that can be quickly accessed in case of an emergency.

followed, that staff training is effective, and that what is learned is used to correct and/or improve the emergency procedures. Records of these drills should be maintained, and improvements in the procedures duly noted whenever they are identified. AZA-accredited institutions must have a communication system that can be quickly accessed in case of an emergency (AZA Accreditation Standard 11.2.4).

AZA-accredited institutions must also ensure that written protocols define how and when local police or other emergency agencies are contacted and specify response times to emergencies (AZA Accreditation Standard 11.2.5).

AZA-accredited institutions which care for potentially dangerous animals must have appropriate safety procedures in place to prevent attacks and injuries by these animals (AZA Accreditation Standard 11.5.3). Large canids are dangerous animals, and each institution should develop their own safety protocols applicable to their facility design, staffing responsibilities, and area operating procedures. These protocols should specifically address animal containment monitoring if/when animals are provided with 24/7 access to outdoor enclosures (e.g., the need for trained animal care staff to be present at all times during any 24-hour period, and after-hour response protocols for gun teams, etc.), but such protocols should be in place whether the animals have 24-hour access to the exhibit or not. For facilities that use hot wire as any part of their secondary containment system, back-up emergency generators should be considered.

Safety protocols should address animal escapes as well as natural disasters relevant to the location of the zoo or aquarium. Protocols should address moving animals at any time of the year if needed, and include crate and transportation availability, as well as an agreement with other zoos and aquariums in the local/extended area in regards to housing displaced canids on a temporary basis, if necessary.

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(11.2.5) A written protocol should be developed involving local police or other emergency agencies and include response times to emergencies.

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(11.5.3) Institutions maintaining potentially dangerous animals (sharks, whales, tigers, bears, etc.) must have appropriate safety procedures in place to prevent attacks and injuries by these animals. Appropriate response procedures must also be in place to deal with an attack resulting in an injury. These procedures must be practiced routinely per the emergency drill requirements contained in these standards. Whenever injuries result from these incidents, a written account outlining the cause of the incident, how the injury was handled, and a description of any resulting changes to either the safety procedures or the physical facility must be prepared and maintained for five years from the date of the incident.

Animal escapes: The following procedures address responses to red wolf and Mexican grey wolf escapes, and should be followed to increase the chance of a quick and safe recovery of an escaped wolf (Waddell, 1998). These protocols can also be further customized by institutions so that there are applicable escape responses for any of the large canid species.

- The relevant AZA Canid SSP Coordinator should be immediately informed of the escape after escape protocols have been initiated by the institution, regardless of time or day. The Coordinator should be called even if the animal has been quickly recaptured, so that details of the escape can be reviewed. These discussions are very valuable to the AZA Canid SSP Programs, should other escapes occur (Waddell, 1998).
- To increase the chances of recapturing an escaped animal within the facility's perimeter fence (when visual contact has been lost), food can be placed at strategic locations with the facility, such as outside of the animal's enclosure, in undisturbed areas, and near natural runways such as old roads, paths, etc. (Waddell, 1998). The food and the area around the food should be checked morning and evening for evidence of the presence of animals, including tracks. Animals that escape an institution's perimeter fence will generally seek shelter (e.g., along wooded streams, old roadways, path margins, etc.), but may also be found in more populous areas. Depending on the species involved, if animals outside of the perimeter fence are not recaptured within 24 hours, regulating agency program personnel (e.g., USFWS) should be contacted by the relevant AZA Canid SSP Coordinator, and they should be accompanied to the site to assist with the capture, as appropriate (Waddell, 1998).
- All institutions should have appropriate capture equipment suitable for use during animal escapes, including 3-4 functional nets (see Chapter 6, section 6.5), catch poles, transfer crates, Cap-Chur® or Telinject® equipment (e.g., gun, jab stick, drugs, etc.), current county road maps,

hand-held radios, and appropriate personnel available to handle any situation at any hour (Waddell, 1998).

- Institutions should develop appropriate channels of communication with local law enforcement and media. Effective and open channels of communication with USDA inspectors can also be very beneficial, and they should be contacted as soon as possible after any animal escapes. If an animal has left the institution, and has not been recaptured within 24 hours, the local community should also be told (Waddell, 1998). In all media contacts, efforts should be made to allay the public's fear to the degree possible (e.g., the animal is shy and afraid of humans, but might respond aggressively to humans if cornered).
- After the escape has been resolved, a follow-up report from the AZA Canid SSP Institutional Representative (IR) should be sent to the relevant AZA Canid SSP Coordinator. This report should address important points such as: 1) how the escape occurred; 2) corrective measures that will be taken; 3) when escape was detected; 4) food provided to attract wolf; 5) news media interactions and response; 6) whether the animal was recaptured and how. This information can be used to evaluate these procedures and minimize the likelihood of subsequent escapes, while maximizing response and efficiency in future escapes (Waddell, 1998).

Animal attack emergency response procedures must be defined and personnel must be trained for these protocols (AZA Accreditation Standard 11.5.3). In many states, the state or county public health department should be notified of any bites from large canids. These public health agencies may dictate the actions to and fate of the biting animal. It is important that each facility have a good relationship with their public health department and share accurate information with them.

Emergency Drills: It is recommended that all institutions include large canids as key species in any animal attack emergency drills, and that appropriate training be provided to animal caretakers involved in any aspect of large canid management and care. Given the wide range of institutional staff and facility set-ups, no specific emergency/animal attack response recommendations can be provided that will be applicable to all zoos and aquariums. It is recommended that all staff members at institutions housing large canids be involved in the process of developing safety procedures, staff training protocols, effective documentation procedures, and documentation templates, which make the most sense for the staff, equipment, and local conditions specific to their institution.

Animal attack emergency drills should be conducted at least once annually to ensure that the institution's staff know their duties and responsibilities and know how to handle emergencies properly when they occur. All drills need to be recorded and evaluated to ensure that procedures are being followed, that staff training is effective, and that what is learned is used to correct and/or improve the emergency procedures. Records of these drills must be maintained and improvements in the procedures duly noted whenever such are identified (AZA Accreditation Standard 11.5.3).

If an animal attack occurs and injuries result from the incident, a written account outlining the cause of the incident, how the injury was handled, and a description of any resulting changes to either the safety procedures or the physical facility must be prepared and maintained for five years from the date of the incident (AZA Accreditation Standard 11.5.3).

Chapter 3. Transport

3.1 Preparations

Animal transportation must be conducted in a manner that adheres to all laws, is safe, and minimizes risk to the animal(s), employees, and general public (AZA Accreditation Standard 1.5.11). Safe animal transport requires the use of appropriate conveyance and equipment that is in good working order.

The permitting requirements for shipping large canids and the specific responsibilities of shippers and receivers are summarized below

AZA Accreditation Standard

(1.5.11) Animal transportation must be conducted in a manner that is safe, well-planned and coordinated, and minimizes risk to the animal(s), employees, and general public. All applicable local, state, and federal laws must be adhered to.

Table 6: Animal transport responsibilities

Responsibilities of shippers	Responsibilities of receivers
• Airline reservation and confirmation. • Contact airline freight manager involved in shipment by phone to discuss nature of the animal and special precautions (e.g., regarding heat, ventilation, sound) that are applicable. • Call to airline on day of travel to confirm times and check for cancellations due to technical problems or weather conditions. • Transport animal to airport 2-3 hours prior to flight, or as directed by the airline. • Contact receiving facility to confirm shipment has been made, and/or to inform them of any changes.	• Call airlines to confirm connections, arrival time, and/or any changes to itinerary (e.g., animal loaded on an earlier arriving flight). • Call shipping institution to confirm safe arrival of animal once animal has arrived and been transported to receiving facility. • Return transport crate to shipping institution if requested.

Forms and permits: Paperwork required to accompany the animal includes, but may not be limited to: two copies of a health certificate from the shipper's veterinarian; two copies of a USDA animal transfer form; and animal health records with the animal(s) studbook and transponder number (Waddell, 1998). USFWS and CITES permits may also be needed, and state-specific permits may be required. Animal data transfer forms, available through the American Association of Zookeepers (AAZK), should also be included. Labels for the outside of the crate showing the shipper's name and address, the receiver's name and address, and any additional instructions, should be attached to the crate (Waddell, 1998).

Pre-shipment testing: Prior to transport, all large canids should undergo a pre-shipment veterinary examination. This examination should include a complete physical examination, CBC/chemistry panel, fecal examination, urinalysis, heartworm test, and thoracic and abdominal radiographs. In addition, the animal should be current on all recommended vaccinations. For more information on physical examinations and recommended vaccinations, see Chapter 6, section 6.4. Records of pre-transport veterinary assessments should be shared with the receiving institution, and veterinarians from both shipping and receiving institutions should be involved in the final decision to transport the animals.

Transport containers: All crates for international air transport should meet USDA (USDA, 2008) and International Air Transport Association (IATA, 2008) requirements for live animal transport. For domestic air transport and ground transport, USDA requirements should be met (USDA, 2008). For domestic air transport, IATA standards are frequently applied by air carriers. The requirements put in place by commercial air carriers should be carefully researched in the planning stage of animal shipments. The IATA Live Animals Regulations document is an annual publication which is routinely updated and so is subject to changes that can affect animal shipments. This publication should be considered a mandatory tool for proper shipping of live animals, and the most recent versions can be ordered from www.iata.org.

IATA requirements: The 2008 IATA Container Requirement #82 states that materials for large canid containers can be wood, metal, synthetic materials, welded mesh, and wire mesh. The frame should be made from solid wood or metal parts bolted or screwed together. It should be constructed so that it

cannot be damaged from continual biting or scratching at the corners. If the total weight of the container plus animal exceeds 60 kg (132 lb), metal bracing should be added to the frame. A sliding door should be included in the crate design. It can be made from the welded mesh ventilation front if required. It should have a secure means of fastening so that it cannot be opened accidentally.

The sides and door should be made of metal or solid wood. The front of the container should be constructed of welded mesh. The mesh should have a diameter that will prevent the animal protruding its nose or paws to the outside (e.g., <2.5 cm/1 in). The whole front should be covered by a sliding shutter that can be raised and lowered to permit feeding and watering. It should have two observation holes of at least 10 cm (4 in) in the upper part, and ventilation holes, with a minimum diameter of 2.5 cm (1 in) , spread over the remainder of the surface in order to provide good ventilation and yet leave the animal in semi-darkness. The roof should be solid wood or metal with ventilation openings over its surface. Crates for African wild dogs should have closed roofs

The main ventilation front should be supplemented by meshed openings along the upper part of the container walls and/or holes with a minimum diameter of 2.5 cm (1 in) spread over the top third of the sides and the whole of the back and top. These holes should be spaced both horizontally and vertically at intervals of approximately 10 cm (4 in) center to center. It is essential that there be ventilation provided in the lower third of the sides for the removal of harmful waste gases. The total ventilated area should be ≥20% of the total surface area of all four sides. More ventilation and the use of larger meshed openings are permitted, but the animal should not be able to protrude its nose or paws to the outside from any opening. If the mesh is fixed to the interior of the container all sharp edges should be protected.

Spacer bars should be made to a depth of 2.5 cm (1 in), and should be present on the sides of the container. Forklift spacers should be provided if the total weight of the container plus the animal exceeds 60 kg. (132 lb) Transport crates should be large enough to allow the animal to stand, sit, lie down naturally, and turnabout freely. A crate of interior dimensions of 101 cm x 69 cm x 76 cm (40 in (l) x 27 in (w) x 30 in (h)) will accommodate most large canids. The shipper should confer with the airline to ensure the dimensions of the selected crate fit within maximum size restrictions specific to the aircraft.

Plastic pet containers: IATA allows rigid, modified plastic pet containers to be used for some of the less destructive large canids, at the discretion of the carrier. Red wolves, grey wolves, and coyotes are regularly and safely transported in modified rigid plastic carriers as described below. Plastic pet containers should not be used for African wild dogs. The following modifications should be made for shipment of wolves and coyotes (IATA 2008):

- The grill door should be covered with a secured and sturdy fixed welded fabric (≤2.54 cm x 2.54 cm/1 in x 1 in), and all ventilation openings covered with wire fabric
- The door of the larger containers should have secure fastenings at the top and the bottom
- A curtain, which can be raised and lowered and does not impede ventilation, should be fixed over the door to reduce light inside the container (see also section 3.2)
- There should be ventilation openings on the rear of the container; extra ventilation openings may have to be made to provide a total ventilation area of ≥20% of the four sides
- Food and water containers should be fixed inside with access from the outside. This is not recommended for red wolves, and a letter of justification by the attending veterinarian for this exemption should be included
- The container should be correctly labeled.

Crate training and medication: Crate training prior to shipment should be considered a standard management procedure for large canids. The routine use of a catch box as part of daily husbandry practices will aid in training an animal to be familiar with confinement, and not consider it to be an aversive experience. This training should eliminate the need for sedation prior to transport (see below). See Chapter 8, section 8.1 for additional information on operant conditioning used with large canids. Crate training is not recommended for red wolves or Mexican gray wolves that may one day be released to the wild. The habituation to animal caretakers required to accomplish crate training may significantly increase the probability of conflicts with humans upon the release of the wolves to the wild.

Large canids should not be sedated for any shipments. If an animal becomes ill during shipment while under sedation, it may not be possible for caretakers to clear its throat of any obstruction safely within the confines of a shipping crate. Sedated animals may also fall and injure themselves when trying to stand

without having a full sense of balance. The equipment should provide for the adequate containment, life support, comfort, temperature control, food/water, and safety of the animal(s).

Transport equipment: Safe animal transport requires the use of appropriate conveyance and equipment that is in good working order. The equipment should provide for the adequate containment, life support, comfort, temperature control, food/water, and safety of the animal(s). If reasonable, equipment such as a noose, medical kit, cellular phone, and nets should be taken along if/when animal caretakers accompany large canids during transport, as these may be needed in the event that any problems arise. All adult large canids should be shipped in separate transport containers. Up to three sibling pups younger than 8 weeks of age may be shipped together in a single container. The container should be large enough for all individuals to stand, turn, and recline comfortably.

Safe transport also requires the assignment of an adequate number of appropriately trained personnel (by institution or contractor) who are equipped and prepared to handle contingencies and/or emergencies that may occur in the course of transport. Planning and coordination for animal transport requires good communication among all affected parties, plans for a variety of emergencies and contingencies that may arise, and timely execution of the transport. At no time should the animal(s) or people be subjected to unnecessary risk or danger.

3.2 Protocols

Transport protocols should be well defined and clear to all animal care staff.

Food and water: Because of the hazard of motion sickness and regurgitation/aspiration, animals should be fasted for 12 hours prior to shipment. Water should be provided until the animal is crated for shipment. A water/food container should be provided in the crate, and should be constructed of durable materials (e.g., stainless steel) to prevent the animal from chewing or consuming it. Large canids can and will chew the small plastic dishes provided with purchased “airline” kennels. If animals are shipped without the USDA required food/water containers within the transport crate (see AWR, 2005), a letter from the shipping institution’s veterinarian is needed that states that they are not recommended. This exemption is needed to prevent delays or refusal to ship by the airline company. The AZA Red Wolf SSP recommends that food/water containers not be provided for red wolf shipments of less than 12 hours duration (Waddell, 1998). However, food and water should be on hand if delays occur during transport, and the shipment takes longer than 12 hours. In general, large canids having access to food and water prior to shipment as described above should not require feeding for 24 hours from their last feeding.

Bedding and substrates: In general, wood shavings are recommended as bedding during transport, but cedar should be avoided because of its strong odor. Straw or certain other agricultural products may be used, but these may not be allowed in some international shipments due to restrictions of the destination country. Bedding requirements of the destination country should be known before animals are shipped internationally. Wood shavings or straw bedding are also sufficient to separate large canids from their urine and feces during transport. In order to prevent urine or feces from spilling out of the crate into the aircraft during air transport, airlines may require a bedding material to cover the bottom of the crate. Wood slats secured to the bottom of the crate are not recommended. Large canids have been known to chew these into splinters.

Temperature, light, and sound: As canids can be susceptible to heat stress during transport, it is recommended that animals be shipped during the cooler months of the year, or at night during the summer months. Under no circumstances should transport crates containing animals be left for any period of time in direct sunlight. Large canids should not be shipped by air when the temperature is above 26.7°C (80°F) due to the risk of heat stress. Many airlines will not accept animal shipments if air temperatures are above 29°C (>84°F) or below 0°C (<32°F) at any point when the animal is on the ground. Where appropriate, a ‘Certificate of Acclimation’ can be provided by the shipping institution’s veterinarian to address any concerns from the airlines. Large canids that are transferred to different areas of the country are best transferred during a time of the year in which the animal will not experience an extreme change in temperature from the local environments of the shipping and receiving institutions. During the ground transportation of large canids, all ground transport vehicles should be well ventilated, and the ambient temperature monitored so that the animals do not become over-heated or chilled.

Placement of transport crates during shipments should avoid areas of excessive noise and commotion. A curtain can be fitted over the doors and windows to reduce light levels, but only if it does

not impede required ventilation. A 60% shade cloth is a good selection. Shade cloth is superior in ventilation capacity to burlap and is recommended by the AZA Red Wolf SSP. Most large canids are calmer in the reduced light of the small, den-like space of a shipping crate.

Animal monitoring: If transit time is expected to exceed 12 hours, it is recommended that an attendant from the shipping facility accompany the animal(s). If reasonable, equipment such as nets, noose, medical kit, and cellular phone should available to caretakers accompanying large canids during transport. Contact details for zoological institutions along the transport route (for ground transport) or at layover locations (for air transport) should also be available in case expert assistance is needed during the shipment, or if there is an unexpected delay.

The maximum amount of time for a large canid to be in transit should not exceed 24 hours. This does not include the time in the crate before transport from the original facility. Whenever possible, non-stop flights should be scheduled between the shipping points. Journeys that require plane changes involve longer layover times for the animal, with additional handling required by the airline, potentially undesirable holding areas (e.g., on the tarmac, within baggage areas), and a longer total crate time for the animal. If transit time exceeds 24 hours, special provisions should be made to ensure that the animal has access to food and water.

Post-transport release: Large canids should be allowed to release themselves from their transport crates immediately upon arrival at their final destination. Animals should first be released into to an unoccupied small (19 m^2/~200 ft^2) holding pen for veterinary evaluation (e.g., visual assessments) and so that they can easily access food and water made available to them. After assessment, they can be released into larger quarantine facilities, if available. A large canid may not choose to venture out from the security of its crate immediately. This is a normal response.

Chapter 4. Social Environment

4.1 Group Structure and Size

Careful consideration should be given to ensure that animal group structures and sizes meet the social, physical, and psychological well-being of those animals and facilitate species-appropriate behaviors. Large canids express a wide variety of social preferences, from seasonal monogamy in maned wolves, to large, multigenerational packs of some dholes, African wild dogs, gray wolves, and red wolves. Coyotes exhibit social structures ranging from single/pair transients to small generational packs. There is social flexibility within many species related to environmental conditions, territory size, prey availability, and prey size. Social relationships within a group are determined by age, sex, breeding season, dominance ranking, and may involve extreme aggression. Dominance interactions in an aggressive predator like a large canid can be lethal.

Red and gray wolves, African wild dog, and dhole: The basic social unit for these species is the breeding alpha pair and young of the year. This grouping works best in zoos and aquariums. The reality of managing a large population of these canids in collaboration with many other zoos is that replicating social conditions observed in the wild is challenging. These species form multigenerational packs in the wild. The benefits of this social structure are far-reaching. The primary litter has the benefit of experiencing and assisting with the growth and development of the secondary litter. The secondary litter likewise can greatly benefit from all aspects of group social interaction including, but not limited to, “aunting” behavior, play, aggression, dominance and submission, vocal communication, hunting, and feeding activities. The only constraint on multigenerational packs in zoos and aquariums is the maturation of the young with the potential for incest or group conflict resulting from the inhibition of dispersal.

Except for maned wolves, short-term groupings of large canids in the wild that are outside of the typical “territorial alpha pair/pack” paradigm are often seen, but appear to be temporary situations; a reflection of the affiliative nature of these species. Besides the basic social unit described above, these species of large canids may be managed as contracepted pairs, contracepted pairs with non-breeding age young, post-reproductive pairs, same-sex groupings, as individuals, or in some other grouping. Fortunately, canid social flexibility permits significant variation in management groupings. Alternative social groupings for red and gray wolves, African wild dogs, and dholes (and some of the problems associated with these groupings) are described below.

Breeding pairs with young: Breeding pairs with young rarely have social incompatibility problems until the pups reach approximately 18 months of age. With the exception of male African wild dogs living in their natal pack, adolescent animals would normally disperse from the family group at this time. Without the ability to disperse, social and sexual maturation in the family pack can result in incest and/or pack conflict. It is best to remove 18-month-old individuals from the pack and keep them separated by sex, except for male African wild dogs living in their natal pack. Contraception of all the reproductively mature members in a large family pack containing adults and their mature offspring has not been effective in reducing pack conflict.

Contracepted pairs: Contracepted pairs work well in zoos and aquariums for the lifetime of that pair. However, see Chapter 7, section 7.6 for the long-term effects of contraception in large canids.

Contracepted alpha pairs with pups: Contracepted alpha pairs with pups may work well for many years, if all the pups are of the same sex. The presence of the alpha pair or a single same-sex parent may reduce the frequency of the conflicts that are sometimes seen with same-sex groupings. If the pups are of both sexes, one same-sex group should be removed from the pack at 18 months of age to prevent breeding.

Same-sex groups: Same-sex groups work best if the individuals are siblings. These groupings usually work better with all-male groups than all-female groups. If same-sex groups of unrelated individuals or individuals of different ages are attempted, animal caretakers should be prepared to remove an individual if group conflict and injury are observed. This approach has been attempted successfully with Mexican wolves, but not with red wolves. If an individual is temporarily removed from a same-sex group, the remaining pack members may re-align their social structure in its absence. If attempts are made at returning this individual to its pack, it may have great difficulty re-establishing itself within the new group structure. There is the potential for significant social conflict and injury in these cases.

Uncontracepted single adult with young: There is a record in Mexican wolves of an uncontracepted single adult female with a male pup of ten months of age resulting in a pregnancy. This grouping is not recommended.

Single animals: Lone individuals are common in the wild. However, the inclination of individual large canids is to be with conspecifics. In zoos and aquariums, a lone large canid will do well by itself, but it will do better if it has a compatible companion.

Post-reproductive pairs: Compatible post-reproductive pairs generally do well for many years.

Maned wolf and coyote: Maned wolves and coyotes are best kept singly, as breeding pairs with young of the year, as contracepted pairs, or sometimes as same-sex sibling groupings. In the wild, maned wolf pairs defend a common territory, but rarely socialize outside of the breeding season. However, in zoos and aquariums, pairs are usually housed together year round, and males provide considerable parental care to the young.

Multi-generational packs of maned wolves are not seen in the wild, and are not recommended in zoos and aquariums. They are not likely to be successful. Formation of non-sibling same-sex groups has rarely been attempted with maned wolves, and has met with limited success. Introducing unrelated same-sex adults is not recommended, and introductions of unrelated same-sex animals less than 2 years of age should be very closely monitored. Same-sex sibling pairs or trios can be housed together for several years. Enclosures should be designed with multiple den sites, feeding stations, and resting/hiding areas to minimize aggression within these types of groups.

Coyotes are rarely bred in North American zoos and aquariums. They are usually non-releasable animals acquired from wildlife rehabilitation centers. It is not recommended to breed coyotes as this will utilize space needed for AZA SSP Program canid species. Contracepted pairs or male groupings of coyotes are the norm in zoos and aquariums. Same-sex groupings are typically small; two or three individuals. Sibling groups work better than trying to assemble a group of unrelated individuals. The problems encountered with Mexican gray wolf and red wolf same-sex groupings may also occur with coyote same-sex groupings, and so pack size should be kept small. Groups of female coyotes are generally unsuccessful.

Optimal group sizes: Table 7 provides a summary of best situations for the social housing of large canids. Certain social groups will be limited by enclosure size, as larger groups need correspondingly larger spaces. Large canids show a great range in social flexibility outside of these recommended group structures.

Table 7: Recommendations for social group composition

Species	Optimal social group	Alternative groupings
Maned wolf, red wolf	Male-female pair and pups (<10 months)	Same-sex sibling pairs or trios for maned wolves; same sex siblings for red wolves.
Mexican gray wolf, dhole	Male-female pair with 1st and 2nd generation litters (<1.5 years)	Litter mates (<1.5 years), or same-sex packs (related or unrelated individuals)
African wild dog	Male-female pair with female pups <1.5 years and male offspring of any age	Litter mates (<1.5 years), or same-sex packs (related or unrelated individuals)
Coyote	Male-female pair	Male sibling groups.

Same-sex group formation: When forming same-sex groups, sibling social groups work best, but tension and aggression may still occur particularly in female groups of some taxa (e.g., coyote), and especially when female groups are in close proximity to mature males. Many factors should be considered when attempting to form a non-sibling same-sex grouping of social canids. Introductions should occur under close supervision in large areas where each animal has the ability to retreat if it feels threatened (see section 4.3 for additional information on introductions). A number of steps can be taken to encourage the successful formation of same-sex packs, including:

- Providing multiple feed, water, and resting sites so that they cannot be monopolized by aggressive or dominant individuals.

- Creating open feeding areas so that all animals feel secure enough to feed.
- Ensuring that all areas are open during an introduction in a manner that provides no opportunity for an animal to be cornered by conspecifics.

The formation of a non-sibling same-sex grouping is most successful if all the animals arrive at the same time and are introduced on neutral ground. To increase the likelihood of success, the animals should be introduced outside of the breeding season. Age composition is also important. An older animal coupled with younger animals (no more than two years apart in age) has been the most effective approach. For Mexican wolves, there has also been some success in mixing subordinate same-sex individuals from large family packs into mixed-age, same-sex groups.

Separation of sexes and young: In the first few months of life, pups are completely dependent upon their dam for milk, warmth, and protection. Pups may also depend on their sire and often other members of the pack to provide additional food, protection, and instruction on social and predatory skills. In the wild, juvenile gray and red wolves, female African wild dogs, and dholes will typically disperse at 18 months of age. Male African wild dogs are an exception to this general rule; young males of this species may remain with the natal pack throughout their lifetime. By 18 months of age, individuals of most large canid species are also sexually mature, and can be socially and functionally independent of their natal pack.

In the wild, coyotes typically disperse in the autumn of their first or subsequent year (these individuals are 'transients'), unless they stay within the pack and maintain resident status. Behavioral suppression by the dominant pair (alphas) is common in natural environments, and this acts to suppress the reproduction of subordinate-ranking individuals that stay with the pack beyond sexual maturation. Reproductive suppression should not be relied on in zoos, however, as there are many examples where this has not occurred. In zoos, sexually mature young are typically removed from the breeding group to prevent incest and to maintain pack stability.

Little is known about the dispersal of young maned wolves in the wild. In zoos and aquariums, young maned wolves are usually separated from the parents before the onset of the following breeding season (at ~10 months of age) in order to prevent interference with subsequent breeding attempts. Siblings may be housed together for several years, although opposite-sex siblings should be separated before they reach two years of age to prevent reproduction.

4.2 Influence of Others and Conspecifics

Animals cared for by AZA-accredited institutions are generally found residing with other animals of their own species, but may also be found residing with animals of other species. Large canid packs are actively territorial. In zoos, the proximity of conspecific groups can influence the behaviors of both groups, but the extent of any disruption is variable. There are many instances of red wolf, Mexican wolf, and African wild dog pairs breeding and successfully rearing young with other breeding conspecifics or other large canid species in nearby enclosures. Although the olfactory capabilities of some large canids are highly attuned and are important to intra-specific communication, the long distance effects of odors from conspecifics and other species on canid husbandry and welfare have not been investigated.

Maned wolf: Maned wolf breeding pairs should be isolated as much as possible from other maned wolves. A solid barrier is recommended between enclosures containing breeding pairs. Maned wolves have successfully bred in close proximity to Mexican wolves and red wolves. Young (<2 years old) can be housed in adjacent enclosures to parents, but more research is needed to determine the potential effects on the parents' subsequent breeding and pup rearing (Maned Wolf SSP, 2007).

Mexican gray wolf, red wolf, African wild dog, dhole, coyote, and gray wolf: The recommendation for Mexican gray wolves and red wolves is a separation of at least 1 m (3 ft) between the enclosures of conspecific breeding pairs/groups. As with the maned wolf, a greater separation between breeding pairs might be prudent. A solid barrier is thought to be effective at minimizing any behavioral disruption that might occur, but this has neither been quantified nor frequently used with red wolves or Mexican gray wolves. The recommendation for proximity of breeding pairs for Mexican gray wolves and red wolves is also applicable for African wild dogs, dholes, coyotes, and gray wolves.

Mixed-species groups: With the exception of the maned wolf, it is not recommended to mix other species of animals with large canids, because they are successful predators with behavioral and physical

hunting adaptations that enable them to kill animals ranging in size from crickets to moose. One of the competitive ecological advantages that pack-forming large canids have over other carnivores is their ability to take on prey many times their own size. Nevertheless, some facilities have mixed gray wolves with bison and beaver with apparent success. Fish might be a more suitable alternative, since they would be mostly out of reach of the canids.

Maned wolf: Maned wolves have been successfully exhibited with tapirs, giant anteaters, and capybara. When mixed-species enclosures are attempted with maned wolves, the enclosure should be designed to meet the needs of all species, including an adequate number of den sites, water features, and feeding stations for all individuals. Hiding spots or escape areas should be provided to minimize chances of injury due to interspecific aggression. Providing each species with a means to avoid the other is an essential aspect of a successful mixed-species exhibit. Enclosures should be large enough to allow individuals to move around without coming into contact with the other species, and designed with a variety of topographical features. Mixed-species exhibits incorporating maned wolves should include all enclosure design elements described in Chapter 2, section 2.1. The majority of maned wolf mixed-species exhibits have involved same-sex pairs of wolves and 1-3 individuals of other species. This model has been successful, and is the recommended strategy.

4.3 Introductions and Reintroductions

Managed care for and reproduction of animals housed in AZA-accredited institutions are dynamic processes. Animals born in or moved between and within institutions require introduction and sometimes reintroductions to other animals. It is important that all introductions are conducted in a manner that is safe for all animals and humans involved.

General introduction procedure: The first step in the introduction process for large canids should be to place the animals in pens next to each other. Physical access should not be allowed initially. Introductions are normally made through howdy units or by utilizing adjacent shift/holding areas separated by mesh or chain-link fencing. The mesh should be small enough to prevent paws or muzzles from going through. An opaque/transparent single line barrier is adequate and desired to encourage interaction between animals without contact. This barrier should be 2.5 cm x 2.5 cm (1 in x 1 in) or smaller steel wire fabric or steel mesh; larger size mesh is not adequate to ensure that a paw or tail cannot be grabbed by the animal in the adjoining pen.

Visual and olfactory contact through a wire-mesh barrier for a period of time prior to physical access is critical. The length of time recommended for limited access is dependent upon the animals' behaviors, but 1-2 weeks is usually sufficient for individuals to become familiar with each other. Three types of behavioral interactions may be observed: friendly interaction, no interaction, or agonistic interaction. Introductions should be delayed if there are extreme agonistic interactions. Friendly interactions or no interactions are a good indicator that the introduction process can continue on to the next stage.

The next step in the introduction process is for each animal or each group to be given separate access to the same common area (one that will be used for the introduction) for a few days, before any physical introductions take place. This allows the new individual(s) time to become familiar with the enclosure. If all animals are new to the enclosure, sufficient time should be allowed for each animal to adapt to the area prior to introduction.

The final step in the process is the physical introduction of animals. Large canids should be physically introduced in an area that facilitates quick and safe separation of the pair/group if undue aggression occurs. Behaviors such as lunging, growling, gaping, and chasing can be expected during the initial introduction period. Wrestling and biting are also seen, and may occur until dominance relationships are established. Once animals are introduced, and if significant aggression is not observed, the animals should be allowed access to the common area together during the day and separated overnight for 1-2 weeks. If there is no significant aggression observed during this time period, the animals may remain together during the day and night. However, animals should be watched closely for wounds and other injuries. Severe aggression or significant injuries may require taking a step back in the process or abandoning the effort entirely.

If two groups of animals are introduced, it is generally best not to separate individuals from either group during the introduction. In some circumstances, it may be advantageous to manipulate group dynamics if animal caretakers are familiar with the behaviors and temperaments of individuals, for example, by temporarily removing particularly dominant and aggressive individuals while the remaining

members of the two groups integrate. However, even short-term separations of familiar animals can lead to alterations in the social hierarchy that can increase social conflict within the group.

It should be noted the introduction process described above is a very conservative approach for an introduction of new pairs, but a very reasonable approach for introducing more than two animals. The introduction could progress at a more rapid rate depending on the animals involved. It is rare for this process to be unsuccessful when introducing pairs. Brief fighting may occur until one individual establishes dominance. Some pairings have proven to be incompatible, however. Aggressive behaviors such as males chasing and being aggressive towards females, as well as females being distressed by the male's presence, have occasionally been documented during introductions. Any continuous significant aggression should not be allowed. The decision to delay or cancel an introduction due to excessive aggression should be made by animal managers and veterinarians.

Introduction of new breeding pairs: Moving maned wolves to accommodate AZA Maned Wolf SSP breeding recommendations has not been found to impact breeding success in a negative manner. It is advisable to move breeding maned wolves several months in advance of the breeding season so they have sufficient time to pass quarantine and adjust to new areas and keepers before introducing them to new mates. It has been noted that bonded pairs kept together for an extended period of time at the same institution have trouble making the adjustment to new surroundings and mates. For maned wolves, introductions of unfamiliar individuals usually proceed smoothly when timed to coincide with the approach of the breeding season.

While Mexican wolf and red wolf introductions to new mates are generally uneventful, interactions should be closely monitored for the first few days to assess compatibility. Although it is rare for introductions to be unsuccessful, there have been a few reports in Mexican wolves and red wolves of a resident wolf being overly territorial to a new animal. In addition, there have been reports of females in this scenario dominating an introduced male, which minimizes the likelihood of successful breeding. Dominance during introductions may be minimized by holding the resident animal in an adjacent holding area, and allowing the new animal solitary access to the enclosure to encourage its adaptation. Physical introductions should always be closely monitored by animal care staff. Some mild aggressive behavior should be expected as the pair works out dominance issues, but severe aggression may warrant separation and continued introduction through a barrier until aggressive behavior subsides.

Separations and reintroductions: The effects of removing pups from their parents or separating adult pairs or groups are highly variable. There have been reports of increased vocalization, pacing, or reduced appetite when bonded pairs have been separated and held apart during the breeding season. Other reports have described similar behavior when females have lost a litter or an individual has lost a mate. The group dynamic may change in the absence of an individual, and the individual may need to re-establish itself within the group if it is eventually reintroduced. It may be necessary to repeat the general introduction procedures as described above for reintroduced animals. In many cases, and particularly with African wild dogs, the removal of a pack member even for a very short time can result in severe injury upon its reintroduction. It is advisable to avoid separation of any pack member unless absolutely necessary.

In cases where a breeding pair of maned wolves has been separated for management purposes (e.g., prior to parturition), the sire can be housed adjacent to dam and pups and remain in visual contact. Bestelmeyer (1999) describes protocols for successful reintroduction of males to dams and pups when the pups are between 2-10 weeks old.

Chapter 5. Nutrition

5.1 Nutritional Requirements

A formal nutrition program is recommended to meet the nutritional and behavioral needs of large canids (AZA Accreditation Standard 2.6.2). Diets should be developed using the recommendations of nutritionists, the Nutrition Scientific Advisory Group (NAG) feeding guidelines (http://www.nagonline.net/Feeding%20Guidelines/feeding_guidelines.htm/), and veterinarians, as well as AZA Taxon Advisory Groups (TAGs), and Species Survival Plans® (SSP) programs. Diet formulation criteria should address the animal's nutritional needs, feeding ecology, and individual and natural histories to ensure that species-specific feeding patterns and behaviors are stimulated.

AZA Accreditation Standard

(2.6.2) A formal nutrition program is recommended to meet the behavioral and nutritional needs of all species and specimens within the collection.

Free-ranging large canids can consume a variety of animals and plants depending upon species, location, habitat, season, and prey availability. Prey species range from large ungulates to medium and smaller prey such as raccoon, nutria, rabbits, birds, eggs, fish, reptiles, and insects. When diets are developed, formulation criteria should address nutritional needs and feeding ecology, as well as individual and natural histories to ensure that species-appropriate feeding patterns and behaviors are stimulated.

Systematically collected data describing the nutritional requirements of non-domestic large canids is limited, and there are varying opinions regarding the best diet for canids housed in zoological institutions. Target nutritional ranges for domestic dogs in Tables 8, 9, and 10 provide a model to consider for application to non-domestic counterparts, although this is an area in need of research (see Chapter 10). Generally the nutritional requirements of non-domestic, large canids can be met by feeding a nutritionally complete, commercial dry dog food. There have been reports of individual large canids exhibiting diarrhea, poor condition, or allergies when fed high cereal dog foods of low to medium energy levels. A nutritionally complete, high-energy extruded dry dog food based on animal protein is less likely to result in these problems, and will maintain most large canids in good condition and with good fecal quality (Lindsey & Hopkins, 1995). Nutritional ranges for the highly omnivorous maned wolf may differ from that of the domestic dog. However, accurate nutritional requirements for maned wolves have not yet been articulated. Most African wild dogs and dholes in North American zoos are fed a nutritionally complete raw meat-based canine diet (see Appendix I).

Non-domestic large canid diets typically contain 20-28% protein, 5-18% fat, and 2-4% crude fiber. Large canids weighing 22-32 kg (48-70 lb) should be fed approximately 1,300-1,800 kcal of metabolizable energy (ME) per day for maintenance in a thermoneutral environment with moderate activity (NRC, 2006). This amount of ME would be supplied by 18 g per kg (0.63 oz per lb) of body weight of a nutritionally complete dry dog food (3.3 kcal ME/g), equating to 0.68-1.13 kg (1.5-2.5 lb) of dry dog food per day per adult animal. Energy requirements will vary with climate, activity level, reproductive status, and the needs of individual animals. Unless prescribed by a veterinarian or nutritionist, vitamin and mineral supplements are not necessary if these general guidelines are followed.

Table 8: American Association of Feed Control Officials (AAFCO) Domestic dog food nutrient profile[a] (www.fda.gov/animalveterinary)

Nutrient	Units DM Basis	Growth and Reproduction Minimum	Adult Maintenance Minimum	Maximum
Protein	%	22.0	18.0	
Arginine	%	0.62	0.51	
Histidine	%	0.22	0.18	
Isoleucine	%	0.45	0.37	
Leucine	%	0.72	0.59	
Lysine	%	0.77	0.63	
Methionine-cystine	%	0.53	0.43	
Phenylalanine-tyrosine	%	0.89	0.73	
Threonine	%	0.58	0.48	
Tryptophan	%	0.20	0.16	
Valine	%	0.48	0.39	
Fat[b]	%	8.0	5.0	
Linoleic acid	%	1.0	1.0	

[a] Presumes an energy density of 3.5 kcal ME/g DM, based on the "modified Atwater" values of 3.5, 8.5, and 3.5 kcal/g for protein, fat, and carbohydrate (nitrogen-free extract, NFE), respectively. Rations greater than 4.0 kcal/g should be corrected for energy density; rations less than 3.5 kcal/g should *not* be corrected for energy.
[b] Although a true requirement for fat per se has not been established, the minimum level was based on recognition of fat as a source of essential fatty acids, as a carrier of fat-soluble vitamins, to enhance palatability, and to supply an adequate caloric density.

Table 9: American Association of Feed Control Officials (AAFCO) Domestic dog food mineral profile[a] (www.fda.gov/animalveterinary)

Minerals	Units DM Basis	Growth and Reproduction Minimum	Adult Maintenance Minimum	Maximum
Calcium	%	1.0	0.6	2.5
Phosphorus	%	0.8	0.5	1.6
Ca:P ratio		1:1	1:1	2:1
Potassium	%	0.6	0.6	
Sodium	%	0.3	0.06	
Chloride	%	0.45	0.09	
Magnesium	%	0.04	0.04	0.3
Iron[c]	mg/kg	80.0	80.0	3000.0
Copper[d]	mg/kg	7.3	7.3	250.0
Manganese	mg/kg	5.0	5.0	
Zinc	mg/kg	120.0	120.0	1000.0
Iodine	mg/kg	1.5	1.5	50.0
Selenium	mg/kg	0.11	0.11	2.0

[c] Because of very poor bioavailability, iron from carbonate or oxide sources that are added to the diet should not be considered as components in meeting the minimum nutrient level.

[d] Because of very poor bioavailability, copper from oxide sources that are added to the diet should not be considered as components in meeting the minimum nutrient level.

Table 10: American Association of Feed Control Officials (AAFCO) Domestic dog food vitamin profile[a] (www.fda.gov/animalveterinary)

Vitamins	**Units DM Basis**	**Growth and Reproduction Minimum**	**Adult Maintenance Minimum**	**Maximum**
Vitamin A	IU/kg	5000.0	5000.0	250000.0
Vitamin D	IU/kg	500.0	500.0	5000.0
Vitamin E	IU/kg	50.0	50.0	1000.0
Thiamine[e]	mg/kg	1.0	1.0	
Riboflavin	mg/kg	2.2	2.2	
Pantothenic acid	mg/kg	10.0	10.0	
Niacin	mg/kg	11.4	11.4	
Pyridoxine	mg/kg	1.0	1.0	
Folic Acid	mg/kg	0.18	0.18	
Vitamin B12	mg/kg	0.022	0.022	
Choline	mg/kg	1200.0	1200.0	

[e] Because processing may destroy up to 90 percent of the thiamine in the diet, allowance in formulation should be made to ensure the minimum nutrient level is met after processing.

Information about the basic gastrointestinal tract function of the domestic dog, as a model for other large canids, can be found in Stevens and Hume (1995), on-line at various veterinary school websites, and by contacting the AZA NAG.

African wild dog: Most African wild dogs in North American zoos are fed approximately 1-1.36 kg (2.2-3 lb) of a nutritionally complete raw meat-based canine diet per adult animal per day, supplemented with knuckle bones and horse, beef, or oxtail bones at least once or twice a week (see Appendix I). Whole prey items such as fetal calves, chicks, rabbits, rats, mice, or deer carcasses are also offered occasionally. If possible, feeding twice daily in zoos may more closely resemble their free ranging feeding habits. Total amounts of food per day depend on activity and physiological state. Male and female African wild dogs weigh between 17-36 kg (37.5 – 79.4 lb), (Kingdon, 1977), with little variation in size between the sexes (Frame et al., 1979). Body mass should be monitored and weights obtained on a routine schedule. Diets can be adjusted based on weight records. Overweight dogs may have their total diet decreased in 5% increments if the diet is still adequate in nutrients. Other options include decreasing calorically dense food items and/or increasing the lower calorie items. See Appendix I for more information.

Maned wolf: Maned wolf nutritional requirements and feeding strategies are more specific than the other large canids with respect to nutritional content, dietary restrictions, and additional items included in the diet. The diet of wild maned wolves has been well-documented, and consists of approximately 50% animal items (e.g., small mammals/rodents, insects, and birds) and 50% plant materials (e.g., fruit such as *Solanum lycocarpum*), as measured by frequency of occurrence in scats (Dietz 1984; Motta-Junior et al. 1996). Maned wolves in zoos and aquariums are highly susceptible to cystinuria, a genetic defect affecting the renal system, resulting in cystine passing into the urine, where it can form crystals and/or stones. If stones form, they can block the urethra and obstruct urinary flow, especially in males. Therefore, it is recommended that maned wolves not be fed a high protein moist or extruded dog food. It is generally believed that an “adult maintenance” dry dog food with 20-25% protein provides adequate nutrition to adults and growing pups alike (Childs-Sanford, 2005). Some zoos feed alkalinizing agents to raise pH in order to avoid cystine calculi, but there is little data confirming its effectiveness. At this point, there is no evidence that low iodine diets (LIDs) are an effective or appropriate diet for maned

wolves. Taurine supplementation has been recommended on a case by case basis, and taurine monitoring is suggested by the AZA Maned Wolf SSP. Studies are underway to more clearly elucidate the nutrient requirements of this highly omnivorous canid. The nutrient composition of diets offered to maned wolves housed at three North American zoos are presented in Appendix I, illustrating the variability of nutrients currently offered (adapted from Phipps and Edwards, 2009).

Dholes: Dholes have only rarely been exhibited in North America, and are typically fed a nutritionally complete raw meat-based diet, supplemented with bones and whole food items such as rabbits and guinea pigs.

Age-related nutritional requirements: The nutritional requirements of large canids can change during the life of individual animals and even seasonally in certain environmental conditions. Assessments should be regularly performed to ensure that the consumed diets meet the nutritional needs of animals as they transition through the following life-stages:

Neonates (birth to weaning): Mother-reared pups will obtain nutritional requirements from lactating females. See Chapter 7, section 7.5 for more information on hand-rearing, and the restrictions and protocols associated with this assisted rearing practice. In the wild, large canid pups are typically weaned at approximately 6 weeks of age, when parents begin regurgitating to pups and they begin eating solid foods. Adding puppy kibble and offering small whole food items to support the nutritional requirements of active growing pups can be attempted, but adult animals may ingest these items before the pups can gain access to them. In all cases, pup weights should be monitored and food amounts should be increased to accommodate the nutritional needs of growing pups. Additional feeding stations are recommended to reduce competition and aggression at feeding time when pups are present. An analysis of growth rate records for large canids within this age range is a potential area of research to obtain mean growth rate data.

Juvenile to adult: Generally, the nutrient requirements for juveniles should follow the recommended guidelines for adults summarized above. The weight of juveniles and adults should be monitored regularly, and diets should be adjusted accordingly based on growth rate and weight gain assessments.

Reproductive adult: Pregnant and lactating females require greater nutritional investment, which warrants close monitoring to accommodate increased food intake. The National Research Council's (NRC, 2006) requirements for domestic dogs provide the following information for the daily energy requirements for bitches in late gestation (4+ weeks after mating until parturition):

ME(kcal) = kcal for maintenance + kcal for gestation
= (130 x BW in kg $^{0.75}$) + (26 x BW in kg)

A lactating female may require up to 3 times the maintenance diet at peak lactation (~4 weeks postpartum), depending on the size of the litter. Daily energy requirements for lactating bitches based on number of puppies and weeks of lactation are (NRC, 2006):

ME(kcal) = kcal for maintenance + kcal for lactation
= (145 x BW kg $^{0.75}$) + (BW kg x (24n+12m) x L)

n = number of puppies between 1 and 4
m = number of puppies between 5 and 8 (<5 puppies m = 0)
L = correction factor for stage of lactation: week 1, 0.75; week 2, 0.95; week 3, 1.1; and week 4, 1.2 (NRC 2006)

Increasing the amount of diet for pregnant and lactating females should take place in 10-20% increments at regular intervals to avoid gastrointestinal distress, for example, by offering additional feedings per day. Increased demand for specific minerals or vitamins is generally met by the increase in the amount of diet, as long as a nutritionally complete diet is used.

It is important to monitor food intake of pregnant and lactating females, especially when females have large litters, and adjust the feeding program accordingly to accommodate their increased energy and nutrient needs. Generally, the normal non-breeding diet of a nutritionally complete, dry dog food (moist canine diet for African wild dog and dhole) should constitute the bulk of the female's diet, and will be sufficient for healthy reproduction. The use of nutrient supplements for females during pregnancy and lactation should be assessed on a case-by-case basis.

Repeated regurgitation by males to feed the pregnant female and subsequently the pups may affect the condition of the sire and/or other members of the pack in socially housed canid species. It is important to monitor the condition of all adults in the family group or pack during reproductively active periods.

Senescent adult: Compromised kidney function is common in older canids. The onset of this condition varies among individuals, and typically requires dietary management as prescribed by a veterinarian on a case-by-case basis.

Seasonal changes in nutritional needs: Generally, specific seasonal changes in nutritional requirements related to ambient temperature are not required. However, during colder months, especially in areas that experience sustained cold weather, food intake should be monitored and adjusted/increased if necessary. Similarly, hotter temperatures can actually increase caloric needs more than colder temperatures. This coupled with the fact that animals tend to reduce feed intake during hot temperature complicates the delivery of required nutrients and energy. In the wild, large canids may adjust food sources seasonally. For example, coyotes are highly carnivorous during cooler seasons and more omnivorous in warmer seasons. Similarly, maned wolves increase the proportion of animal protein in the diet during the cooler dry season when small mammals are more abundant (Dietz, 1984). Seasonal changes in body appearance in large canids are most often associated with seasonal shedding and fur growth for winter pelage, although winter pelage does not necessarily reflect an accurate assessment of body condition. These changes are not related to nutritional requirements.

5.2 Diets

The formulation, preparation, and delivery of all diets must be of a quality and quantity suitable to meet the animal's psychological and behavioral needs (AZA Accreditation Standard 2.6.3). Food should be purchased from reliable, sustainable and well-managed sources. The nutritional analysis of the food should be regularly tested and recorded.

AZA Accreditation Standard

(2.6.3) Animal diets must be of a quality and quantity suitable for each animal's nutritional and psychological needs. Diet formulations and records of analysis of appropriate feed items should be maintained and may be examined by the Visiting Committee. Animal food, especially seafood products, should be purchased from reliable sources that are sustainable and/or well managed.

Sample diets: There are varying opinions of the best diet for non-domestic large canids in zoos and aquariums, and identifying the most appropriate dietary approach is an area in need of research. The primary dietary component for red wolves, gray wolves, and coyotes should consist of a nutritionally complete and balanced commercial domestic dog or wild canid product. Foods labeled as complete and balanced for domestic dogs should meet the standards established by AAFCO (American Association of Feed Control Officials). African wild dog and dhole are typically fed meat-based diets. The AZA Maned Wolf SSP recommends feeding a nutritionally complete adult maintenance dog kibble (20-25% protein), preferably not soy-based, supplemented with a variety of fruits and vegetables (Phipps and Edwards, 2009). Supplements should not exceed 30% of the dry matter intake. Consultation with program managers for species-specific nutritional guidelines and for examples of commercial products is recommended to ensure that the most up-to-date information of nutritional requirements is considered for diet formulation.

Except for maned wolves, periodic supplementation of maintenance diets with commercial meat-based products and whole prey items (e.g., mice, rats, rabbits, guinea pigs, quail, etc.) can be considered for large canids, based on recommendations made by institution veterinarians and nutritionists. Organ or muscle meats may also be considered, although certain organs, like liver, are generally not recommended for carnivores (D. Schmidt, personal communication). Some organs can store large quantities of minerals and could change the nutritional composition of the diet. Questions about the suitability of dietary items for large canids can be directed to an AZA NAG member or AZA Large Canid SSP Veterinary/Nutrition Advisor. Supplemental meat/prey-based items should not constitute a canid's primary diet, as they are generally not considered to be nutritionally complete. The diet of maned wolves can be supplemented with 2-3 small whole prey items such as mice or quail several times a week, based on recommendations made by institution veterinarians and nutritionists.

Gray and red wolves, African wild dog, and dhole: These species are predators and scavengers of large prey, principally ungulates (Sillero-Zubiri et al., 2004). In the wild, one prey item may feed an entire pack, with individuals gorging themselves for days and then fasting for several days before their next meal. Red wolves typically prey on smaller game than other species in this group, although deer are important prey

items in the wild. As indicated above, gray and red wolves are typically fed a base diet of nutritionally complete, meat-based dry dog food in zoos and aquariums (Lindsey & Hopkins 1995). Supplemental feed items (e.g., prepared meats, bones, and carcasses) may be provided periodically, but should be limited to avoid interfering with the balanced composition of the principal diet. A good target is 90-95% base diet and 5-10% supplemental food enrichment (M. Griffin, personal communication).

African wild dog diets can be supplemented with knuckle bones and horse, beef, or oxtail bones at least once or twice a week. Whole prey items such as fetal calves, chicks, rabbits, rats, mice, or deer carcasses can also be provided. Consideration should be given to feeding African wild dogs separately, if possible, in order to monitor food intake for individual animals. Dholes are typically fed a nutritionally complete horse- or beef-based meat diet supplemented with bones and whole food items such as rabbit and guinea pigs. Whenever bones are fed to this group of species, they should be very large, such as beef or horse leg bones, or knuckle bones, as this will ensure the health of the animals as they gnaw, chew, and feed on them. Bones can help to keep the animals' teeth clean in addition to providing enrichment and increasing activity levels.

These large canids can generally be fed once per day. Evening feeding is sometimes preferred in order to reduce scavenging of food items by birds and ants, and to reduce the exposure of food to high daytime temperatures. Evening feedings can also provide animals that are less habituated to the presence of humans (e.g., zoo and aquarium visitors) with a better opportunity to feed. Depending on the staff and feeding location at each institution, evening feeding may have the drawback of not providing animal caretakers with much time to assess levels of aggression or competition between pack members during feeding, or to monitor more specifically individual appetites and food intake. The relative importance of these conflicting concerns should be judged by each institution. Red wolves are routinely fasted one day per week except when there are young present or during sustained periods of cold weather (~4.4°C/40°F) (Waddell, 1998).

Maned wolf and coyote: The natural diets of the omnivorous and opportunistic maned wolf and coyote are highly variable depending on region, season, and food availability (Dietz, 1984; Gese & Bekoff, 2004). Neither is typically considered a predator of large animals, although both occasionally kill deer. Coyotes are avid scavengers, but will routinely take small rodents, rabbits, reptiles, birds, bird eggs, and a variety of invertebrates. Both species eat fruits of all kinds. The fruit of the ever-bearing shrub lobeira (*Solanum lycocarpum*; "fruit of the wolf") is an important part of the maned wolf's diet year round in Brazil's savannas. Field studies of the maned wolf show that their wild diet is evenly split between animals and plants (Dietz 1984).

Diets fed to maned wolves and coyotes in zoos and aquariums should consist of a nutritionally complete, dry dog food, with supplemental fruits, vegetables, and whole prey items such as mice, small rats, quail, or chicks. Dry dog foods can be offered freely to these animals, as they will not spoil readily and over-consumption is generally not a concern. All food offered should be completely changed out on a daily basis. Once- or twice-daily feedings of dry dog food for these species is also appropriate. Other food items such as fresh fruits, vegetables, whole prey, or other perishables may be offered with the base diet, or as additional feedings (especially during periods of hot weather) to minimize food spoilage and promote more species-appropriate foraging and feeding behavior (see below). If there is aggressive competition among coyotes for supplemental food items, they can be discontinued with no negative nutritional effects, based on recommendations made by institution veterinarians and nutritionists.

Food and water presentation: The type and intensity of competition over food among individual animals should be carefully monitored for all large canid species. Individual food containers should be available for each animal housed within an enclosure. To avoid aggressive competition over food, multiple feeding locations (up to one location per animal) with food of equal palatability and nutritional value may need to be provided based on levels of aggressive competition and injuries observed. The location of feeding sites should be carefully considered to ensure that an animal cannot be accidentally trapped if approached by a more dominant or aggressive conspecific. One approach that can be considered to limit agonistic interactions among conspecifics is to distribute feeding sites and containers widely within enclosures, utilizing visual barriers and enclosure topography to limit the visibility of the various feeding areas among conspecific as they are feeding. There have been some reports of the successful use of communal feeders for dry kibble for groups of large canids (Waddell, 1998). Regardless of the feeding approach used for social groups of large canids, the group should be carefully monitored for agonistic

interactions. Weight and/or condition of each animal should also be monitored to ensure that individuals are obtaining enough food, and not receiving significant injuries in trying to do so.

There are a number of acceptable food containers or structures in which food can be presented to large canids. Food can be provided in stainless steel bowls, directly on an open cleanable surface such as a concrete feeding pad, or on feeding trays that are elevated off the ground and provide cover to keep the food dry. The specific approach used should be determined based on the needs and temperaments of the animals, the facility design, and other institution-specific considerations. The proper disinfection and cleanliness of the food bowls or feeding areas should be maintained (AWR, 2005). Food containers should be cleaned and disinfected daily (AWR, 2005).

Fresh, clean, drinking water should be available at all times for large canids (AWR. 2005). All water containers should be cleaned daily and disinfected weekly (AWR, 2005). Potable water can be provided in a stainless steel 7.6 L (2 gal) bucket. Gray wolves can chew on and damage a galvanized steel bucket, and most other large canids can easily damage plastic containers. Water containers can be clipped and secured to fences within the enclosure to prevent the animals from tipping over the container, and can be clipped high enough to prevent animals from urinating in them. However, water containers should be accessible to all individuals within a group (e.g., pups, adults, and geriatrics), and the location of the water container should reflect the needs of the animals. If the canids pull at or play with the water containers secured to the enclosure containment barriers to the extent that it may damage the integrity of the barrier, a pool or tub that provides drinking water may be a better alternative. Enclosures with more than two animals should consider multiple containers in order to satisfy the water needs of all the animals in the enclosure without increasing levels of aggression. In freezing climates, a stainless steel heated pet bowl (4.7 L, 1.25 gal) that is carefully secured, and with the electric cord well protected, will provide constant access to water at freezing temperatures. Institutions that experience prolonged periods of below-freezing conditions should have back-up systems available to ensure that the animals have access to drinkable water.

Food variability: Supplements beyond the principal diet are nutritionally unnecessary for large canids, but will provide opportunities for animals to perform species-appropriate foraging and feeding behaviors. Providing additional food items for enrichment (see Chapter 8, section 8.2) on a periodic and/or random basis is very desirable, as long as certain precautions are taken. Feeding large quantities of prepared meats or whole carcasses, such as rabbit, mice, deer or feeding a large volume of fruit can interfere with the balanced composition provided by the principal diet. Feeding whole prey items as the primary diet can also make switching to dry kibble more difficult for the animals, especially if the animal is moved to another facility that does not offer the same prey items. Prepared meats can be used to entice finicky eaters to eat more of the balanced diet, or for administering oral medications to the animals. Bones such as beef or horse shank and knucklebones can also be fed, and these items provide valuable enrichment, promote good dental health, and may aid in strengthening cranial muscle and bone. When feeding whole carcasses (see below), it is important that the source of the carcass is reputable, and that it is not contaminated.

Carcass feeding: Whole or partial carcasses are often fed to large canids, mainly for behavioral enrichment or for training prior to reintroductions to the wild. Facilities have reported feeding the following: rabbit (domestic and wild), chicken, turkey, pheasant, rats, mice, pig, white-tailed and mule deer, cattle, goat, sheep, elk, horse, donkey, and fish. Large carcasses should not be fed to maned wolves, as diets high in animal protein have been associated with cystinuria in this species (Bovee et al., 1981) (see Chapter 6, section 6.6 for additional information on this disorder).

To reduce possible exposure to endoparasites and other pathogens, healthy carcasses are preferable to those from animals of unknown health history, such as road-kills. The AZA NAG recognizes the feeding of animal carcass as a practice desired by some AZA institutions to stimulate activity and normal feeding behavior. The AZA NAG cautions institutions that choose to carcass-feed about numerous hazards (pathogenic and parasitic) that exist for collection carnivores (e.g., Harrison et al., 2006). Precautions are necessary to ensure the carcass is wholesome. All institutions responsible for feeding carnivores in zoos and aquariums should be aware of and follow the USDA “Proper Diets for Large Felids” Policy #25 (USDA AWA Section13, 9 CFR, Subsection F, Section 3.129 issued 13 October 1998). This policy states in part:

> "The feeding of road kills should be discouraged. If they are used, they should be fresh, wholesome, and fed as soon as possible. The carcass should be removed when spoilage begins, or 12 hours after it has been placed into the enclosure. If not immediately fed, it should be processed into smaller pieces and frozen for future use. Sick animals, or animals that have died of illness or unknown causes, should not be used for food. Animals euthanized with chemical euthanizing agents should not be used for food because of the danger of poisoning. When food animals have been euthanized by gunshot, the lead shot should be removed to prevent lead poisoning from ingestion of the pellets. Downer animals exhibiting signs of central nervous system disorders, including dairy and beef cows, horses, other livestock (particularly sheep), and wildlife species, should not be used for food because of the risk of transmissible spongiform encephalopathies. This includes animals suffering from scrapie and any chronic wasting disease. If the downer animals were clearly harvested because of physical injuries only, they may be used for food when properly processed. In addition, animals known or suspected of being affected with Johne's disease should not be fed to large felids."

Even though policy #25 is written specifically for large felids, the AZA NAG recommends this policy be applied to all carnivores. In addition to this policy, the AZA NAG strongly recommends that institutions that choose to carcass feed exercise caution and employ wholesome feeding practices including the acquisition of fresh killed carcass, appropriate handling of the carcass to ensure rapid cool down and minimal bacterial contamination of the meat, and the removal of head, spinal column, hide, and internal organs if the carcass is not that of a neonate collected at birth. Finally, and most importantly, unless the carcass is that of a neonate collected at birth and fed fresh, or is from a USDA inspected facility, the institution should freeze the carcasses solid and properly defrost it prior to offering to an animal to minimize potential parasite exposure for collection animals. Carcasses should be stored and handled in a manner designed to minimize spoilage or contamination.

The AZA NAG urges that institutions that choose to carcass feed acquire the carcass from USDA inspected facilities. The AZA NAG only condones carcass feeding as part of a feeding program that ensures the diet of the animal is nutritionally balanced and wholesome. The feeding of road kill should be done only under close veterinary consultation or supervision; extreme caution should be taken if animal carcasses of unknown history are fed. These carcasses should be inspected for signs of communicable disease, for freshness, and for any signs of contamination such as antifreeze at the road-kill site, or lead shot in a hunter-provided carcass. State permits and property owner authorizations may be needed if road-kill collection is utilized.

In recent years, the potential risks associated with carnivores eating ungulates that may have prion-associated diseases (e.g., chronic wasting disease, bovine spongiform encephalopathy) have become a concern. When feeding carcasses that come from an area in which prion-associated diseases occur, it is strongly recommended that the brain stem and spinal cord be removed, or that only muscle meat from the carcasses be fed. It may be best not to use wild deer carcasses in areas where chronic wasting disease or tuberculosis is endemic in wild deer herds. In all cases, the best approach is not to offer carcasses if any doubts exist, or to only provide carcasses from USDA inspected facilities.

A distinction should be made between acceptable carcass-feeding practices for large canids that will never be released to the wild, and potential release candidates. Potentially releasable animals should not be fed carcasses of domestic animals, as the released animals' familiarity with domestic livestock may lead to animal/livestock owner conflicts after release. If possible, carcasses from prey species that will be found in release areas should be fed to release candidates. Large canids that will never be released to the wild may be periodically fed other carcasses.

Promoting hunting and foraging: Several different approaches to presenting food items in a way that promotes species-appropriate foraging and feeding behaviors have been used with large canids (in addition to carcass feeding), including:

- Varying feeding regime (feast and famine)
- Hiding food items in enclosure
- Varying the time food is given each day
- Providing novel food items
- Spreading out diet presentation over the course of day
- Using several feed pans or stations

- Burying food
- Hanging food items from trees/branches
- Presenting food items where animals cannot see them being placed/hidden
- Using logs with holes drilled for food items
- Providing frozen food items

Maned wolves and coyotes with access to outdoor yards are also often seen eating insects, small rodents, birds, frogs, and grass. The feeding of 20-30 live crickets once or twice per week can also stimulate feeding activity and offers some dietary variability in these species. With the precautions described above, and veterinarian/nutritionist approval, the following food items can be considered to promote species-appropriate foraging and feeding behaviors in maned wolves, coyotes, and possibly the other large canids:

- Mealworms
- Pinkies
- Ice blocks
- Cherries
- Blueberries
- Snow
- Smelt
- Blackberries
- Melons
- Trout
- Orange
- Raw meat diet for canines
- Dog kibble
- Ice cubes with meat or blood from meat.
- Antlers
- Live crickets
- Large bones
- Hair from game native to release area
- Bones from game native to release area
- Live prey, game native to release area
- Meat from game native to release area
- Pumpkins

Depending on institutional enrichment and nutritional programs, the addition of plant material (e.g., fruits, vegetables, browse, etc.) may be considered for large canids. Vegetables and fruits fit for human consumption are considered safe for inclusion in maned wolf diets. Maned wolves typically consume planted or naturally occurring grasses found in exhibit areas. Favorite fruits, such as grapes, are sometimes offered as a training reward. Veterinarians should be consulted, as certain plant items can be toxic to canids. Toxic plant listings can be found online. If browse plants are used within the animal's diet or for enrichment, all plants should be identified and assessed for safety. The responsibility for approval of plants and oversight of the browse-feeding program should be assigned to at least one qualified individual within each institution. The program should identify whether the plants have been treated with any chemicals or grew near any point sources of pollution, and if the plants are safe for the species. If animals have access to plants in and around their exhibits, there should be a staff member responsible for ensuring that toxic plants are not available.

AZA Accreditation Standard

(2.6.1) Animal food preparations must meet all local, state/provincial, and federal regulations.

Food preparation must be performed in accordance with all relevant federal, state, or local regulations (AZA Accreditation Standard 2.6.1). Meat processed on site must be processed following all USDA standards. The appropriate hazard analysis and critical control points (HACCP) food safety protocols for the diet ingredients, diet preparation, and diet administration should be established for the taxa or species specified. Diet preparation staff should remain current on food recalls, updates, and regulations per USDA/FDA. Remove food within a maximum of 24 hours of being offered, unless state or federal regulations specify otherwise, and dispose of per USDA guidelines.

5.3 Nutritional Evaluations

Physical and dietary assessments that focus on the nutritional needs of individual animals should include information about an individual's age, normal activity level, reproductive status, and disease/parasite presence, while recognizing that variation between individuals is not uncommon. Additionally, direct inspection of weight and body condition, coat condition, eating habits, stool quality, and standard veterinary measures should be included in gauging an individual's nutritional health. Recommended values for serum and tissue nutrient levels are unknown but are likely similar to that of the domestic dog. This is an area in need of research and is currently being studied by the AZA Maned Wolf SSP. Overall activity levels should be monitored daily to note any changes in behavior or temperament.

Any deviation from normal stool quality should be noted. Animals that appear lethargic or distressed, or that are chronically overweight or underweight may have underlying parasite or disease issues that require veterinary examination and dietary management, if warranted. In general, excess weight in large canids is uncommon if the dietary recommendations outlined in sections 5.1 and 5.2 are followed.

Stool quality should be monitored daily with general appearance compared to the gastrointestinal grading sheet as described by Waddell (1998) for red wolf and summarized without pictures in Table 11 below. A similar fecal consistency score sheet has been developed for maned wolves.

Table 11: Gastrointestinal grading sheet for red wolves (adapted from Waddell 1998)

Stool grading	Description
Grade 1	Greater than ⅔ of feces in a defecation are liquid. Feces have lost all form, appearing as a puddle or squirt.
Grade 2	Soft/liquid feces are an intermediate between soft and liquid feces, with approximately equal amounts of soft and liquid feces within the defecation.
Grade 3	Greater than ⅔ of feces in a defecation are soft. The feces retain enough form to pile, but have lost their firm cylindrical appearance.
Grade 4	Firm/soft feces are an intermediate between the grades of firm and soft, with approximately equal amounts of firm and soft feces within the defecation.
Grade 5	Greater than ⅔ of feces in a defecation are firm. They have a cylindrical shape with little flattening.

Grades 3 to 4 are considered typical in healthy red wolves. Changes in an individual animal's stool quality should be reported to the veterinarian, and chronic diarrhea should be reported to the relevant AZA Canid SSP Veterinary Advisor.

The body condition of large canids could be determined by developing a standardized body condition scoring system, or by regular weighing. Currently, there is no standardized body condition scoring system for wild canids. Training large canids for regular weighing is the most accurate approach, and is recommended as part of a husbandry training program (see Chapter 8, section 8.1 for more information on operant conditioning). However, the training of canids that are part of a release or reintroduction program, such as the red wolf and Mexican gray wolf programs is prohibited in order to prevent human habituation by animals that may be released to the wild. This prohibition is by agreement between the AZA Canid SSPs and the United States Fish and Wildlife Service (USFWS).

Health status: The extent to which moist diets contribute to gingivitis and plaque build-up in some large canids has not been determined. However, periodic use of whole prey items, bones, etc., appears to improve oral health. Physical exams should include a thorough inspection and cleaning of teeth (see Chapter 6, section 6.4 for more information on preventative health care). Food allergies have been reported in individual maned and red wolves, requiring comprehensive allergy panels and specific dietary management. In red wolves, cases of environmental and food allergies have been confirmed using the Heska Serology test (Heska Veterinary Diagnostic Laboratories, Fort Collins, CO). These animals empirically responded to elimination of the offending allergen.

Three significant medical disorders associated with diet and nutrition have been identified in large canids. Gastric volvulus (stomach torsion) has been documented in Mexican gray, red, and maned wolves. Cystine calculi in the urinary tract are a significant health problem for maned wolves in North American zoos and aquariums. Intermittent diarrhea has also been reported by several facilities holding Mexican gray, red and maned wolves, and may be associated with Inflammatory Bowel Disease (IBD). More information on these diseases and disorders can be found in Chapter 6, section 6.6.

Chapter 6. Veterinary Care

6.1 Veterinary Services

Veterinary services are a vital component of excellent animal care practices. A full-time staff veterinarian is recommended, however, in cases where this is not practical, a consulting/part-time veterinarian must be under contract to make at least twice monthly inspections of the animal collection and to any emergencies (AZA Accreditation Standard 2.1.1). Veterinary coverage must also be available at all times so that any indications of disease, injury, or stress may be responded to in a timely manner (AZA Accreditation Standard 2.1.2). All AZA-accredited institutions should adopt the guidelines for medical programs developed by the American Association of Zoo Veterinarians (AAZV) www.aazv.org/associations/6442files/zoo_aquarium_vet_med_guidelines.pdf.

AZA Accreditation Standard

(2.1.1) A full-time staff veterinarian is recommended. However, the Commission realizes that in some cases such is not practical. In those cases, a consulting/part-time veterinarian must be under contract to make at least twice monthly inspections of the animal collection and respond as soon as possible to any emergencies. The Commission also recognizes that certain collections, because of their size and/or nature, may require different considerations in veterinary care.

Table 12: Veterinary Advisors to the Large Canid AZA SSP Programs (2011)

AZA Maned Wolf SSP	Veterinary Advisor	Elizabeth Hammond	vet@lioncountrysafari.org
AZA Red Wolf SSP	Veterinary Advisor	Holly Reed	bongovet@msn.com
AZA Mexican Wolf SSP	Veterinary Advisor	Carlos Sanchez	carlos.sanchez@czs.org
AZA African Wild Dog SSP	Veterinary Advisor	Michael Briggs	mbbriggs@apcro.org

Institutions or individuals with questions pertaining to other veterinary resources (e.g. American Association of Zoo Veterinarians) or species specific veterinary internships or training programs are encouraged to contact the appropriate AZA SSP Veterinary Advisor or AZA SSP Coordinator.

AZA Accreditation Standard

(2.1.2) So that indications of disease, injury, or stress may be dealt with promptly, veterinary coverage must be available to the animal collection 24 hours a day, 7 days a week.

Protocols for the use and security of drugs used for veterinary purposes must be formally written and available to animal care staff (AZA Accreditation Standard 2.2.1). Procedures should include, but are not limited to: a list of persons authorized to administer animal drugs, situations in which they are to be utilized, location of animal drugs and those persons with access to them, and emergency procedures in the event of accidental human exposure.

AZA Accreditation Standard

(2.2.1) Written, formal procedures must be available to the animal care staff for the use of animal drugs for veterinary purposes and appropriate security of the drugs must be provided.

Veterinarians at each institution should be involved in formulating their own institutional protocols for the storage and use of drugs that could be used in the care and management of large canids. Given the wide variation in the staff and equipment available to veterinarians at different institutions, no specific large canid recommendations can be made. Drugs commonly used in the treatment of large wild canids are the same as those commonly used in domestic dog veterinary practices.

AZA Accreditation Standard

(1.4.6) A staff member must be designated as being responsible for the institution's animal record-keeping system. That person must be charged with establishing and maintaining the institution's animal records, as well as with keeping all animal care staff members apprised of relevant laws and regulations regarding the institution's animal collection.

Animal recordkeeping is an important element of animal care and ensures that information about individual animals and their treatment is always available. A designated staff member should be responsible for maintaining an animal record keeping system and for conveying relevant laws and regulations to the animal care staff (AZA Accreditation Standard 1.4.6). Recordkeeping must be accurate and documented on a daily basis (AZA Accreditation Standard 1.4.7). Complete and up-to-date animal records must be retained in a fireproof container within the institution (AZA Accreditation Standard 1.4.5) as well as be duplicated and stored at a separate location (AZA Accreditation Standard 1.4.4).

All pertinent health information for large canids should be recorded as per institutional protocols in ARKS and MedARKS. ISIS reference ranges for large canid physiological values (Teare, 2002) are

provided in Appendix J. Also see Drag (1991) for Mexican wolf. When the new Zoological Information Management System (ZIMS) becomes widely available, it is recommended that institutions make full use of this resource. ZIMS provides the opportunity to record key animal behavior information along with health records, including data collected on the onset or elimination of abnormal stereotypic behavior, response to enrichment initiatives, conspecifics, or heterospecifics. The AZA Canid TAG and AZA Canid SSP Programs can be contacted for more information on current research being undertaken on large canids for which institutional records may be valuable (see also Chapter 10 for additional information).

Health, medical, dietary, reproductive, and mortality records for each animal should be kept in accordance with each institution's recordkeeping system. Written daily reports should be maintained indicating significant events regarding the animals' general condition, food consumption, bowel habits, social interactions, etc. Specific information that should be collected in large canid records include (adapted from AZA Maned Wolf SSP, 2007):

- Veterinary health problems, treatments (including medication, dosage amount and duration, results, etc.), and tranquilizations (type, amount, effect, etc.).
- Reproductive data including dates and signs of breeding behavior, copulation dates and frequency, birth dates, survivorship, parent- or hand-reared, etc.
- Diet composition, amounts fed, times of day fed, favorite food items, 'treat' foods used, etc.
- Behavioral interactions with conspecifics (especially during introductions), abnormal or unusual behavior(s), seasonal variations, normal behavioral activity budgets for individuals.
- Enrichment initiatives developed, goals associated with enrichment, successes and failures of items offered, types of items (e.g., food, toys, 'furniture', plant species, etc.). See Chapter 8, section 8.2 for more information on enrichment documentation.

Beyond maintaining medical records in accordance with USDA regulations and AZA accreditation standards, some AZA Canid SSP programs within the AZA Canid TAG may maintain additional records required through formalized agreements that involve government-owned species (e.g., USFWS, ICMBio (Brazil)). These records are often available through studbook datasets and institutional records maintained in ARKS and MedARKS. Additionally, other permits or documents that are commonly maintained in association with large canids may include: endangered species permit, captive bred wildlife permit, cooperative agreements, vendor profiles, and breeding loan agreements. Copies of all pertinent records should accompany each animal whenever it is transferred to another facility (Waddell, 1998). Copies of the records should also be provided to the relevant AZA Canid SSP Coordinator upon request, or whenever the institution has significant information to report. Records of births, deaths, and transfers should be reported promptly to the relevant AZA Canid SSP Coordinator.

6.2 Identification Methods

Ensuring that large canids are identifiable through various means increases the ability to care for individuals more effectively. Animals must be identifiable and have corresponding ID numbers whenever practical, or a means for accurately maintaining animal records must be identified if individual identifications are not practical (AZA Accreditation Standard 1.4.3).

AZA Accreditation Standard

(1.4.3) Animals must be identifiable, whenever practical, and have corresponding ID numbers. For animals maintained in colonies or other animals not considered readily identifiable, the institution must provide a statement explaining how record keeping is maintained.

In large canids, tattoos and ear tags have largely been replaced with a microchip transponder (Passive Integrated Transponder – PIT tag), although the AZA Maned Wolf SSP still recommends tattooing the international studbook number on the inner thigh (left for female, right for male) of each animal – in addition to the use of PIT tags (AZA Maned Wolf SSP, 2007). The AZA Mexican Wolf SSP Management Group recommends that institutions use a transponder and reader system that can read a wide variety of transponders, Contact program managers or the AZA Canid TAG Chair for more information or specific recommendations regarding transponder manufacturers. Because of the evolution of microchip systems and vendors, compatibility among systems is a subject that is difficult to address in this manual. However, the AZA Canid TAG does advocate review of this issue to develop future recommendations. When transponders are used, all PIT tag numbers should be provided to the relevant AZA SSP Coordinator and

Studbook Keeper, and should be included in accompanying documents when a large canid is transferred to another facility.

Generally, it is not necessary to anesthetize large canids to implant a PIT tag, although this can be easily accomplished during routine physical exams when large canids are anesthetized. The most common subcutaneous site to insert the microchip is between the shoulder blades, or to the left of the midline in the shoulder area. African wild dogs that are trained to use a restraint device may be implanted in the hip or shoulder for easier reading of the transponder. Canid pups can be PIT tagged in conjunction with scheduled vaccinations.

AZA member institutions must inventory their large canid population at least annually and document all large canid acquisitions and dispositions (AZA Accreditation Standard 1.4.1). Transaction forms help document that potential recipients or providers of the animals should adhere to the AZA Code of Professional Ethics, the AZA Acquisition/Disposition Policy (see Appendix B), and all relevant AZA and member policies, procedures and guidelines. In addition, transaction forms must insist on compliance with the applicable laws and regulations of local, state, federal and international authorities. All AZA accredited institutions must abide by the AZA Acquisition and Disposition policy (Appendix B) and the long-term welfare of animals should be considered in all acquisition and disposition decisions. All species owned by an AZA institution must be listed on the inventory, including those animals on loan to and from the institution (AZA Accreditation Standard 1.4.2). See Appendix K for examples of acquisition and dispositions forms.

AZA Accreditation Standard

(1.4.1) An animal inventory must be compiled at least once a year and include data regarding acquisitions and dispositions in the animal collection.

AZA Accreditation Standard

(1.4.2) All species owned by the institution must be listed on the inventory, including those animals on loan to and from the institution. In both cases, notations should be made on the inventory.

6.3 Transfer Examination and Diagnostic Testing Recommendations

The transfer of animals between AZA-accredited institutions or certified related facilities due to AZA or Breeding and Transfer Plan recommendations occurs frequently as part of a concerted effort to preserve these species. These transfers should be done as altruistically as possible, and the costs associated with specific examination and diagnostic testing for determining the health of these animals should be considered.

Pre-shipment exams should be conducted before transport to assure that the animal is well to travel, inform the receiving institution of the animal's health status, and determine any pre-shipment or during transit treatments. Prior to the exams, the veterinarian of the receiving institution should be contacted for a list of their pre-shipment test requests. At this time any preexisting conditions should be discussed so that the receiving vet can prepare for the animal's arrival and treatment needs. It is helpful if a full set of medical records can be sent to the receiving institution prior to shipment. In addition, the Department of Agriculture's State Veterinarian of the receiving state should be contacted for information on test requirements necessary for the animal to enter the state, statements that need to appear on the health certificate, and the acquisition of permit numbers. For a list of the basic examination procedures and diagnostic tests that should be performed during a pre-shipment exam, see Section 6.4, 6.5 and Appendix C,

AZA Accreditation Standard

(2.7.1) The institution must have holding facilities or procedures for the quarantine of newly arrived animals and isolation facilities or procedures for the treatment of sick/injured animals.

AZA Accreditation Standard

(2.7.3) Quarantine, hospital, and isolation areas should be in compliance with standards or guidelines adopted by the AZA.

6.4 Quarantine

AZA institutions must have holding facilities or procedures for the quarantine of newly arrived animals, and isolation facilities or procedures for the treatment of sick/injured animals (AZA Accreditation Standard 2.7.1). All quarantine, hospital, and isolation areas should be in compliance with AZA standards/guidelines (AZA Accreditation Standard 2.7.3; Appendix C). All quarantine procedures should be supervised by a veterinarian, formally written and available to staff working with quarantined animals (AZA Accreditation Standard 2.7.2). If a specific quarantine facility is not present, then newly acquired animals should be kept separate from the established collection to prohibit physical contact, prevent disease transmission, and avoid aerosol and drainage contamination. If the receiving institution lacks

appropriate facilities for quarantine, pre-shipment quarantine at an AZA or American Association for Laboratory Animal Science (AALAS) accredited institution may be applicable. Local, state, or federal regulations that are more stringent than AZA Standards and recommendations have precedence.

It is recognized that the size and design of quarantine areas will vary among institutions. The ability to shift quarantined canids to outdoor runs with natural light and substrate is preferred (and recommended for new quarantine area designed for large canids), but may not be feasible at all institutions. It is recommended that some form of small den structure be available within the quarantine enclosure to allow an animal to seek refuge (see Chapter 2, section 2.1, and Chapter 7, section 7.4 for additional information on dens).

AZA Accreditation Standard

(11.1.2) Training and procedures must be in place regarding zoonotic diseases.

AZA institutions must have zoonotic disease prevention procedures and training protocols established to minimize the risk of transferable diseases (AZA Accreditation Standard 11.1.2) with all animals, including those newly acquired in quarantine. Keepers should be designated to care only for quarantined animals if possible. If caring for both quarantined and resident animals of the same class is unavoidable, they should care for the quarantined animals only after caring for the resident animals. Equipment used to feed, care for, and enrich animals in quarantine should be used only with these animals. If this is not possible, then all items should be appropriately disinfected, as designated by the veterinarian supervising quarantine, before use with resident animals.

Quarantine procedures: The recommended quarantine period for large canids is a minimum of 30 days, unless otherwise directed by the attending veterinarian. Ideally, the animal should be held in a separate quarantine area away from the resident population. Appropriate disinfectants for equipment and footbaths should be used, as directed by the attending veterinarian, to reduce the spread of infectious agents. The use of disposable items, change of clothes, gowns, masks, gloves, etc. may also be required during quarantine by the attending veterinarian. Disinfectants such as 0.5% sodium hypochlorite (bleach) or chlorhexidine diocetate should be used in footbaths and to disinfect equipment and food dishes. When possible, animal staff caring for resident canids, mustelids, or viverrids, should avoid contact with quarantined canids. If additional mammals of the same order are introduced into a quarantine area containing large canids, the minimum quarantine period should begin over again. However, the addition of mammals of a different order to those already in quarantine will not require the re-initiation of the quarantine period.

AZA Accreditation Standard

(11.1.3) A tuberculin testing and surveillance program must be established for appropriate staff in order to ensure the health of both the employees and the animal collection.

A tuberculin testing and surveillance program must be established for animal care staff as appropriate to protect both the health of both staff and animals (AZA Accreditation Standard 11.1.3). Depending on the disease and history of the animals, testing protocols for animals may vary from an initial quarantine test to yearly repetitions of diagnostic tests as determined by the veterinarian. Animals should be permanently identified by their natural markings or, if necessary, marked when anesthetized or restrained (e.g., tattoo, ear notch, ear tag, etc.). Release from quarantine should be contingent upon normal results from diagnostic testing and two negative fecal tests that are spaced a minimum of two weeks apart. Medical records for each animal should be accurately maintained and easily available during the quarantine period.

Quarantine health assessment: Quarantine is an appropriate time to perform a complete physical exam, update vaccinations, and address any pre-shipment issues noted in the previous institution's medical records (see section 6.4, Table 6 for recommended veterinary assessments and procedures during physical exams). During the quarantine period, specific diagnostic tests should be conducted with each animal (see Appendix C for AZA quarantine policy). A complete physical, including a dental examination, should be performed. Animals should be evaluated for ectoparasites and treated accordingly. Blood should be collected, analyzed and the sera banked in either a -70ºC (-94°F) freezer or a frost-free -20ºC (-4°F) freezer for retrospective evaluation. Large canid species ISIS Reference Ranges for physiological data values can be found in Appendix J.

Fecal samples should be collected and analyzed for gastrointestinal parasites and the animals should be treated accordingly. Vaccinations should be updated as appropriate, and if the vaccination history is not known, the animal should be treated as immunologically naive and given the appropriate series of

vaccinations. Animals should be permanently identified by their natural markings or, if necessary, marked when anesthetized or restrained (e.g., tattoo, ear notch, ear tag, etc.), if this has not been performed previously (See chapter 6.2). Medical records for each animal should be accurately maintained and easily available during the quarantine period. Mortalities during quarantine are considered extremely rare for these taxa.

At the discretion of the veterinarian, it is recommended that release from quarantine be contingent on a minimum of two negative fecal examinations completed at least one week apart using a centrifugal concentration technique. Upon completion of the quarantine period, and if the animals are determined to be in good health, they may be introduced to the resident collection. If the diet at the previous institution differs from the new diet, care should be taken to transition the animal slowly. Staff should ensure that a supply of the previous institution's diet is on hand during quarantine.

Rabies: Rabies is a zoonotic disease caused by a rhabdovirus, transmitted primarily through contamination of a bite wound by the infected animal's saliva. The virus infects the neurological system and produces progressive, reportedly agonizing and eventually fatal disease. Based on the 2008 recommendations of the CDC's Advisory Committee for Immunization Practices, rabies pre-exposure prophylaxis with human diploid cell culture vaccine should be provided to staff handling non-domestic large canids in outdoor open zoo enclosures. The initial rabies prophylaxis series consists of three vaccinations given at 0, 7, and 21 or 28 days. Vaccinated staff should have a serum sample tested for rabies virus neutralizing antibody at lease every two years. If the titer is less than complete neutralization at a 1:5 serum dilution, the person also should receive a single booster dose of vaccine. State or local health departments may provide names and addresses of laboratories performing rabies serological testing. Many county or state public health departments require that all bites from large canids be reported. This includes bites to the public on zoo grounds. Facilities should be familiar with their public health department's requirements and be prepared to respond to a public health department reaction to a bite at the facility. Public health departments may not be aware that large canids in zoos are vaccinated off-label with domestic dog, killed-virus rabies vaccine and that animals can be quarantined for observation by a veterinarian after a bite incident.

Separation and isolation during quarantine: Large canids are typically shipped alone as single animals. The negative effects of social isolation on these social species during the 30-day quarantine period may be difficult to mitigate. During quarantine, large canids may display increased pacing, abnormal behaviors, and initial refusal to eat. Institutional or program approved enrichment items (e.g., whole prey items, bones, scents, foliage, logs, etc.) may be considered to address any abnormal behavioral responses shown by the animal during quarantine. The AAZK 'Animal Data Transfer Forms' should be reviewed to gauge an individual's temperament, favorite food items, enrichment preferences, etc. If possible, when multiple individuals (e.g., siblings) are shipped to a new facility, they should be quarantined together to reduce stress. The behavior of the animals should be closely monitored to ensure that aggression is not observed between the animals.

AZA Accreditation Standard

(2.5.1) Deceased animals should be necropsied to determine the cause of death. Disposal after necropsy must be done in accordance with local/federal laws.

If a large canid should die in quarantine, a necropsy should be performed, and the subsequent disposal of the body must be done in accordance with any local or federal laws (AZA Accreditation Standard 2.5.1). Necropsies should include a detailed external and internal gross morphological examination and representative tissue samples form the body organs should be submitted for histopathological examination. Normal and abnormal gross and histopathological results of wild large canid examinations are consistent with typical domestic dog histopathological results. A generic large canid necropsy protocol can be found in Appendix G, and the AZA Maned Wolf SSP Necropsy Protocol is presented in Appendix H. AZA SSP Program Leaders or Veterinary Advisors should be consulted for species-specific necropsy protocols; see Waddell (1998), AZA Mexican Wolf SSP (2009), and AZA Maned Wolf SSP (2007).

6.5 Preventive Medicine

AZA-accredited institutions should have an extensive veterinary program that must emphasize disease prevention (AZA Accreditation Standard 2.4.1). The American Association of Zoo Veterinarians (AAZV) has developed an outline of an effective preventive veterinary medicine program that should be implemented to

AZA Accreditation Standard

(2.4.1) The veterinary care program must emphasize disease prevention.

ensure proactive veterinary care for all animals (www.aazv.org/associations/6442/files/zoo_aquarium_vet_med_guidelines.pdf). Equipment needs for conducting preventive husbandry procedures may include but are not limited to: squeeze cage; equipment for anesthesia/monitoring, radiographs, dentals, and fecal exams, etc.

Daily visual assessments: All large canids should be observed daily for general physical and behavioral indicators of health. Food and water intake, general appearance, behavior, and stool quality should all be monitored and recorded in daily reports, as patterns may indicate underlying medical issues. Stool quality may vary among individuals and may be affected by activity level, stress, parasites, infection, bowel disease, food allergy, or food changes (see Chapter 5, section 5.3 for a gastrointestinal grading scale that can be used with large canids). Dramatic changes in an individual animal's behavior, food and water consumption, or stool quality should be reported to the attending veterinarian.

Physical examinations: An individual animal's medical records should be reviewed annually. Each facility's veterinarian should visually inspect animals on a routine basis and, if warranted, conduct a physical exam under anesthesia. Table 13 provides recommendations for physical examinations that are applicable to large canids.

Table 13: Recommendations for pre-shipment, quarantine, and annual physical exams

Procedure	Notes
Blood collection	• For CBC and serum banking • Reticulocyte count (e.g., maned wolf) • Serum chemistry • Thyroid/T4 (e.g., red wolf) • Heartworm test
Storage of serum and whole blood	• Serological screening of antibodies to common canine pathogens • Potential genetic analysis
Radiographs	• Thoracic and abdominal
Dental care	• Teeth are examined, ultrasonic scaling and polished at low speed as needed
Opthalmological exam	---
Vaccinations and deworming	• See Tables 7 & 8
Fecal exam	• Direct and using the centrifugal concentration technique • Cryptosporidium/Giardia IFA • Rectal swab for enteric pathogen screening
Body weight	---
Urine analysis	• pH and microscopic exam for cystine crystals (e.g., maned wolf)
Visual inspection	• Ears, eyes, and teeth, palpation (including testes, prostate, and mammary gland)
Auscultation	• Heart and lungs
Identification check	• Transponder chips or tattoos should be checked for readability annually – and preferably each time the animal is handled.

Mexican gray wolf: Drag (1991) found that 4-24 week old Mexican gray wolf pups showed progressive age-related increases in PCV, hemoglobin concentration, mean cell volume, and RBC counts, similar to those seen in domestic dog pups (*Canis familiaris*). The hematological indices in adult wolves (e.g., older than 24 weeks) were comparable to those of the adult domestic dog, except with higher PCV, hemoglobin concentration, and RBC counts. For more information on hematological indices for Mexican gray wolves see Drag (1991).

Parasite control: Daily removal of feces and old food from enclosures and removal of standing water should be considered standard procedures to minimize parasite occurrence. Fecal exams (direct and using the centrifugal concentration technique) and ELISA test for intestinal parasites (e.g., *Cryptosporidium spp*., *Giardia spp*.) should be performed as recommended by the attending veterinarian based on risk and past history. The same assessments should be performed by the veterinarian if a significant infection is documented in an enclosure, or for follow up fecal exams with an infected animal. A thorough parasite assessment and surveillance is suggested just prior to breeding season to eliminate infections in pregnant or lactating females.

Common internal parasites for large canids include ascarids, *Trichuris* spp., cestodes, and strongyles. Standard anthelmintics at canine dosages have been successfully used to treat internal parasites (see Table 14). Large canids should be tested for heartworm, and appropriate treatments administered by the attending veterinarian if test results are positive. All animals in heartworm endemic areas should be tested annually and placed on prophylaxis using selamectin, ivermectin, or milbemycin. Animals with a negative test result should receive avermectin on a monthly basis at standard canine dosages.

Large canid pups can be de-wormed, if needed, at 10-14 days of age with pyrantel pamoate at 5 mg/kg (2.27 mg/lb). If early treatment is not required, routine de-worming can start in conjunction with other scheduled vaccinations. Table 14 provides recommendations for medication and dosages suitable for the treatment of internal parasites in adult large canids. Attending veterinarians at each institution should determine appropriate treatment for large canids.

Table 14: Suggested treatment for internal parasites in large canids

Parasite	Treatment and dosage
Roundworms (ascarids) & hookworms	• Fenbendazole (Panacur): 50 mg/kg orally for 3 days. • Pyrantel pamoate (Nemex, Strongid): 5 mg/kg orally, repeat in 3 weeks. • Ivermectin: 0.2 mg/kg IM or orally – one treatment.
Whipworms (*Trichuris* spp.)	• Fenbendazole (Panacur): 50 mg/kg orally once a day for 3 days, repeat dosing in 3 weeks and 2 months.
Tapeworms	• Praziquantel (Droncit): 1 dose in oral and injectable forms, based on animal weight (not for use in pups less than 6 weeks of age). Repeat after 1-2 weeks if heavily infected.
Coccidia	• Sulfadimethoxine: 50 mg/kg orally on day 1, then 25 mg/kg orally daily for 14-20 days. • Amprolium: 100 mg/kg orally for 7-10 days.
Giardia spp.	• Metronidazole: 50 mg/kg orally once a day for 5 days.
Heartworm prevention[1]	• Ivermectin* • Milbemycin* • Selamectin*
Heartworm treatment	• Treatment as determined by attending veterinarian.

[1] Monthly treatment with selamectin, ivermectin, or milbemycin should be used during mosquito season.
* Attending veterinarian should determine appropriate dosage for all heartworm prevention treatments.

In some geographic areas, external parasites such as fleas and ticks can be found on large canids. Infestations can be managed by dusting or spraying areas used by the animals for resting with products containing carbaryl or pyrethrins. Treating individual animals using products such as Advantage or Frontline at domestic dog recommended dosages has been effective in ectoparasite control. Flea control may require comprehensive management programs involving area sprays or foggers. Systemic treatments may also be needed to supplement premise management programs. Keeping outdoor enclosures well-trimmed may also help control numbers of ectoparasites without chemicals, although the effectiveness of this approach has not been tested.

Fly bites, especially to ear tips, can be a common problem for large canids. Daily removal of feces and old food from enclosures, and removal of standing water should be considered standard operations to minimize attracting flies. Fly traps have met with some success, especially in smaller spaces and when used in conjunction with other measures. Spraying enclosures with insecticide sprays such as Dursban® or permethrins, combined with daily removal of feces and prompt removal of uneaten meat, will also help control fly populations. Label directions should be followed for all insecticidal sprays, and local and federal regulations should be adhered to when pesticides are used. Fly repellent gels can be applied to ears during normal physical examinations as a short-term repellant. Insecticide misting systems have also had some success at certain facilities. Proper disinfection, sanitation, and chemical treatment in all enclosures and holding areas will also have beneficial effects, if consistently maintained. Biological control of biting insects (e.g., introducing parasitic wasps) has been used successfully, and can be considered as part of a comprehensive pest management program.

Maned wolf: Insecticide applications can be made in and around maned wolf enclosures. There are many chemicals available, both primary insecticides and newer growth regulator compounds that have low toxicity potential when used correctly. Examples of insecticides that have been used effectively include diazinon, piperonyl butoxide, natural and synthetic pyrethrins, carbamates, and chlorpyrifos. Maned wolf enclosures should be treated by removing the animals, applying chemicals safe to use in the primary enclosures, and then cleaning the enclosure to avoid exposure prior to returning the maned wolves. Standard canine dosages of domestic dog products have also been used successfully on maned wolves for ectoparasite control, and as part of a comprehensive parasite control program.

AZA Accreditation Standard

(11.1.2) Training and procedures must be in place regarding zoonotic diseases.

As stated in the Chapter 6.4, AZA institutions must have zoonotic disease prevention procedures and training protocols established to minimize the risk of transferable diseases (AZA Accreditation Standard 11.1.2) with all animals. Keepers should be designated to care for only healthy resident animals, however if they need to care for both quarantined and resident animals of the same class, they should care for the resident animals before caring for the quarantined animals. Care should be taken to ensure that these keepers are “decontaminated” before caring for the healthy resident animals again. Equipment used to feed, care for, and enrich the healthy resident animals should only be used with those animals.

Animals that are taken off zoo/aquarium grounds for any purpose have the potential to be exposed to infectious agents that could spread to the rest of the institution’s healthy population. AZA-accredited institutions must have adequate protocols in place to avoid this exposure (AZA Accreditation Standard 1.5.5).

Although a tuberculin testing and surveillance program is a requirement for institutions housing animals that can be affected by tuberculosis, there are no requirements or recommendations associated specifically with large canids, or for staff members working directly with large canids. Animal caretakers may require tuberculin testing if they also work with other species within their institutions. Recommendations for a testing and surveillance programs should be developed by each institution, and by AZA TAGs that include species most affected by this disease.

Vaccinations: Currently, there are no consistent recommendations for vaccination protocols for all large canid species, and the AZA Canid SSP programs have developed different recommendations as applicable for their species. Vaccination protocols are also dependent on institutional preventive medicine programs that are specific to local environmental disease prevalence. For example, multivalent vaccines have been and continue to be used successfully in a number of large canid species without problems being reported, but some AZA Canid SSP programs have elected to use monovalent doses. As a result of these variations in treatment approach, it is difficult to recommend a specific vaccination regime to accommodate all large canids. The AZA Canid TAG recognizes this important topic as an area in need of additional research. Tables 15 and 16 provide suggestions for adults and pups based on current vaccination recommendations used for maned wolves, and Mexican gray and red wolves. See text below tables for African wild dog vaccine information.

Table 15: Vaccine guidelines for adult canids. **Note**: Program veterinary advisors should be consulted as recommendations are periodically updated.

Disease**	Vaccine	Notes
Canine distemper [a]	• Red wolf: Galaxy D® (Schering Plough) • Maned wolf: Merial Purevax Ferret distemper vaccine • Mexican gray wolf: Vanguard® 5 (Pfizer)	Modified live mammalian cell origin – SQ Merial: recombinant canary pox vector All annually or a titer check
Parvo virus	• Vanguard 5® • Duramune Max 5 (Fort Dodge) • Red wolf: Recombitek CPV (Merial) • Maned wolf: Duramune Max PV (Fort Dodge)	All annually or a titer check Recombitek w/ Galaxy D
Rabies	• Imrab®3 (Merial) • Grey and maned wolves: Imrab®1 (Merial)	Killed virus. Booster every 3 years for Imrab3 and annually for Imrab1
Leptospirosis[b]	• Leptoferm 5-way® (Smith Kline Beecham) • Lepto Shield 5 (Grand Laboratories, Inc.)	Recommended in endemic areas or during an outbreak. Vaccinate annually or every 6 months per veterinarian or a titer check
Corona virus	• Duramune® CV-K (Fort Dodge Labs Inc.)	Killed virus. Used only if recommended for your area.

[a] 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.

[b] Maned wolf and red wolf: Vaccination for leptospirosis may be considered in areas where the disease is common, however, the safety and efficacy of the available products is unknown.

** Note: Hepatitis and Parainfluenza vaccines are recommended by the AZA Mexican Wolf SSP.

Table 16: Vaccination schedule for large canid pups. **Note:** Program veterinary advisors should be consulted as vaccination schedules can vary.

Disease**	Vaccine	Schedule (weeks)
Canine distemper [a]	• Maned wolf: Merial Purevax Ferret distemper vaccine • Red wolf: Galaxy D® • Mexican gray wolf: Vanguard® 5 (Pfizer) • African wild dog: Merial's PUREVAX recombinant canary pox vector vaccine	Begin at 6-9 weeks, booster every 2-3 weeks through 16-20 weeks
Parvo virus [b]	• Vanguard® 5 • Duramune Max 5 (Fort Dodge) • Recombitek CPV (Merial) • Maned wolf: Felovax PCT (Fort Dodge) until titer is >1:80, then MLV product, e.g., Duramune Max PV (Fort Dodge)	Begin at 6-9 weeks, booster every 2-3 weeks through 16-20 weeks
Rabies	• Imrab® 3 • Gray and Maned wolves: Imrab® 1	16 weeks. Booster at one year then booster every 3rd year for Imrab3 & annually for Imrab1
Leptospirosis [c]	• Leptoferm 5-way® • Vanguard 5 CPV	16 weeks, then annually or titer check

[a] 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.
[b] Maned wolf pups should not receive a modified live parvovirus vaccine until titer is >1:80.
[c] Maned wolf and red wolf: Vaccination for leptospirosis may be considered in areas where the disease is common, however, the safety and efficacy of the available products is unknown.

** Note: Hepatitis and parainfluenza vaccines are recommended by the AZA Mexican Wolf SSP.

Vaccinating adults and pups against coronavirus or lyme disease may be warranted in areas where these diseases are common, however, the efficacy and safety of the available products are unknown. As killed vaccines do not provide complete protection, every effort should be made to minimize exposure to infectious disease (Montali et al., 1983).

African wild dog: The safety and efficacy of vaccines against the diseases that threaten African wild dogs are often unsatisfactory. Inactivated rabies vaccines have caused seroconversion in some free-range and *ex situ* African wild dogs (Gascoyne et al., 1993), but others have failed to seroconvert (Visee,1996), or failed to establish sustained immunity (G. Thomson, personal communication; P. Kat, personal communication). Some free-range African wild dogs that have been vaccinated against rabies have subsequently died of rabies (Kat et al., 1995; Scheepers & Venzke, 1995; Woodroffe et al., 1997). Problems have also occurred with vaccines used against canine distemper. While modified live vaccines have brought about seroconversion in some cases (Spencer & Burroughs, 1992), in others they have either failed to produce protective antibody levels (van Heerden et al., 1980), or have induced distemper and death (McCormick, 1983; van Heerden et al., 1989; Durchfeld et al., 1990). Vaccine induced distemper can be avoided by using killed vaccines, but studies on maned wolves, bush dogs, fennec, kit, and crab-eating foxes in zoos indicate that such vaccines rarely cause seroconversion (Montali et al., 1983; Woodroffe et al., 1997). Modified live vaccines against parvovirus have brought about seroconversion in African wild dogs managed in zoos (Spencer & Burroughs, 1990; Woodroffe et al., 1997).

6.6 Capture, Restraint, and Immobilization

The need for capturing, restraining and/or immobilizing an animal for normal or emergency husbandry procedures may be required. All capture equipment must be in good working order and available to authorized and trained animal care staff at all times (AZA Accreditation Standard 2.3.1).

AZA Accreditation Standard

(2.3.1) Capture equipment must be in good working order and available to authorized, trained personnel at all times.

Due to the variety of large canids and the number of institutions that manage these species, the AZA Canid TAG cannot provide detailed capture and restraint operations that will be appropriate for all institutions. AZA Canid SSP Coordinators should be consulted if specific questions arise. It is also difficult to characterize or predict the response of canids to capture and restraint procedures. A general capture and restraint protocol that is applicable to all large canids is described below. In order to achieve a successful and timely capture, keepers familiar with the habits and temperament of the animals are vitally important to capture and restraint procedures. Appropriate animal keepers, curators, and veterinary staff should coordinate all captures in advance, and in a well-planned manner. Ambient temperature and humidity should always be considered when planning these operations. Captures should not be scheduled during periods of high humidity and temperature, if possible, although care is still required to prevent heat stress whatever the ambient temperature (e.g., even at low temperatures). Capture and restraint procedures should be discontinued any time the health and safety of the animal is in question.

During any large canid capture and restraint procedure, there are many factors to consider including but not limited to: enclosure design and size; holding and shifting capabilities; animal habits and temperament; number of animals per enclosure; an animal's prior capture/restraint experience; staff experience; reason for capture and restraint (e.g., what procedures are being done); the need for immobilization; and the involvement of the animal in an institutional training program.

For many large canids, procedures such as annual physical exams, inoculations, or blood collection, may be performed without anesthesia if these procedures have been trained as part of a husbandry training program (see Chapter 8, section 8.1 for more details). AZA Canid SSP Coordinators should always be consulted if specific questions arise. More thorough exams, such as pre-shipment and quarantine physicals, and more involved medical procedures, will require capture and immobilization of the animals.

Capture in a confined area: It is common for many large canids to seek out or be easily encouraged to run into a den or other confined area such as a holding or shift area. Once in this confined space, the animal can be restrained with a catchpole, net, or v-stick by personnel familiar with the animal's habits/temperament, and proficient in the use of restraint equipment. Most basic procedures (e.g.,

inoculation, blood collection, palpation) can be accomplished without removing the animal from the den or holding area. Unless the animal is to be crated, it can be handled and then immediately released. If crating is required (e.g., for transport to the veterinary hospital) the animal can be 1) immobilized in the confined area and placed in a crate, 2) encouraged into the crate, or 3) guided into the crate with a catch-pole until secured. If multiple animals are being handled, each captured animal should remain confined until all animals are processed to minimize confused identifications and stress to the animals.

Capture in an open area: Large canids that cannot be captured in a confined area may require a larger, coordinated effort with multiple staff members and restraint equipment, depending on the enclosure size. An example of a technique that is appropriate for gray and red wolves is provided below.

Personnel should form a line and move in a coordinated manner to corner or work the animal into a desirable location within the enclosure for capture. The animals should always be given the opportunity to run into appropriate dens that can then be secured. Animals that will not go into the den should be captured first to avoid prolonged running and possibly running into pen mates. If the animal cannot be worked into a corner of the pen for capture, the animal will require netting as it runs the perimeter of the pen. The animal should not be chased. Staff members should position themselves so that they can maneuver their nets in front of the animal as it runs past.

Once the animal is in the net, the caretakers should position themselves to follow through with the animal's movement and place the net flat against the ground as soon as possible. To avoid further stress and possible injury from excessive struggling, the animal should be quickly and carefully restrained by another member of the catch team using a tool such as a v-stick. Unless the animal is to be crated, it can be handled at the point or location of capture and then immediately released. If the animal is to be crated from the net, the crate should be positioned along a fence line with the opening of the net abutted to the opening of the crate, allowing the animal to move directly from the net into the crate. A catchpole can also be used to guide the animal into the crate. When capturing a large pack, it is recommended that each animal caught in this manner be confined to a den area or crate until all the members of the pack have been processed.

Capture equipment: The following types of equipment may be used in the capture of large canids:

Catch box: 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 transfer doors routinely used by animals, will facilitate many handling needs that arise. Conditioning, coercing, or crowding an animal into a crate can reduce the stress associated with routine vaccinations, administration of tranquilizers, transport, or preparation for other procedures.

Catchpole: The type of catchpole recommended is a 1.5 m (5 ft) pole. The ends have swivels that help to prevent the noose from twisting down. It is also recommended to add additional padding around the end of the catchpole to prevent tooth breakage in case the animal bites the pole. Prior to use, the plastic coating on the cable should be examined for wear or damage as this can cause the cable to stick inside the pole and fail to release properly. Catchpoles should be inspected monthly for wear. Replacement cable kits are available. It is advisable to have cable cutters available during a capture in case of an emergency.

Nets: A hoop net consisting of a 1.2-1.5 m (4-5 ft) handle, a hoop opening of 0.9-1 m (36-40 in) , and a 2.5-3.75 cm (1-1.5 in) mesh nylon net with a depth of 1.2 m (4 in) is recommended. The mesh size of the net should be small enough to prevent feet and legs or noses from pushing through the net, causing injuries. Multiple nets in good condition should be available at all times, and should be inspected monthly.

V-stick: A metal pole approximately 1.1 m (42 in) long with a cross bar at the top and a Y-shaped fork at the bottom can also be used with large canids. The v-stick is utilized by placing the fork across the back of the neck of the animal just in front of the shoulders, and applying a downward pressure – making sure it contacts the ground on either side of the neck. This is typically done after the animal has been secured by the catchpole or net. Care should be taken to avoid placing pressure on the larynx or trachea if the animal rolls.

Muzzles: Depending on the procedure (e.g., short v. long duration), animal temperament, and staff experience, muzzles can be useful for some large canids (e.g., grey and red wolves).

Restraint device: Conditioning animals to enter crates or squeeze cages by placing them in the entrance to a den or other routinely used transfer door is an effective technique for moving animals to another enclosure, restraining animals for routine medical procedures, and administering anesthesia for non-routine procedures. See Chapter 8, section 8.1 for additional information.

Capture: Stress and overheating are the two primary concerns when capturing large canids. The procedure should be discontinued any time the health and safety of the animal is in question. Ambient temperature and humidity should always be considered when planning these capture and restraint procedures, although care is still required since capture stress can occur regardless of weather conditions. Initial indications of capture stress in large canids are excessive panting and drooling, white frothy foam corporal around the mouth, reddened eyes, pale gums due to poor capillary refill times (≥2 seconds), and vomiting (in extreme cases). Respiration, heart rate, and rectal temperature should be continuously monitored by the veterinary staff during restraint. A rectal temperature should be taken, recorded, and continuously monitored for animals that have been captured and restrained (normal temperature is 37.7±1ºC/100±2ºF).

A temperature of 40ºC (104ºF) requires immediate attention. Administration of cool (not cold) intravenous fluids (Lactated Ringers solution or Normosol-R) is effective at lowering the temperature of the animals in this case. Additional cooling methods include application of cool water, wet towels, or wrapped ice packs between the rear legs in the groin region, and applying isopropyl alcohol onto the footpads and ear flaps. Once overheated, an animal cannot cool down enough on its own while in a kennel or crate, even in an air-conditioned room. Hyperthermia should be addressed prior to kenneling, and the animal closely monitored afterwards. If the symptoms of overheating are observed and the animal has not yet been captured, attempts to capture should cease immediately. The veterinarian should determine if further attempts to capture can resume or should be postponed until another time.

Capture myopathy (CM): Capture myopathy has been reported in large canids (e.g., Mexican gray wolf) and should be treated as a medical emergency. CM is a muscle disease associated with the stress of capture, restraint, and transportation. It is a syndrome that occurs in free-ranging mammals and birds and those managed in zoos and aquariums. Four clinical syndromes of CM have been observed in animals: capture shock, ataxic myoglobinuric, ruptured muscle, and delayed-peracute (Fowler, 1993). Affected animals may die within 1-6 hours post-capture or may live for several days and show muscular stiffness of the hind legs. No reliably effective treatments are available for capture myopathy. The recommended treatment is supportive care, including intravenous fluids, sodium bicarbonate to combat metabolic acidosis, corticosteroids, vitamin E and selenium supplements, calcium channel blockers, and antibiotics (Fowler, 1993). Many of the pathologic changes of CM are irreversible. Despite supportive measures, animals suffering from capture myopathy often die, so careful handling and reduced stress during capture, restraint, and transport are essential. The following guidelines provide recommendations for all planned capture events to reduce the likelihood of capture myopathy:

- Immobilizations should be scheduled during early morning hours when temperature and humidity are lowest
- Blindfolds should be used to decrease visual stimulation
- All personnel involved should work quietly. Unnecessary conversation and noise should be avoided
- Temperature, pulse, and respiration rates should be checked repeatedly during all restraint procedures
- Animals with temperatures >40ºC (104°F) should be cooled down with water and receive IV fluids if the condition is severe
- Animals showing increased heart rates (>250 bpm), or species prone to capture myopathy, can be given a prophylactic treatment of one liter Lactated Ringer's solution containing 1,000mEq sodium bicarbonate.

Anesthesia: Hand-injection of anesthetic drugs when animals are manually restrained or in a squeeze cage is the common method of achieving chemical immobilization in large canids. Remote delivery systems may also be used (e.g., pole syringe, dart gun). In all cases, AZA Canid SSP Veterinary Advisors or Coordinators should be consulted if specific questions arise. Table 17 provides a summary of common anesthetic regimes used for large canids.

Table 17: Successful anesthetic regimes used for large canids

Anesthesia[1]	Notes[2]
Butorphanol (0.4 mg/kg) mixed with medetomidine (0.04 mg/kg) IM	- Reverse with Naloxone (0.02 mg/kg) and Atipamizole (0.2 mg/kg).
Telazol (2-3 mg/kg) mixed with medetomidine (0.02-0.03 mg/kg) IM	• Reverse with atipmezole (same dosage as medetomidine) • Supplement with oxygen. • Induction is rapid & smooth; recovery is slow but smooth. • Mild decrease in heart rate, respiratory depression not seen.
Ketamine (4-5 mg/kg) mixed with medetomidine (50-70 mcg/kg) IM	• Reverse with atipmezole • Supplement with oxygen. • Wait at least 45 minutes after inducing anesthesia before reversing the medetomidine.
Telazol (± 5 mg/kg) IM	---
Telazol (2.5 mg/kg) mixed with Ketamine (3.5 mg/kg) IM	---
Ketamine (6-8 mg/kg) mixed with xylazine (1.1 mg/kg) IM – maned wolf	---

[1] Dose and regimen selected should take into account factors including age, health, and environmental conditions. Supplemental anesthesia with isofluorane, sevoflurane, or halothane are commonly used for extended procedures.
[2] Also see Larsen et al. 2001.

6.7 Management of Diseases, Disorders, Injuries and/or Isolation

AZA-accredited institutions should have an extensive veterinary program that manages animal diseases, disorders, or injuries, and the ability to isolate these animals in a hospital setting for treatment if necessary. Large canid keepers should be trained for meeting the animal's dietary, husbandry, and enrichment needs, as well as in restraint techniques, and recognizing behavioral indicators animals may display if their health becomes compromised (AZA Accreditation Standard 2.4.2). Protocols should be established for reporting these observations to the veterinary department. Large canid hospital facilities should have x-ray equipment or access to x-ray services (AZA Accreditation Standard 2.3.2), contain appropriate equipment and supplies on hand for treatment of diseases, disorders or injuries, and have staff available that are trained to address health issues, manage short and long term medical treatments and control for zoonotic disease transmission.

AZA Accreditation Standard

(2.4.2) Keepers should be trained to recognize abnormal behavior and clinical symptoms of illness and have knowledge of the diets, husbandry (including enrichment items and strategies), and restraint procedures required for the animals under their care. However, keepers should not evaluate illnesses nor prescribe treatment.

The following medical conditions have been commonly identified and documented in species of large canids:

Stomach torsion: Gastric volvulus (stomach torsion) has been documented in Mexican gray, red, and maned wolves. This condition should be treated as a medical emergency. Some concern has been expressed that feeding dry dog food may contribute to this condition. However, no studies have established a cause and effect relationship between food type and volvulus. It is believed that stomach torsion may be related to pacing or strenuous exercise on a full stomach, and so care should be taken not to run or excite large canids for at least two hours after they have eaten. Additionally, large canids that have been fasted prior to medical procedures should be fed a smaller ration of their diet following the procedure.

Ulcerative pododermatitis: Footpad ulceration can be a problem in neonates, and is typically characterized by abrasions of the footpads and abdomen. It is suspected that neonates move within the den box, pushing aside bedding (e.g., straw, shavings), resulting in abrasions of their soft footpads and

abdominal skin on the rough den box floor. An affected pup may initially appear bright and alert, but at a later stage be found depressed and lethargic, which can be an indication of sepsis. Infected foot sores are usually associated with *Staphylococcus spp*. overgrowth and septicemia. Footpad lesions may not appear until a couple of days after birth, and so continuous early monitoring is important. Successful treatments have included topical agents (e.g., diluted chlorhexidine solution) and/or systemic antibiotics (e.g., oral amoxicillin). Preventive management recommendations include adding a substantial layer of dirt to the den box prior to whelping, or inserting a thick, rubberized mat (e.g., horse stall mat) to the den box, topped with straw.

Progressive Retinal Atrophy (PRA): PRA is a disease in which the retinal cells gradually die until the animal is completely blind. This may occur over the course of months or years. In domestic dogs this disease is inherited. The early signs of this disease are night blindness or poor vision in dimming light, unusually dilated pupils, and an increase in reflectivity of the eyes (eye shine). Cataract development may occur secondarily to PRA. A few cases of PRA have been documented in Mexican gray and red wolves (Acton et al., 2000; Zangerl et al., 2007). If PRA is suspected, examination by a veterinary ophthalmologist is recommended, and program veterinary advisors and/or coordinators should be consulted.

Bladder/uterine infections: Excessive vaginal discharge during the breeding season, continued spotting after the season, spotting shortly after parturition, or spotting at any other time of the year may indicate that the animal has a uterine or bladder infection. In all large canids, uterine infections can be fatal, but can be effectively treated if detected early. Institutional veterinarian or program veterinary advisors should be consulted for treatment options.

Inflammatory Bowel Disease (IBD): Intermittent diarrhea has been reported by several facilities holding Mexican gray, red, and maned wolves. Stress, dietary factors, and parasitism are considered likely underlying mechanisms in this disease. Reports of chronic diarrhea, active gastrointestinal disease (e.g., eosinophilic infiltrates, lymphocytic plasmacytic enteritis), and thickened bowel on physical exam or necropsy have raised questions concerning the cause of these problems in large canids. Pathologists have suggested that the IBD is due to chronic antigenic stimulation (e.g., parasitism, food allergy, chronic infection). When working up an animal with gastrointestinal abnormalities, diagnostics should include serum analysis in the fasted animal for canine trypsin-like immunoreactivity (TLI), cobalamine, and folate (in addition to CBC and chemistry panel), as well as standard fecal tests to define the etiology. In advanced cases, intestinal biopsies should be considered for definitive diagnosis.

Reproductive system tumors: Ovarian tumors, especially dysgerminomas, are found quite frequently in adult female maned wolves (Munson & Montali, 1991). Sertoli cell tumors have been seen in male maned wolves. Ovarian and sertoli cell tumors have also been documented in the red wolf (Acton et al., 2000).

Cryptorchidism: Unilateral and bilateral cryptorchidism has been reported in several Mexican gray and red wolves, and rarely in maned wolves. Bilaterally cryptorchid wolves appear to be non-reproductive. There is some debate regarding the reproductive fitness of monorchid males, although semen collection has indicated they are generally reproductively competent. Program coordinators should be consulted if cryptorchidism is detected; the AZA Maned Wolf SSP does not recommend breeding cryptorchid animals.

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

Cystinuria: Cystinuria has been documented in a significant number of maned wolves (Dietz, 1984). Both zoo-managed and free-ranging maned wolves have been shown to excrete excessive amounts of cystine and other dibasic amino acids in their urine. Cystine calculi in the urinary tract are a significant health problem. These stones can predispose maned wolves to urinary tract infections. Additionally, urethral stones can lead to obstruction and secondary rupture of the bladder, especially in males. Urethral obstruction due to cystine calculi may present as an animal straining (as in constipation), and should be

considered an emergency situation. Surgery may be indicated to remove stones. Untreated urethral obstruction can lead to bladder rupture and death. Many institutions have treated cystinuria with Thiola (no longer available in the U.S.), U/D diet, and urine alkalinization, all with varying success. It is generally agreed that diet contributes to this medical condition and that limiting the amount of animal protein in the diet and increasing the urine pH may reduce the formation of cystine calculi. Further research is warranted to determine nutrient needs of maned wolves.

Tumors: Nasal and brain tumors have occurred with greater than expected frequency in Mexican gray wolves. This observation is being further investigated.

Proliferative gingivitis: Proliferative gingivitis with associated tooth loss has been reported in maned wolves of all ages. Regular dental cleaning (see Table 6, section 6.4) and avoiding moist diets may slow the progress of this disease.

Separation and isolation: If it is necessary to separate a large canid from its social group for medical reasons, institutional or program approved enrichment items (e.g., whole prey items, bones, scents, foliage, logs, etc.) may be considered for diversion during treatment periods (as approved by veterinarian). When a large canid is separated for medical reasons with the intention of returning it to the group, the length of separation, gender of the separated animal, family group sex ratio, family group social dynamics, and time of year (e.g., breeding season) should be carefully considered. In some cases, and particularly with African wild dogs, if an animal is separated from the group (whether due to aggression or for medical reasons), it may not be possible to integrate that individual back within the group successfully.

In situations where individuals are temporarily separated from the pack for medical reasons that do not require isolation, a transparent single line barrier can be set up between adjacent enclosures to encourage interaction without contact. This barrier should be 2.5 cm x 2.5 cm (1 in x 1 in) or smaller steel wire fabric or steel mesh; larger size mesh is not sufficient to prevent a paw or tail from being bitten by animals in the adjoining enclosure.

Medical management of neonates and geriatrics: The AZA Canid SSPs have different philosophies associated with handling newborn neonates and pups, and program coordinators should be consulted if questions arise (see also Chapter 7, section 7.5 for issues relating to hand-rearing). Minimizing handling frequency by combining procedures is recommended for all large canid species. For example, obtaining weights or implanting transponders can occur in conjunction with scheduled vaccinations. Ideally, pups should be examined thoroughly by the attending veterinarian within the first two weeks following birth; however, AZA Canid SSP Program Coordinators should be consulted for specific recommendations. Pups should be sexed and examined for general physical condition and the presence of congenital defects (e.g., cleft palate, imperforate anus, umbilical problems, heart defects, etc.). Male pup testicles should be checked when one of the last series of vaccinations is given to confirm that both testicles have descended. Testicles should be descended no later than 6 months of age.

It is common for older large canids to experience arthritis, and this may require specific medical management approaches and enclosure modifications to meet the needs of these animals. Geriatric animals should have access to a warm, dry den with sufficient bedding or proper application of supplemental heat. The attending veterinarian can also prescribe anti-inflammatory medications as determined by the health status of the animal. It is very important to monitor coat condition for geriatric animals. Tooth wear should also be monitored, and a routine dental cleaning schedule followed. Older animals unable to compete with enclosure mates for food may need to be isolated at mealtime.

AZA Accreditation Standard

(1.5.8) The institution must develop a clear process for identifying and addressing animal welfare concerns within the institution.

AZA-accredited institutions must have a clear process for identifying and addressing large canid animal welfare concerns within the institution (AZA Accreditation Standard 1.5.8) and should have an established Institutional Animal Welfare Committee. This process should identify the protocols needed for animal care staff members to communicate animal welfare questions or concerns to their supervisors, their Institutional Animal Welfare Committee or if necessary, the AZA Animal Welfare Committee. Protocols should be in place to document the training of staff about animal welfare issues, identification of any animal welfare issues, coordination and implementation of

appropriate responses to these issues, evaluation (and adjustment of these responses if necessary) of the outcome of these responses, and the dissemination of the knowledge gained from these issues.

Given the wide variety of zoos and aquariums that house large canids, the provision of specific recommendations for the best approaches to take in order to communicate animal welfare issues effectively within every institution is not possible. All animal caretakers that work with large canids should be aware of institutional protocols in place for them to identify, communicate, and address potential animal welfare issues that are associated with the care and management of these animals. Specific signs of stress in grey wolves include: pacing, spinning or twirling, increase in aggression or submission, over grooming, excessive licking or chewing, diarrhea, hair loss, decreased appetite and/or weight loss, reproductive failure, or maternal neglect (AZA Mexican Wolf SSP, 2009). Additional overt signs of compromised health can include lethargy, difficulty urinating or defecating, ataxia, etc. Animal caretakers should be aware of these indicators, and those specific to the other large canid species, and these should be included within any animal welfare monitoring and communication protocols.

AZA-accredited zoos and aquariums provide superior daily care and husbandry routines, high quality diets, and regular veterinary care, to support large canid longevity; In the occurrence of death however, information obtained from necropsies is added to a database of information that assists researchers and veterinarians in zoos and aquariums to enhance the lives of large canids both in their care and in the wild. As stated in Chapter 6.4, necropsies should be conducted on deceased large canid to determine their cause of death, and the subsequent disposal of the body must be done in accordance with local, state, or federal laws (AZA Accreditation Standard 2.5.1). Necropsies should include a detailed external and internal gross morphological examination and representative tissue samples from the body organs should be submitted for histopathological examination. Normal and abnormal gross and histopathological results of wild large canid examinations are consistent with typical domestic dog histopathological results. The AZA and American Association of Zoo Veterinarians (AAZV) website should be checked for any AZA Canid SSP Program approved active research requests that could be filled from a necropsy.

AZA Accreditation Standard

(2.5.1) Deceased animals should be necropsied to determine the cause of death. Disposal after necropsy must be done in accordance with local/federal laws.

Euthanasia and necropsies: Euthanasia should be performed in a humane and compassionate manner, utilizing the techniques and procedures outlined in the 2007 American Veterinary Medical Association's Guidelines on Euthanasia, and the American Association of Zoo Veterinarians 2006 Guidelines for Euthanasia of Non Domestic Animals. Each institution housing large canids should have a euthanasia protocol in place that is developed by the veterinary team, in case euthanasia becomes necessary in a particular situation. The AZA Animal Welfare Committee also encourages each institution to develop a process to determine when elective euthanasia might be appropriate from a quality of life perspective, taking into account behavioral, medical, social, nutritional, and animal caretaker perspectives.

Examples of approaches used by institutions are available from the AZA Animal Welfare Committee. When a large canid's health is compromised enough to consider euthanasia, the attending veterinarian and AZA SSP institutional representative (IR) should attempt to contact the appropriate AZA SSP Coordinator to discuss the case. Euthanasia decisions should be made on a case-by-case basis and always with the welfare of the individual animal in mind and in concert with the overall objectives of the AZA SSP Program. In emergency cases, the AZA SSP's IR and veterinarian should follow their institutional animal euthanasia policy.

Various cancers (carcinoma, lymphoma, sarcoma), old age related disease (e.g. renal), splenic/gastric torsions are examples of death causes that are not uncommon for this taxa. Necropsies should include a detailed external and internal gross morphological examination, and representative tissue samples from the body organs should be submitted for histopathological examination. Institutional veterinarians typically submit histopathological samples to laboratories they work with on a routine basis or can contact the appropriate AZA SSP Veterinary Advisor for alternatives. A generic large canid necropsy protocol can be found in Appendix G. AZA SSP Program Leaders or Veterinary Advisors should be consulted for species-specific necropsy protocols; see also Waddell (1998), AZA Mexican Wolf SSP (2009), AZA Maned Wolf SSP (2007).

Chapter 7. Reproduction

7.1 Reproductive Physiology and Behavior

It is important to have a comprehensive understanding of the reproductive physiology and behaviors of the animals in our care. This knowledge facilitates all aspects of reproduction, artificial insemination, birthing, rearing, and even contraception efforts that AZA-accredited zoos and aquariums strive to achieve.

The following table (Table 18) provides a summary of reproductive data for maned wolves, Mexican gray wolves, red wolves, dholes, and African wild dogs:

Table 18: Reproductive information for maned, Mexican gray, red wolves, dholes, and African wild dogs (AWD)

	Maned wolf	Mexican gray	Red wolf	Dholes	AWD
Reproductive cycle	Monestrous	Seasonally Monestrous	Seasonally Monestrous	Monestrous[1]	Monestrous
Usual age of 1st reproduction	22-36 months	21-22 months	21-22 months	22-24 months	21-22 months
Copulation (N. America)	Sept-Jan	Jan-Apr	Feb-Mar	Nov-Dec	Aug-Oct
Length of estrus	5-10 days	5-7 days	5-7 days	+5-7 days	3-7 days
Gestation (from first day of copulation to parturition)	63-67 days	60-63 days	60-63 days	60-62 days	69-72 days
Parturition (N. America)	Nov-March	April-May	April-May	Jan-Feb	Oct-Jan
Mean litter size	2-5	4-5	4-5	4-10	7-10

[1] There is some question about the reproductive cycle in dhole, as they have been reported as monestrous (M.Franke, personal communication) and seasonally polyestrous (Durbin et al., 2004). Studies are underway to elucidate reproduction in dhole (N. Songsasen, personal communication).

Although large canids may reach sexual maturity during the first year, it is more typical for both males and females to begin reproducing during the second breeding season, when they are approximately 22 months old.

In female wolves, physiological changes that indicate estrus may include vaginal swelling (pink in color) and discharge (pink or bloody before, clear during, and thick and yellow at the end of estrus). Enlargement of the testes in the weeks leading up to the breeding season is observed in males. The testes decrease in size near the end of the breeding season and remain reduced in size throughout the non-breeding season. These physiological changes may be difficult to observe through the fur covering the genital area in both sexes.

Behavioral changes associated with the onset of estrus include increased frequency of synchronized activities between males and females (e.g., reclining and moving together), the male following the female (or vice versa), the male sniffing and licking the female's anogenital area, obvious mounting and coupling, and the female moving her tail to the side when the male approaches ("presenting or tail deflection"). Animals should be observed outside the breeding season in order to establish baseline levels of social interactions (e.g. compatibility, aggression). Sexual behaviors (e.g., females presenting to males and copulation) are not observed outside of the estrous period. As copulation may not be observed during the estrous period, it is crucial that affiliative behaviors be monitored to aid in predicting possible parturition dates. See Appendix F for examples of behavioral ethograms for large canids.

Reproductive hormone monitoring: Non-invasive measurements of reproductive steroids excreted in feces have been validated to assess the reproductive status of both male and female canids (Wasser et al. 1995, Velloso et al. 1998, Walker et al. 2002, Songsasen et al. 2006, Carlson & Gese, 2008). Longitudinal fecal steroid analysis may be useful in planning introductions of potential breeding pairs. Cycling females show an estrogen surge just prior to estrus, followed by a sustained rise in progesterone

levels. Progesterone remains high throughout pregnancy and also during pseudopregnancy (non-pregnant luteal phase). Further research is currently underway to distinguish male fecal steroids from female (AZA Maned Wolf SSP, 2007). Similarly, in maned wolves (N. Songsasen, unpublished data) and red wolves (Walker et al., 2002), male testosterone levels are significantly higher during the reproductive season, dropping rapidly after the breeding season. Endocrine monitoring of hormonal changes associated with estrus and ovulation using fecal hormone assays can also help diagnose reasons for reproductive failure in large canids. Similarly, fecal samples can be analyzed for cortisol as a possible indicator of stress, as stress can negatively influence reproductive success.

Pregnancy diagnosis using a commercially available serum relaxin assay has been validated for Mexican wolves and is likely to be effective in red wolves as well, but has not been tested adequately in other large canids (Bauman et al., 2008). The assay is not accurate until at least 30 days post-breeding. Research is currently underway to develop a non-invasive urine assay to determine pregnancy status in maned wolves. It is possible that this assay may also be accurate for other large canid species after it has been appropriately validated.

7.2 Assisted Reproductive Technology

The practical use of artificial insemination (AI) with animals was developed during the early 1900s to replicate desirable livestock characteristics to more progeny. Over the last decade or so, AZA-accredited zoos and aquariums have begun using AI processes more often with many of the animals residing in their care. AZA Studbooks are designed to help manage animal populations by providing detailed genetic and demographic analyses to promote genetic diversity with breeding pair decisions within and between our institutions. While these decisions are based upon sound biological reasoning, the efforts needed to ensure that transports and introductions are done properly to facilitate breeding between the animals are often quite complex, exhaustive, and expensive, and conception is not guaranteed.

AI is an increasingly popular technology that is being used to meet the needs identified in the AZA Studbooks without having to re-locate animals. Males are trained to voluntarily produce semen samples and females are being trained for voluntary insemination and pregnancy monitoring procedures such as blood and urine hormone measurements and ultrasound evaluations, although these strategies have not been reported for species covered in this manual and may conflict with policies established for certain AZA SSP programs. Techniques used to preserve and freeze semen have been achieved with a variety of, but not all, taxa and should be investigated further. Sperm has been banked via electro-ejaculation from many individual Mexican gray and red wolves, but sample quality has not been verified by AI. All recent advances in cryopreservation methodology for domestic dogs should be evaluated with sperm from non-domestic large canids. The development of effective artificial insemination procedures and protocols is a high priority for the AZA Canid TAG (see Chapter 10, section 10.2).

As many of the large canid species are managed as small populations, assisted reproductive techniques such as AI can be a potential reproductive tool to address animal transport logistics. These techniques may be especially important to maximize genetic management given the small size of many zoo populations, and the reproductive behavioral strategies shown by the animals. However, at this time AI is not considered a consistently reliable alternative to natural mating. Further, the costs and logistics of monitoring the female estrus cycle (e.g. via fecal hormones) and performing AI may restrict the application of these techniques to a few institutions.

Semen collection and cryopreservation procedures have been performed with gray wolves (Seager et al., 1975), red wolves (Goodrowe et al., 1998), maned wolves (Leibo & Songsasen, 2002), and Mexican gray wolves (Zindl et al., 2006). Treating generic female gray wolves with Ovuplant® (short-acting deslorelin) to induce estrus has resulted in successful live births by natural mating and intravaginal AI (Asa et al., 2006). Similar research with female maned wolves is currently underway. As reported in Thomassen & Farstad (2009), insemination of three Mexican gray wolves' natural estrus resulted in live births following non-surgical intrauterine AI. There has been one instance with red wolves in which pups were born following AI timed by fecal progestin analysis (Goodrowe, unpublished results). Although there has been some success with freezing semen from African wild dogs in zoos using domestic dog cryopreservation protocols (Hermes et al., 2001; Johnston et al., 2007), there are currently no reports of successful births resulting from AI techniques for this species (Thomassen & Farstad, 2009).

Sperm: In general terms, sperm can be collected from the epididymis, the testis, or from ejaculate (Thomassen & Farstad, 2009). The collection of testicular sperm has not been applied to large canids at this time (Farstad & Kraugerud, 2006). As epididymal sperm can also be collected post-mortem, this

source can play an important role in maintaining gametes from genetically important animals in populations of large canids. In large non-domestic canids, training animals to obtain an ejaculate via manual stimulation may not be feasible or permissible (e.g., with red and Mexican wolves). In these cases, electroejaculation of immobilized canids has been performed using methods developed in cats (Platz & Seager, 1978).

Analyses of semen samples collected by electroejaculation from 55 Mexican gray wolves revealed lower quality sperm, especially measures of motility and morphology compared to generic gray wolves. However, the difference was shown to be directly correlated with inbreeding coefficient due to breeding only within lineages early in the recovery program. Samples from males of lineage crosses had semen quality equivalent to that of the generic wolves (Asa et al., 2007). Lockyear (2006) examined a red wolf database of fresh ejaculate characteristics spanning 14 years which showed a high degree of variability in red wolf semen quality in relation to age and inbreeding coefficient.

Sperm storage: Large canid AZA SSPs continue to examine pre- and post-thaw sperm quality, semen extender, and freeze-thaw protocols to optimize semen banking and assisted reproductive technologies. AZA Canid TAG/SSP reproductive advisors (see Chapter 10) should be contacted for current information. Epididymal sperm may be freeze-stored (e.g. liquid nitrogen) to preserve valuable gametes for breeding populations of animals, and is often necessary if sperm are collected from animals postmortem. However a variety of factors (e.g., time of year, age, health status, length of time from postmortem to examination of epididymal sperm, etc.) may preclude obtaining samples suitable for banking.

Artificial Insemination techniques: The three main approaches that can be used to perform AI with canids are summarized below with specific details on each procedure provided by Thomassen & Farstad (2009).

- Semen is deposited in the vagina
- Semen is deposited in the uterus by transcervical catheterization or by surgery
- Intratubal AI is performed with surgery

For large canids involved in reintroduction programs, training is prohibited for animals to undergo non-sedated AI procedures, and sedated insemination may be the only available option (Thomassen & Farstad, 2009).

Because of the unique nature of the endocrinology of the estrous cycle in female canids, the timing for performing AI can be challenging with non-domestic canids. In many mammals, there is a precise time interval between a rise in estrogens or LH and ovulation. In the domestic dog, ovulation is indicated by a rise in progesterone, with a broad time frame of 3-5 days after an initial elevation; serum progesterone levels measured over consecutive days enable relatively accurate prediction of ovulation and timing for AI. It is assumed that non-domestic large canids follow a similar endocrine pattern. In non-domestic canids, daily blood samples can be more difficult to collect and as a result, this method provides challenges for successful AI (see non-invasive hormone monitoring above).

One method that avoids the need for regular monitoring to detect time of ovulation is to induce a timed ovulation. Ovuplant® (Peptech Animal Health), a small implant containing the GnRH agonist deslorelin, has been used successfully to induce estrus and ovulation in generic gray wolves, with birth of pups following natural mating or artificial insemination (Asa et al., 2006). Results of Ovuplant stimulation with Mexican wolves have been more variable (Asa et al., unpublished). Whether this is due to what appears to be their generally lower fertility compared to generic gray wolves or some other unidentified problem is unknown. The success of AI in African wild dogs housed in zoos and aquariums, for example, can also be limited by the challenges associated with managing the complex social structures and interactions within packs that are often necessary to perform assisted reproductive procedures (Thomassen & Farstad, 2009). In some cases, the presence of dominant or aggressive females, or the proximity to males, can affect the reproductive behavior in males and females. The ability to collect viable sperm from male large canids using electroejaculation can also be dependent on the structure of the male's social group and the time of year (Johnston et al., 2007).

In addition to learning more about the reproductive physiology of large canids, the potential success of AI can also be increased by researching female anatomy (e.g., ultrasonography of ovaries, vaginal speculum and endoscope to observe vaginal crenulation, laparoscopic surgery, postmortem examination) (Thomassen & Farstad 2009). This knowledge can help in the design of appropriately shaped intrauterine catheters used in AI procedures, as there can be subtle differences in the internal morphology of the

reproductive tract between species. Additional questions related to assisted reproduction should be directed to the AZA Canid TAG reproductive advisors.

7.3 Pregnancy and Parturition

It is extremely important to understand the physiological and behavioral changes that occur throughout an animal's pregnancy. Pregnancy may be difficult to determine from a visual assessment in large canids, because there are typically only minor changes in physical appearance, although variability exists among individuals. Physical changes may include abdominal swelling in the last 2-3 weeks, nipple development, milk in teats 1-2 weeks pre-delivery, and hair loss around the nipple area. However, these signs may also appear in pseudopregnant females. It is important to monitor the female's food intake during pregnancy, and adjust the feeding program accordingly to compensate for increased energy requirements (see Chapter 5, section 5.1). Behavioral changes in pregnant females may include increased digging, restlessness several days prior to birth, aggression towards males, and possible aggression towards keepers. Hormonal pseudopregnancy is normal in non-pregnant large canids, with individuals often exhibiting similar behavioral changes to those seen in pregnant females.

As noted above, a serum relaxin assay has been used successfully in large canids to determine pregnancy, and a non-invasive urine assay to determine pregnancy is currently under development. It is also possible to use radiographs or ultrasound to determine pregnancy in maned wolves. Ultrasound has been used successfully to determine pregnancy 30 days post-copulation, while radiographs are effective in the final 3 weeks of pregnancy, when fetal bone formation occurs. The use of ultrasound or radiographs is considered safe for confirmation of pregnancy, although these techniques are not commonly used.

The degree of veterinary management for pregnant canids should be evaluated by the individual institution, and guided by the comfort level of the female. Birth complications such as spontaneous abortions or stillbirths can occur in large canids, and the female should be closely monitored by animal care and veterinary staff. If other medical issues arise that are not life threatening to the female, the potential effects that treatment may have on the unborn pups should be weighed against the severity of the problem.

Parturition and pup-rearing: For all species of large canids, the breeding pair should not be separated before, during, or after parturition. Both parents typically participate in the rearing of the young. Separation can result in an anxious mother, leading to pup loss or poor pup development. Bestelmeyer (2000) found that female maned wolf pups raised by both parents in zoos and aquariums had a higher rate of survival, and were more likely to raise their own young as adults.

Reports of a dam consuming pup(s), while not considered common, does occur. Whether an innate mechanism by the dam allows detection of neonate abnormalities, or environmental, physiological, or behavioral stress leads to infanticide is speculative and requires further research. In many social canids, some or all members of the pack are typically involved in rearing the pups, including den and pup defense, food regurgitation to mother and pups, and grooming and playing with pups. In African wild dog packs, temporarily separating the alpha male/female pair from the remainder of the pack is recommended for parturition. The alpha pair can then be slowly reintroduced back to the remainder of the pack once the pups begin to emerge from the den.

Depending on litter size, red wolf neonates (on average) weigh between 255-350 g (0.56-0.77 lb) (Waddell, 1998). The pups' eyes open between 10-14 days, and will begin to wander out of the den by about four weeks, generally staying near the den's entrance. Pups are typically weaned at about 8-9 weeks, depending on the temperament of the dam (Waddell, 1998).

Although there are instances in the wild and in zoos and aquariums of multiple female gray wolves, dholes, and African wild dogs giving birth within the same pack, this is not typical. In at least one reported instance with dholes and African wild dogs, there was pup stealing and other disruptive behavior noted between the two mothers and others in the pack (Thomas et al., 2006).

7.4 Birthing Facilities

As parturition approaches, animal care staff should ensure that the mother is comfortable in the area where the birth will take place, and that this area is "baby-proofed." A key factor in encouraging all large canid species to breed in zoos is to provide surroundings in which they feel comfortable and secure. The animals should not be unduly disturbed throughout the breeding season, whelping, and pup-rearing periods. There should be no major changes in the routine to which the animals have become

accustomed. It is also important to keep stress levels at a minimum at all times, as it has been observed that animals believed to have experienced stress throughout the year may not reproduce successfully during the breeding season.

In the wild, female wolves and dholes may excavate underground dens or make shallow depression dens or digs. In zoos, it is important to offer all species several options for denning (see below), whether above or below ground, and including artificial structures or dirt mounds in which to dig (see also Chapter 2, section 2.1). However, accessibility to the denning area by animal staff should always be considered when opportunities for denning are provided. Since maned wolves and some African wild dogs give birth during the winter, adequate heat should be provided in whelping areas to maintain temperatures above 7°C (45°F). Dholes are cold tolerant animals and typically do not require supplemental heat, however, they should be provided with shelter. They will use a heated house/shelter during the night if given access during colder winter months.

Natural dens: Large canids will occasionally dig their own dens, and may be encouraged to do so by providing the proper substrate in the enclosure. Although allowing animals to dig natural dens is thought to encourage and strengthen species-appropriate behavior and skills, animal-made dens can make the task of inspecting, removing, treating, or monitoring adults and pups difficult and dangerous. There is also the possibility of the animal-made den collapsing or creating other problems. The length, depth, and location of the den, and the soil type, should all be considered when deciding whether to fill in the den or allow its continued use by the animals.

Artificial dens: For aboveground dens, the following measurements can be used as a guideline for den box construction: 0.9 m x 1.5 m x 0.9 m (4 ft x 5 ft x 4 ft) high with a slight slope to the back of the den box to allow for water runoff. Accessibility by animal staff should be incorporated into den box design. Dust-free straw, stall mats, or cedar chips may be used for bedding. Hay is not recommended because of the risk to pups of inhaling small particles (AZA Maned Wolf SSP, 2007). Underground dens constructed of polyethylene, a material that is water proof, retain body warmth, and stand up to biting and chewing without breaking or cracking have also been utilized. The provision of multiple dens will allow the dam the opportunity to select her preferred option, and provides her with the opportunity to move the pups if she chooses.

Prior to parturition, all enclosure boundaries and fencing should be carefully examined, especially around gates, to ensure that there is appropriate containment for the pups. It may be necessary to add some sections of smaller dimension wire near the base of gates (e.g., on the hinge and lock sides) to prevent pups from squeezing through (see also Chapter 2, section 2.2). Water levels within the enclosure should also be evaluated, and may need to be lowered/drained (see Chapter 1, section 1.3; and Chapter 2, section 2.2 for more information).

Females may not allow the male access to the den when occupied by the pups. At approximately 4-6 weeks of age, when the pups start venturing away from the den, the male (and other pack members in the social species) will take a more active role in pup care by feeding, guarding, and socializing with the pups. All efforts should be made to allow the animals to exhibit natural behaviors and to restrict human-animal interaction. The animals' seclusion should be respected by animal care staff, and disturbance kept to an absolute minimum. Human presence at this critical time may cause animal aggression towards staff, den/pup abandonment, and/or infanticide with or without consumption. Weighing of the pups and other veterinary assessments should be performed when the pups are removed for regularly scheduled vaccinations.

During the first weeks after a birth, some institutions close the exhibit to public viewing to minimize disturbance. Depending on the animals, a facility may also decide not to clean or enter an enclosure around the time of parturition. The use of remote video cameras to monitor the den box during the period of expected whelping can help to establish whether pups are born (to distinguish pregnancy from pseudo-pregnancy), and whether they are born live, without having to disturb the animals. Cameras with a wide angle lens that work under low light conditions are recommended in den boxes. The ability to detect/record sound is also advantageous. When used, all electronic equipment should be mounted out of reach of the animals.

7.5 Assisted Rearing

Although mothers may successfully give birth, there are times when they are not able to properly care for their offspring, both in the wild and in *ex situ* populations. Fortunately, animal care staff in AZA-accredited institutions are able to assist with the rearing of these offspring if necessary.

Hand-rearing is not recommended for many large canids, and is generally not compatible with the goals of species recovery programs. Prior approval by the USFWS is required to hand-rear red or Mexican gray wolves. For all large canids, species-appropriate socialization is critical given the importance that dominance interactions generally play in normal day-to-day interactions. Although parent-rearing is usually recommended for maned wolves, hand-rearing has occasionally been an option for the offspring of the most genetically valuable pairs when circumstances warrant (e.g. pup abandonment or injury). In all cases, when hand-rearing is considered, protocols from appropriate husbandry manuals should be reviewed in consultation with the respective AZA SSP Program Coordinator. AZA Maned wolf SSP hand rearing protocols can be found in Appendix L. AZA Mexican wolf SSP hand rearing guidelines are in Appendix M. Cross-fostering pup(s) for rearing by surrogate parents with their own pups has been a useful rearing option (when necessary) for red wolves (Waddell, personal communication; also see Kitchen and Knowlton, 2006) and has occurred within the same facility, between institutions, and in free-ranging populations. This strategy has been used to balance large or small litters, provide a single pup the social benefit of being raised with siblings, or when a source litter parent has a known history of rearing neglect.

There are various predictors and indicators that can be used to determine the likelihood of females harming or abandoning pups, and these should be carefully considered in terms of the potential need to hand-rear the litter. Females with a past history of inappropriate maternal care should be carefully monitored. Indicators of potential rearing issues (as observed via video or firsthand) include:

- Excessive restlessness on the part of the female, including standing up and lying down frequently, and going in and out of the den repeatedly
- Excessive carrying of pups in and out of the den by the dam
- Persistent and frequent licking and mouthing of the pups
- The dam lying apart from the pups, and making no effort to pull them close to her body
- Pups that appear lethargic and make no attempt to move close to dam. Pups should appear strong and vigorous. If they do not, this indicates that they are getting cold and weak
- Lack of regular nursing; nursing activity should be observed every 2-3 hours in neonates (Brady & Ditton, 1979).

7. 6 Contraception

Many animals cared for in AZA-accredited institutions breed so successfully that contraception techniques are implemented to ensure that the population remains at a healthy size. The development of safe, reversible methods for contracepting over-represented animals is a research priority for the AZA 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 canids (Moresco et al., 2009) and large felids (Munson, 1993). Instead, the AZA Wildlife Contraception Center recommends GnRH (gonadotropin releasing hormone) agonists, such as Suprelorin® (deslorelin) implants or Lupron Depot® (leuprolide acetate), 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. Especially in seasonally breeding species, GnRH treatment needs to be initiated well before estrus and ovulation are anticipated (3-4 months) and before males are expected to be producing viable sperm.

Following is general information on contraceptive options for canids. More details and ordering information can be found at www.stlzoo.org/contraception. The AZA Canid SSP Coordinators should be consulted for species-specific contraception recommendations.

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 should be granted by the AZA Canid SSP Coordinators and/or AZA Canid TAG.

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. However, the risk to females housed with vasectomized males is no greater than being housed alone or with other females.

Gonadotropin releasing hormone (GnRH) agonists: GnRH agonists (e.g., Suprelorin® implants or Lupron Depot®) 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. These agents first stimulate the reproductive system, which can result in estrus and ovulation in females or temporary enhancement of testosterone and semen production in males. Down-regulation follows the initial stimulation. The stimulatory phase can be prevented in females by daily megestrol acetate administration for one week before and one week after implant placement.

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

Although GnRH agonists can also be an effective contraceptive in males, they are more commonly used in females, because monitoring efficacy in females by suppression of estrous behavior or gonadal steroids in feces is usually easier than ensuring continued absence of sperm in males, since most institutions cannot perform regular semen collections. Suprelorin® has been tested primarily in domestic dogs and cats, whereas Lupron® has been used primarily in humans but should be as effective as Suprelorin® as the GnRH molecule is identical in all mammalian species.

If 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, weight gain, and symptoms of diabetes mellitus. Other progestins are also likely to cause these side effects, although data are not available for 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 grey wolf, proestrus (based on blood in vaginal smears) begins an average of 6 weeks before estrus. This means that some individual females may show elevated estradiol levels 2 months or more prior to 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, because the stimulated endometrium is only sloughed during parturition following pregnancy. Non-fertile cycles are more likely to exacerbate deleterious effects because 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.

Androgen: Mibolerone is a synthetic androgen in pill form that is approved for female dogs, but it may stimulate aggressive behavior and 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.

Chapter 8. Behavior Management

8.1 Animal Training

Classical and operant conditioning techniques have been used to train animals for over a century. Classical conditioning is a form of associative learning demonstrated by Ivan Pavlov. Classical conditioning involves the presentation of a neutral stimulus that will be conditioned (CS) along with an unconditioned stimulus that evokes an innate, often reflexive, response (US). If the CS and the US are repeatedly paired, eventually the two stimuli become associated and the animal will begin to produce a conditioned behavioral response to the CS.

Operant conditioning uses the consequences of a behavior to modify the occurrence and form of that behavior. Reinforcement and punishment are the core tools of operant conditioning. Positive reinforcement occurs when a behavior is followed by a favorable stimulus to increase the frequency of that behavior. Negative reinforcement occurs when a behavior is followed by the removal of an aversive stimulus to also increase the frequency of that behavior. Positive punishment occurs when a behavior is followed by an aversive stimulus to decrease the frequency of that behavior. Negative punishment occurs when a behavior is followed by the removal of a favorable stimulus also to decrease the frequency of that behavior.

AZA-accredited institutions are expected to utilize reinforcing conditioning techniques to facilitate husbandry procedures and behavioral research investigations.

Routine husbandry of large canids can be accomplished successfully using a range of management approaches. As the Mexican gray and red wolf programs manage wolves for possible reintroduction, training techniques utilized for other large canids are not considered appropriate for these species. Shifting, feeding, cleaning, and administration of oral medications are daily procedures that can be completed regardless of the management approach.

Other large canids 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, and can be trained to target to a buoy (or other object), which can then be utilized for training other non-routine husbandry procedures (e.g., shifting, standing on a scale, presentation of body parts). Large canids have also been trained to position various body parts (e.g., paws, ears, etc.) for inspection and treatment of wounds in protected contact settings, and can be trained to open their mouths to allow inspection of teeth, tongue, and other mouth parts.

Advanced operant conditioning training programs can also train individuals for blood collection and hand-injections. Conditioning animals to enter crates or squeeze cages by placing them in the entrance to a den or other routinely used transfer door is an effective technique for moving animals to another enclosure, restraining animals for routine medical procedures, and administering anesthesia for non-routine procedures. See Chapter 6, section 6.5 for additional information about capture and restraint techniques. To prevent animals from associating crates and squeeze cages with only negative experiences, training animals to enter these areas should be included in the daily management or the animals, as appropriate, and should involve positively reinforcing the animals for entering and remaining calm within the crates or squeeze cages.

Managing aggression: Managing aggression within a group of socially competitive predators can be challenging, with no clearly defined procedure or predictable outcome. In most cases, attempts can be made to manage aggression within a group by providing opportunities for animals to escape or get out of sight of conspecifics within enclosures, modifying group composition by taking into account ages, relationships, sex, and season, and by minimizing competitive situations during daily management. Hormonal management of aggression through administration of deslorelin (see Chapter 7, section 7.6) has been tried in Mexican gray wolves, but the results of this trial are not yet clear, and additional research may be needed.

8.2 Environmental Enrichment

Environmental enrichment, also called behavioral enrichment, refers to the practice of providing a variety of stimuli to the animal's environment, or changing the environment itself to increase physical activity, stimulate cognition, and promote natural behaviors. Stimuli, including natural and artificial objects, scents, and sounds are presented in a safe way for the large canid to interact with. Some suggestions include providing food in a variety of ways (i.e., frozen in ice or in a manner that requires an animal to

solve simple puzzles to obtain it), using the presence or scent/sounds of other animals of the same or different species, and incorporating an animal training (husbandry or behavioral research) regime in the daily schedule.

Enrichment programs for large canids should take into account the natural history of the species, individual needs of the animals, and facility constraints. The large canid enrichment plan should include the following elements: goal-setting, planning and approval process, implementation, documentation/record-keeping, evaluation, and subsequent program refinement. The large canid enrichment program should ensure that all environmental enrichment devices (EEDs) are "large canid" safe and are presented on a variable schedule to prevent habituation. AZA-accredited institutions must have a formal written enrichment program that promotes large canid-appropriate behavioral opportunities (AZA Accreditation Standard 1.6.1).

AZA Accreditation Standard

(1.6.1) The institution must have a formal written enrichment program that promotes species-appropriate behavioral opportunities.

Large canid enrichment programs should be integrated with veterinary care, nutrition, and animal training programs to maximize the effectiveness and quality of animal care provided. AZA-accredited institutions must have specific staff members assigned to oversee, implement, train, and coordinate interdepartmental enrichment programs (AZA Accreditation Standard 1.6.2).

AZA Accreditation Standard

(1.6.2) The institution must have a specific staff member(s) or committee assigned for enrichment program oversight, implementation, training, and interdepartmental coordination of enrichment efforts.

A variety of approaches to promote species-appropriate behaviors in large canids (and provide them with choice and control over their environment) have been provided throughout this manual. It is recommended that enrichment initiatives be developed, implemented, and evaluated in a systematic way, using information available from published and Internet resources, such as the American Association of Zoo Keepers (AAZK – www.aazk.org), www.enrichmentonline.org, and www.animalenrichment.org. The following tables (Tables 19 and 20) provide examples of enrichment items utilized by AZA-accredited institutions housing large canids. With approval from institution veterinarians and managers, the following enrichment initiatives can be utilized within enrichment programs to promote foraging, feeding, object manipulation, and investigation in large canids:

Table 19: Enrichment initiative ideas for use with maned wolves (adapted from Daley & Lindsey, 2000; Cummings et al., 2007)

Olfactory enrichment [1]	**Physical objects**
• Common herbs and spices • Perfume [2] • Animal scents and lures (commercial formulations) • Feces or urine from other species [2]	• Favored food item hidden within exhibit • Large bones • Large rawhide chew toys • Ice blocks containing food items • "Boomer®" or other safe balls, for short durations to avoid habituation • Coconuts • PVC pipe with small rocks or seeds inside • Sticks and small branches • Feathers

[1] Only small amounts of scent are needed.
[2] Approval from veterinarians should be sought prior to using these enrichment initiatives.

Olfactory enrichment provided to Mexican gray wolves can increase the complexity within their zoo environments, provide them with experience of natural scents, and promote species-appropriate investigation and foraging. The effects of some odors or scents may be under-appreciated in canid husbandry, and can be very enriching. With approval from area veterinarians and managers, the following types of olfactory enrichment can be provided:

Table 20: Suggested olfactory enrichment for Mexican gray and red wolves

Urine[1] and Feces[1] from	Other scents[2]	Spices
Wolves	Vinegar	Allspice
Coyotes	Orange	Onion
Foxes	Chamomile	Chives
Black bears	Mint	Paprika
Cougars/pumas	Vanilla	Cinnamon
White-tailed deer	Lemon	Sage
Mule deer	Peppermint	Cumin
Lagomorphs	Almond	Ground cloves
	Anise	Coriander
	Pecan	Rosemary
	Banana	
	Strawberry	
	Maple	
	Honey	

[1] There are possible medical risks associated with presentation of urine and feces (e.g., bison brucellosis). All urine should be autoclaved, and feces should be free of parasites.
[2] No perfumes or aftershaves, or any other non-natural scents should be used with Mexican grey or red wolves.

The hides (with hair) of deer acquired from USDA-approved venison farms have been provide to African wild dogs as a form of enrichment to promote investigation, object manipulations, and species-appropriate hunting/foraging behaviors. The hides should be kept frozen and then thawed just before being provided to the animals, if approved by area veterinarians and managers. For more information on carcass-feeding, including some of the associated risks and concerns with this activity, see Chapter 5, section 5.2.

Some large canids (e.g., gray wolves and coyotes) can be naturally frightened by new objects and situations (neophobic) (Musiani & Visalberghi, 2001; Mettler & Shivik, 2007). Exposing animals from a young age to a complex varied environment and making exhibit modifications in small steps may help minimize neophobic responses in sensitive species. Complex and varied rearing environments are critical for animals considered for possible reintroduction attempts.

8.3 Staff and Animal Interactions

Animal training and environmental enrichment protocols and techniques should be based on interactions that promote safety for all involved.

Large canids are generally managed in one of two styles. Either the keepers never enter the same space with the animals (i.e., protected contact), and the animals are trained to shift between enclosures (in addition to other trained husbandry behaviors – see section 8.1); or the animals are not trained and the keepers enter enclosures with the canids to shift them or perform other husbandry procedures. Animal training is not permitted with Mexican gray or red wolves based on the potential for returning some of these animals to the wild. African wild dogs are usually trained under protected contact settings. Where appropriate for the species, all training should occur in protected contact situations to ensure the safety of the keeper staff. With the exception of red and Mexican gray wolves, large canids should be trained to shift into holding areas while the main enclosure is serviced and cleaned by animal caretakers. All enclosures should have easily accessible holding areas that permit this to occur (see Chapter 2, section 2.1 for additional information on enclosure design). Positive reinforcement (e.g., providing food rewards) has proven to be an effective training technique for large canids, however, hand-feeding in free contact situations is not recommended.

Red wolf and Mexican gray wolf: As red and Mexican gray wolves are part of a recovery program that will result in some of the animals being released to the wild, no formalized animal training should be utilized with these species. These wolves should not be trained to shift between enclosures, as the process of training an animal to shift generally involves encouraging it to come close to the keepers. This management practice would result in encouraging wolves to approach humans, which is prohibited with red and Mexican gray wolves. Any habituation to human proximity should be discouraged for these species. Special consideration should always be taken while handling and interacting with them to reduce their likelihood they will become habituated or socialized with humans. During handling and restraint for medical exams or procedures, for example, the tendency to stroke, pet, or scratch the animals behind the

ears should be avoided. It is essential that wolves involved in release programs find the experience of contact or close proximity to humans extremely distasteful. The habituation of pre-release animals to humans decreases their fear and increases the likelihood of human conflict after they have been released. It is important that all animal caretakers have a clear understanding of the goals of the Mexican grey and red wolf recovery programs, and are periodically reminded of each program's objectives: encouraging natural behavior in the wolves, avoiding the socialization of wolves to humans, and discouraging the habituation of wolves to human proximity.

It is a recommended practice that keepers enter the enclosures of red wolves and Mexican gray wolves for enclosure maintenance and servicing. For keeper safety, it is a sound management practice to have more than one person accessing an enclosure at a time. Keepers should have a rake, a shovel, or similar implement when entering the enclosure, and should maintain visual contact with the wolves at all times while inside the enclosure. In general, wolves become ill at ease when intently stared at from within their 'territory'. Should a wolf approach a keeper, whether out of curiosity or aggression, the wolf should be rebuffed by the caretakers by directly, confidently, threateningly, and aggressively approaching the wolf with whatever tool is in hand while looking directly at it and sternly saying "no" in a firm voice. If the animals sense fear in their keepers, they may begin "testing" certain keepers with a close approach or a threatening lunge, and may eventually become aggressive and unmanageable. Only under rare circumstances should the keeper retreat from a wolf. After a few tests the animal will usually give up such confrontations. Keepers should take care that such activities do not become a game for the animals. These interactions should be effective from the start.

When moving around inside an enclosure, keepers should remain together at all times, and should move in a circular route. This allows the wolves to avoid the keepers, and at the same time maintains the greatest amount of space between animal and human. Keepers can compromise their own safety by cutting through the center of an enclosure or failing to stay together, leading to the possible separation of the animals from each other, and increasing confusion and levels of stress. Repeatedly using the same circular pattern should reduce the wolves' stress level as they learn to recognize a familiar feeding and cleaning routine. This illustrates that while no formalized animal training approaches are permitted with these species, elements of animal training (albeit inadvertent) are inescapable in the zoo environment.

Maned wolf: In general, maned wolves will avoid humans, and enclosures can be serviced without shifting animals out of them. However, pairs with young can often become very defensive, and hand-reared animals can become quite unpredictable. In both cases, shifting animals for enclosure servicing is recommended.

Dhole: Dhole exhibits may be serviced without shifting animals, however, a minimum of two keepers should be present in the enclosure during the breeding and whelping season.

African wild dog: For keeper safety, it is recommended that African wild dogs be trained to shift off exhibit for the keepers to clean, service, and restock the enclosures. If keepers need to enter enclosures with African wild dogs, they should do so as a group, and with deliberation and caution.

8.4 Staff Skills and Training

Large canid staff members should be trained in all areas of large canid behavior management. Funding should be provided for AZA continuing education courses, related meetings, conference participation, and other professional opportunities. A reference library appropriate to the size and complexity of the institution should be available to all staff and volunteers to provide them with accurate information on the behavioral needs of the animals with which they work.

Keepers should be carefully selected for their knowledge and temperament and should be aware of the overall purpose of managing these animals (e.g., future release of Mexican gray wolves and red wolves). Keepers should maintain control over the animals by conveying a calm assertive attitude at all times. The animals should recognize that the human is the "pack leader." Wolves that sense fear in their handlers may begin 'testing' how much control they have over daily situations, and may eventually become more aggressive and unmanageable during restraint and handling procedures. A thorough knowledge of the natural history of the species is essential to understanding an individual animal's behavior.

Chapter 9. Program Animals

9.1 Program Animal Policy

AZA recognizes many public education and, ultimately, conservation benefits from program animal presentations. AZA's Conservation Education Committee's Program Animal Position Statement (Appendix D) summarizes the value of program animal presentations.

For the purpose of this policy, a program animal is described as an animal presented either within or outside of its normal exhibit or holding area that is intended to have regular proximity to or physical contact with trainers, handlers, the public, or will be part of an ongoing conservation education/outreach program.

Program animal presentations bring a host of responsibilities, including the welfare of the animals involved, the safety of the animal handler and public, and accountability for the take-home, educational messages received by the audience. Therefore, AZA requires all accredited institutions that give program animal presentations to develop an institutional program animal policy that clearly identifies and justifies those species and individuals approved as program animals and details their long-term management plan and educational program objectives.

AZA's accreditation standards require that the conditions and treatment of animals in education programs must meet standards set for the remainder of the animal collection, including species-appropriate shelter, exercise, sound and environmental enrichment, access to veterinary care, nutrition, and other related standards (AZA Accreditation Standard 1.5.4). In addition, providing program animals with options to choose among a variety of conditions within their environment is essential to ensuring effective care, welfare, and management. Some of these requirements can be met outside of the primary exhibit enclosure while the animal is involved in a program or is being transported. For example, housing may be reduced in size compared to a primary enclosure as long as the animal's physical and psychological needs are being met during the program; upon return to the facility the animal should be returned to its species-appropriate housing as described above.

AZA Accreditation Standard

(1.5.4) A written policy on the use of live animals in programs should be on file. Animals in education programs must be maintained and cared for by trained staff, and housing conditions must meet standards set for the remainder of the animal collection, including species-appropriate shelter, exercise, social and environmental enrichment, access to veterinary care, nutrition, etc. Since some of these requirements can be met outside of the primary enclosure, for example, enclosures may be reduced in size provided that the animal's physical and psychological needs are being met.

9.2 Institutional Program Animal Plans

AZA's policy on the presentation of animals is as follows: AZA is dedicated to excellence in animal care and welfare, conservation, education, research, and the presentation of animals in ways that inspire respect for wildlife and nature. AZA's position is that animals should always be presented in adherence to the following core principles:

- Animal and human health, safety, and welfare are never compromised.
- Education and a meaningful conservation message are integral components of the presentation.
- The individual animals involved are consistently maintained in a manner that meets their social, physical, behavioral, and nutritional needs.

AZA-accredited institutions which have designated program animals are required to develop their own Institutional Program Animal Policy that articulates and evaluates the program benefits (see Appendix E for recommendations). Program animals should be consistently maintained in a manner that meets their social, physical, behavioral, and nutritional needs. Education and conservation messaging must be an integral component of any program animal demonstration (AZA Accreditation Standard 1.5.3).

AZA Accreditation Standard

(1.5.3) If animal demonstrations are a part of the institution's programs, an education and conservation message must be an integral component.

The AZA Canid TAG recommends the following key messages for AZA member institutions to use in their educational efforts relating to wild large canids:

1. Wild canids serve as key predators in a wide range of environments.
2. Wild canids face significant and urgent challenges to survival in the wild.
3. Scientists around the world, many affiliated with AZA institutions, are dedicated to learning more about wild canids in order to conserve them.

4. Wild canid conservation depends on local community members and other stakeholders valuing wildlife.
5. AZA-accredited zoos and aquariums play a key role in the conservation of wild canids.

Given the dangerous and carnivorous nature or large canids, these species should not be involved in conservation/education programs outside of their enclosures, but may be involved in animal training demonstrations that zoo or aquariums visitors can observe, whether out on exhibit or during 'behind the scenes' tours. The provision of enrichment to large canids in the view of the public could also be considered an educational program based on the definition of 'program animals' provided in section 9.1.

Animal care and education staff should be trained in program animal-specific handling protocols, conservation and education messaging techniques, and public interaction procedures. These staff members should be competent in recognizing stress or discomfort behaviors exhibited by the program animals and should be able to address any safety issues that arise.

Animal program protocols: Only animal caretakers that have received training within the institution relevant to working with large canids should be involved in any animal training demonstrations, and specific protocols should be developed and implemented to ensure that animal care staff remains safe and focused on the animals during any demonstrations. The presence of zoo or aquarium visitors should not distract animal caretakers during protected contact interactions with the animals. Although unlikely with large canids kept within their enclosures, animal care staff should be competent in recognizing stress or discomfort behaviors exhibited by any animals used in programs/demonstrations (e.g., increased aggression between animals, abnormal stereotypic behaviors), and be able to communicate these issues effectively using institution-specific animal care protocols so that welfare or safety concerns can be specifically addressed. Animal care staff members involved in large canid 'programs' should be trained in conservation and education messaging techniques, and public interaction procedures.

Chapter 10. Research

10.1 Known Methodologies

AZA believes that contemporary large canid management, husbandry, veterinary care and conservation practices should be based in science, and that a commitment to scientific research, both basic and applied, is a trademark of the modern zoological park and aquarium. AZA-accredited institutions have the invaluable opportunity, and are expected, to conduct or facilitate research both in *in situ* and *ex situ* settings to advance scientific knowledge of the animals in our care and enhance the conservation of wild populations. This knowledge might be achieved by participating in AZA Taxon Advisory Group (TAG) or Species Survival Plan® (SSP) Program sponsored research, conducting original research projects, affiliating with local universities, and/or employing staff with scientific credentials (AZA Accreditation Standard 5.3).

AZA Accreditation Standard

(5.3) Institutions should maximize the generation of scientific knowledge gained from the animal collection. This might be achieved by participating in AZA TAG/SSP sponsored research when applicable, conducting original research projects, affiliating with local universities, and/or employing staff with scientific credentials.

Research methodologies and goals: Research investigations, whether observational, behavioral, physiological, or genetically based, should have a clear scientific purpose with the reasonable expectation that they will increase understanding of large canids, and may provide results which benefit the health or welfare of animals in wild populations. Many AZA-accredited institutions incorporate superior positive reinforcement training programs into their routine schedules to facilitate sensory, cognitive, and physiological research investigations, and these types of programs are strongly encouraged by the AZA, where appropriate for the species in question (i.e. not for Mexican gray wolves and red wolves).

AZA SSP Programs are encouraged by the AZA to develop a prioritized list of research projects of interest to the program that will provide guidelines for scientists seeking support from the AZA SSP. Project proposals are distributed to the AZA SSP Management Committee for approval, and once approved, institutions in the AZA SSP should make every effort to accommodate the researcher's needs. Projects may also need approval from an institutional research committee or IACUC. Collaborations between AZA SSP programs and institutions and agencies outside of AZA are common. Examples include USFWS (Mexican gray wolf and red wolf programs), ICMBio and Procarnivoros (Maned wolf SSP), African Wild Dog Conservancy and Botswana Wild Dog Research Project (African Wild Dog SSP). Additional information and current lists of priority research goals can be obtained from individual Program Leaders.

There are a number of scientific advisors working closely with the AZA Canid & Hyaenid TAG and the large canid AZA SSP Programs to identify, prioritize and conduct research studies of both wild and managed canid populations (Table 21).

Table 21: Scientific Advisors to the AZA Canid & Hyaenid TAG and SSP programs

AZA Program Name	Advisor Type	Advisor Name	Contact Email
Canid & Hyaenid TAG	Reproductive Advisor	Cheryl Asa	asa@stlzoo.org
Canid & Hyaenid TAG	Reproductive Advisor	Karen Goodrowe	Karen.goodrowe@pdza.org
Canid & Hyaenid TAG	Reproductive Advisor	Nucharin Songsasen	songsasenn@si.edu
Canid & Hyaenid TAG	Veterinary Advisor	Clay Hilton	chilton@birminghamzoo.com
Canid & Hyaenid TAG	Nutrition Advisor	VACANT	
Maned Wolf SSP	Reproductive Advisor	Nucharin Songsasen	songsasenn@si.edu
Maned Wolf SSP	Veterinary Advisor	Elizabeth Hammond	vet@lioncountrysafari.org
Maned Wolf SSP	Pathology Advisor	D McAloose	dmcaloose@wcs.org
Maned Wolf SSP	Nutrition Advisor	Mark Edwards	msedward@calpoly.edu
Red Wolf SSP	Reproductive Advisor	Karen Goodrowe	Karen.goodrowe@pdza.org
Red Wolf SSP	Pathology Advisor	VACANT	
Red Wolf SSP	Veterinary Advisor	Holly Reed	bongovet@msn.com
Mexican Wolf SSP	Reproductive Advisor	Cheryl Asa	asa@stlzoo.org
Mexican Wolf SSP	Pathology Advisor	VACANT	
Mexican Wolf SSP	Veterinary Advisor	Carlos Sanchez	carlos.sanchez@czs.org
African Wild Dog SSP	Reproductive Advisor	Cheryl Asa	asa@stlzoo.org
African Wild Dog SSP	Pathology Advisor	Michael J. Kinsel	mkinsel@lumc.edu

African Wild Dog SSP	Veterinary Advisor	Michael B. Briggs	mbbriggs@apcro.org

Current Research: The following research studies that are currently underway focus on increasing our understanding of large canid behavior, health, and reproduction, and benefit from the collaborative efforts of AZA institutions housing large canids.

Conservation research: Wild populations of large canids have been the subjects of intensive research by field biologists for many years. Much of what we know about canid behavior and biology is the result of pioneering studies of wild wolves, maned wolves, African wild dogs and other large canid species. The contribution of AZA programs to field conservation efforts has been a more recent development. AZA SSP programs now include goals and priorities for initiating, supporting and/or conducting field studies and conservation initiatives in range countries. Some examples of current AZA program involvement in large canid conservation research include:

- Studying the ecology and health of maned wolves in Brazil and Bolivia (Nucharin Songsasen, Rogerio de Paula, Frederico de Lemos, Sharon Deem, Ellen Bronson, Louise Emmons).
- Supporting conservation initiatives in the range countries to resolve conflicts between African wild dogs and people.
- Supporting African wild dog census surveys in range countries.
- Genetics studies of red wolf free-ranging population in Eastern North Carolina (L. Waits, J. Bohling).

For additional information about canid field studies consult the AZA TAG or SSP Program Leaders or contact the chairs of the IUCN SSC Canid Specialist Group (Claudio Sillero-Zubiri Claudio.sillero@zoo.ox.ac.uk) or Wolf Specialist Group (Dave Mech, mechx002@umn.edu).

Reproductive biology and assisted reproduction technologies: Understanding canid reproductive biology has been the focus of numerous studies in managed canids. Endocrine hormone cycles have been delineated in most large canids and current efforts are focusing on the development of assisted reproduction techniques. For example, Dr. Cheryl Asa and Dr. Juan Arturo Rivera conduct a variety of research related to assisted reproduction technologies, such as semen and ova collection and storage, estrus evaluation, and artificial insemination in Mexican and gray wolves. They also maintain frozen semen and ova banks for the AZA Mexican wolf SSP. Similar research is underway in wild and *ex situ* populations of maned wolves, under the supervision of Dr. Nucharin Songsasen and Dr. Karen Goodrowe for the AZA Red Wolf SSP.

Contraception pathology monitoring and research: Dr. Dalen Agnew at Michigan State University conducts comprehensive pathologic examinations of female reproductive tracts to detect deleterious effects associated with contraceptives. The results of these analyses become part of the AZA Contraceptive Advisory Group Pathology Database and provide important information about contraceptive safety that is used to make informed decisions for annually updated recommendations. A new study investigating the incidence of pyometra in contracepted females has been initiated by Dr. Cheryl Asa and Karen Bauman.

Necropsy tissues banking: The University of California at Davis banks selected tissues from necropsies of Mexican gray wolves according to AZA SSP protocols. The purpose of this banking is to support future pathology research. The Maned wolf and red wolf AZA SSP Necropsy Protocols request that institutions bank selected tissues and provide complete necropsy reports to the AZA SSP veterinary and pathology advisors. Records of all necropsy reports are maintained by the AZA MWSSP and AZA RWSSP veterinary and pathology advisors for future reference.

Carcass banking: All post necropsy carcasses of Mexican gray wolves that die in the USA are sent to The Museum of Southwestern Biology at the University of New Mexico, Albuquerque, NM, for permanent storage. This banking will support a variety of future research. Carcasses are sent according to AZA SSP protocols to the museum Collection Manager, Jon Dunnum.

Blood banking: Blood samples from every Mexican gray wolf are stored at the University of New Mexico for genetic data and historical value. Samples are collected according to AZA SSP protocols and sent to Dr. Cheryl Parmenter.

Nutrition in maned wolves: Dr. Elizabeth Hammond, AZA MWSSP vet advisor, is conducting a study aimed at characterizing circulating nutritional parameters in managed maned wolves. Samples from 34 animals are currently being analyzed to measure amino acids, fatty acids, minerals, vitamins A and E, cholesterol and triglycerides in an ongoing effort to establish a standardized diet for this uniquely omnivorous large canid. AZA MWSSP Nutrition Advisor Mark Edwards is collaborating with colleagues in the animal feed industry to formulate a diet specifically for maned wolves in zoos.

Inbreeding surveillance: Dr. Philip Hedrick and Dr. Rich Fredrickson maintain a program of inbreeding surveillance for the AZA Mexican wolf SSP. Similarly, Dr. David Rabon is examining the effects of inbreeding on reproductive success, performance, litter size and survival in managed red wolves.

Rabies vaccine efficacy in gray wolves: Dr. Krista Wenning is collecting data concerning the effectiveness of domestic dog rabies vaccine in gray wolves.

Determining an effective vaccine interval program for red wolves: Dr. Kathy Larson and Dr. Holly Reed; For more information about current research projects, contact the AZA Canid & Hyaenid TAG and Canid SSP Program Leaders (Table 22).

Table 22: AZA Canid TAG & SSP Program Leaders (2010)

AZA Program Name	Program Leader	Contact Email
Canid & Hyaenid TAG	Jack Grisham	grisham@stlzoo.org
African Wild Dog SSP	Michael Quick	mquick@scz.org
Maned Wolf SSP	Melissa Rodden	roddenm@si.edu
Mexican Wolf SSP	Peter Siminski	psiminski@livingdesert.org
Red Wolf SSP	Will Waddell	will.waddell@pdza.org

Research investigations, whether observational, behavioral, physiological, or genetically based, should have a clear scientific purpose with the reasonable expectation that they will increase our understanding of the species being investigated and may provide results which benefit the health or welfare of animals in wild populations. Many AZA-accredited institutions incorporate superior positive reinforcement training programs into their routine schedules to facilitate sensory, cognitive, and physiological research investigations and these types of programs are strongly encouraged by the AZA.

AZA-accredited institutions are required to have a clearly written research policy that identifies the types of research being conducted, methods used, staff involved, evaluations of the projects, the animals included, and guidelines for the reporting or publication of any findings (AZA Accreditation Standard 5.2). Institutions must designate a qualified individual to oversee and direct its research program (AZA Accreditation Standard 5.1). If institutions are not able to conduct in-house research investigations, they are strongly encouraged to provide financial, personnel, logistical, and other support for priority research and conservation initiatives identified by Taxon Advisory Groups (TAGs) or Species Survival Plans® (SSP) Programs.

AZA Accreditation Standard

(5.2) Institutions must have a written policy that outlines the type of research that it conducts, methods, staff involvement, evaluations, animals to be involved, and guidelines for publication of findings.

AZA Accreditation Standard

(5.1) Research activities must be under the direction of a person qualified to make informed decisions regarding research.

Red wolf and Mexican gray wolf research: The Red Wolf and Mexican Wolf Federal Recovery programs and collaborators receive numerous requests for cooperation and collaboration with researchers. As federally-owned and endangered animals controlled by the USFWS, a formal policy has been developed by the USFWS to address research proposals involving red or Mexican gray wolves. All research requests received by the AZA Red Wolf or Mexican Wolf SSP will require a formal proposal to be reviewed and approved by USFWS recovery program personnel and the AZA SSP Management Group before being implemented at any holding facility. The merit of all research proposals will be determined by the USFWS recovery program personnel in the context of advancing recovery program goals and objectives. All holding facilities are expected to cooperate with USFWS approved and AZA SSP Management Group approved research proposals to the best of their abilities.

Additional expectations of researchers conducting approved projects include:

- Any biomaterial provided from any endangered red wolf or Mexican gray wolf will remain the property of the USFWS. Sample biomaterial remaining after research is completed should be

returned to the USFWS or be made available to other researchers when instructed to do so by the USFWS.

- The USFWS and the AZA Red Wolf SSP or the AZA Mexican Wolf SSP and all cooperating institutions that participate in approved research are expected to be acknowledged in any publication, and should be given the opportunity to review the results, reports, and/or publications generated from research prior to publication. Authorship of publications is the prerogative of the principal investigator, but significant participation by AZA collaborators should be worthy of secondary authorship.

10.2 Future Research Needs

The Large Canid Care Manual is a dynamic document that will need to be updated as new information is acquired. Knowledge gaps have been identified throughout the manual and are included in this section to promote future research investigations. Knowledge gained in these areas will maximize AZA-accredited institutions' capacity for excellence in animal care and welfare, as well as enhance conservation initiatives for large canid species. The following areas have been identified as knowledge gaps within this manual:

Chapter 1. Ambient Environment

Section 1.4. Sound and Vibration: A more complete understanding of the sensitivity of large canids to sounds and vibrations and the effects on their well-being is an area in need of.

Chapter 2. Habitat Design and Containment

Section 2.1. Space and Complexity: Although the olfactory capabilities of some large canids are highly attuned and are important to intra-specific communication, the long distance effects of odors from conspecifics and other species on canid husbandry and welfare have not been investigated. Additional research is needed to determine whether olfactory stimuli have positive or negative effects on the health and behavior of individual animals. A preliminary study of olfactory communication in zoo and aquarium red wolves has been investigated by Rabon (2009); www.lib.ncsu/theses/available/etd-07062009-125414.

Section 2.1. Space and Complexity: In maned wolves, young (<2 years old) can be housed in enclosures adjacent to parents after they have been weaned and physically separated, but more research is needed to determine the potential effects on the parents' subsequent breeding and pup rearing (AZA Maned Wolf SSP, 2007). Additionally, little is known about the dispersal of young maned wolves in the wild, and field studies that can describe the dispersal process in more detail may prove beneficial to the management of these animals in zoos.

Section 2.1. Space and Complexity: It is recommended that breeding pairs of Mexican gray wolves and red wolves be separated from the enclosures of conspecific breeding pairs/groups by at least 1 m (3 ft) . A solid barrier is thought to be effective at minimizing any behavioral disruption that might occur, but this has neither been quantified nor frequently used with red or Mexican gray wolves. Additional research investigating the role played by different barriers may provide useful information for future management of breeding groups.

Chapter 4. Social Environment

Section 4.1. Group Structure and Size: The AZA African Wild Dog SSP and AZA Mexican Wolf SSP encourage studies to improve our ability to manage large social groupings in order to increase display value, utilize space more effectively and provide a more enriching environment for managed canids. Developing strategies to manage multi-generational packs and same-sex groups is also a priority.

Section 4.2. Influence of Others and Conspecifics: Olfactory sensitivity in the domestic dog and presumably other large canids may be underappreciated in zoo canid management and needs research attention.

Chapter 5. Nutrition

Nutritional research: Systematically collected information on the nutritional requirements of non-domestic large canids is limited, and there are varying opinions on the best diet for these species of canids in zoos. This is an area in need of research, and collaborations between the AZA Nutrition Advisory Group and

the AZA Canid TAG and SSP nutrition advisors to identify valuable research approaches will be very helpful. The following topics need further investigation:

Section 5.1. Nutritional Requirements: An analysis of growth rate records for large canids is an area of potential research in order to obtain mean growth rate data.

Section 5.1. Nutritional Requirements: Since it is generally agreed that diet contributes to cystinuria in maned wolves, and that limiting the amount of animal protein in the diet and increasing the urine pH may reduce the formation of cystine calculi, further research is warranted to determine the specific nutrient needs of maned wolves so that appropriate diets can be formulated.

Section 5.2. Diets: There are varying opinions on the best diet for non-domestic large canids in zoos and aquariums. Identifying the most appropriate dietary approach is an area in need of.

Section 5.2. Diets: The extent to which a single feeding of the entire daily allotment of food may promote loose stool in maned wolves and coyotes has not been objectively studied, and additional research would be useful.

Section 5.2. Diets: The extent to which moist diets contribute to gingivitis and plaque build-up in some large canids has not been determined. It is possible that periodic use of whole prey items and bones can improve oral health, but additional research on this topic is necessary

Section 5.3 Nutritional Evaluation: For animals that are not involved in husbandry training programs where weights can be taken on a regular basis, the body condition of large canids could be determined by developing a standardized body condition scoring system. Currently, there is no standardized body condition scoring system for wild canids. This system could be beneficial for assessments of animals in the wild, or those being reintroduced back to the wild.

Chapter 6. Veterinary Care

Section 6.2 Identification Methods: Compatibility among different brands of microchip transponders requires further investigation before a specific system can be recommended by the AZA Canid TAG and Canid SSPs.

Section 6.5 Preventative Medicine: Multivalent vaccines have been and continue to be used successfully in a number of large canid species without problems being reported, but some AZA Canid SSP programs have elected to use monovalent doses. Based on these different approaches, it is difficult to recommend a specific vaccination regime to accommodate all large canids. The AZA Canid TAG recognizes this important topic as an area needing additional research. Disease prevalence and mortality data on animals given multi- and monovalent vaccinations should be directly compared to look for patterns and trends. There is also an opportunity to determine the efficacy and safety of vaccines that can be used with pups against coronavirus or lyme disease, as this information is not currently known.

Section 6.5 Preventive Medicine: Keeping outdoor enclosures well-trimmed may help to control numbers of ectoparasites without the use of potentially harmful chemicals. However, the effectiveness of this approach has not been objectively tested, and more information is needed.

Section 6.7. Management of disease: Additional studies are needed to determine the effects of and treatment for infectious diseases that affect both wild and managed populations of large canids.

Chapter 7. Reproduction

Continuing efforts to elucidate the physiological mechanisms that determine reproductive success is a priority for the AZA Canid & Hyaenid TAG. Examples of specific topics related to large canid reproduction that have been identified for further investigation, including:

Section 7.1 Reproductive Physiology: There is still some question about the reproductive cycle in dholes, as they have been reported as monoestrous and seasonally polyestrous. Studies are needed to elucidate which is more accurate.

Section 7.1 Reproductive Physiology: Further research is needed to characterize more accurately conceptive cycles from non-conceptive cycles in maned wolves (pseudopregnancies), and to distinguish male fecal steroids from female.

Section 7.1 Reproductive Physiology: Although bilaterally cryptorchid canids appear to be non-reproductive, there is continuing debate regarding the reproductive fitness of monorchid males. Semen collection has indicated they are generally reproductively competent, but there are little data measuring reproductive successes in monorchid males.

Section 7.2 Assisted Reproductive Technology: More information is needed to determine semen quality after freezing and thawing in large canids, as the cryotolerance of sperm can be very different depending on the species. The following topics relating to large canid reproduction need further investigation:

- Find a suitable extender for fresh sperm.
- Assessments of morphology, motility, speed, survival, and longevity of spermatozoa.
- In-vitro zona penetration tests.
- Flow cytometry to assess sperm chromatin structure state and acrosome integrity.
- Genome banking: Identify appropriate freeze–thaw protocols and storage conditions; identify locations for genome banks; develop databases to monitor genome banks; coordinate efforts within AZA and internationally.

Section 7.3. Pregnancy. Additional research is needed to develop a non-invasive urine assay to determine pregnancy status in maned wolves. Preliminary test suggest that this assay may also be accurate for other large canid species, and further validation will be needed.

Section 7.6. Contraception: Continue development of safe, reversible contraceptives. Additional research is needed to determine appropriate dosages and duration of efficacy for GnRH (gonadotropin releasing hormone) agonists, such as Suprelorin® (deslorelin) implants or Lupron® (leuprolide acetate), used as contraceptives in large canids.

Chapter 8. Behavior Management

Section 8.1 Enrichment and training: Compile enrichment and training strategies that contribute to improved management of large canids.

Hormonal management of aggression: The hormonal management of aggression through administration of GnRH agonists, e.g. deslorelin, has been tested in Mexican gray and maned wolves, but the results are not yet clear, and additional research is needed.

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Appendix A: Accreditation Standards by Chapter

The following specific standards of care relevant to large canids are taken from the AZA Accreditation Standards and Related Policies (AZA 2010) and are referenced fully within the chapters of this animal care manual:

General Information

(1.1.1) The institution must comply with all relevant local, state, and federal wildlife laws and regulations. It is understood that, in some cases, AZA accreditation standards are more stringent than existing laws and regulations. In these cases the AZA standard must be met.

Chapter 1

(1.5.7) The animal collection must be protected from weather detrimental to their health.

(10.2.1) Critical life-support systems for the animal collection, including but not limited to plumbing, heating, cooling, aeration, and filtration, must be equipped with a warning mechanism, and emergency backup systems must be available. All mechanical equipment should be under a preventative maintenance program as evidenced through a record-keeping system. Special equipment should be maintained under a maintenance agreement, or a training record should show that staff members are trained for specified maintenance of special equipment.

(1.5.9) The institution must have a regular program of monitoring water quality for collections of fish, pinnipeds, cetaceans, and other aquatic animals. A written record must be maintained to document long-term water quality results and chemical additions.

Chapter 2

(1.5.2) Animals should be displayed, whenever possible, in exhibits replicating their wild habitat and in numbers sufficient to meet their social and behavioral needs. Display of single specimens should be avoided unless biologically correct for the species involved.

(10.3.3) All animal enclosures (exhibits, holding areas, hospital, and quarantine/isolation) must be of a size and complexity sufficient to provide for the animal's physical, social, and psychological well-being; and exhibit enclosures must include provisions for the behavioral enrichment of the animals.

(11.3.3) Special attention must be given to free-ranging animals so that no undue threat is posed to the animal collection, free-ranging animals, or the visiting public. Animals maintained where they will be in contact with the visiting public must be carefully selected, monitored, and treated humanely at all times.

(11.3.1) All animal exhibits and holding areas must be secured to prevent unintentional animal egress.

(11.3.6) Guardrails/barriers must be constructed in all areas where the visiting public could have contact with other than handleable animals.

(11.2.3) All emergency procedures must be written and provided to staff and, where appropriate, to volunteers. Appropriate emergency procedures must be readily available for reference in the event of an actual emergency. These procedures should deal with four basic types of emergencies: fire, weather/environment; injury to staff or a visitor; animal escape.

(11.6.2) Security personnel, whether staff of the institution, or a provided and/or contracted service, must be trained to handle all emergencies in full accordance with the policies and procedures of the institution. In some cases, it is recognized that Security personnel may be in charge of the respective emergency (i.e., shooting teams).

(11.2.4) The institution must have a communication system that can be quickly accessed in case of an emergency.

(11.2.5) A written protocol should be developed involving local police or other emergency agencies and include response times to emergencies.

(11.5.3) Institutions maintaining potentially dangerous animals (sharks, whales, tigers, bears, etc.) must have appropriate safety procedures in place to prevent attacks and injuries by these animals. Appropriate response procedures must also be in place to deal with an attack resulting in an injury. These procedures must be practiced routinely per the emergency drill requirements contained in these standards. Whenever injuries result from these incidents, a written account outlining the cause of the incident, how the injury was handled, and a description of any resulting changes to either the safety procedures or the physical facility must be prepared and maintained for five years from the date of the incident.

Chapter 3

(1.5.11) Animal transportation must be conducted in a manner that is safe, well-planned and coordinated, and minimizes risk to the animal(s), employees, and general public. All applicable local, state, and federal laws must be adhered to.

Chapter 5

(2.6.2) A formal nutrition program is recommended to meet the behavioral and nutritional needs of all species and specimens within the collection.

(2.6.3) Animal diets must be of a quality and quantity suitable for each animal's nutritional and psychological needs. Diet formulations and records of analysis of appropriate feed items should be maintained and may be examined by the Visiting Committee. Animal food, especially seafood products, should be purchased from reliable sources that are sustainable and/or well managed.

(2.6.1) Animal food preparations must meet all local, state/provincial, and federal regulations.

(2.6.4) The institution should assign at least one person to oversee appropriate browse material for the collection.

Chapter 6

(2.1.1) A full-time staff veterinarian is recommended. However, the Commission realizes that in some cases such is not practical. In those cases, a consulting/part-time veterinarian must be under contract to make at least twice monthly inspections of the animal collection and respond as soon as possible to any emergencies. The Commission also recognizes that certain collections, because of their size and/or nature, may require different considerations in veterinary care.

(2.1.2) So that indications of disease, injury, or stress may be dealt with promptly, veterinary coverage must be available to the animal collection 24 hours a day, 7 days a week.

(2.2.1) Written, formal procedures must be available to the animal care staff for the use of animal drugs for veterinary purposes and appropriate security of the drugs must be provided.

(1.4.6) A staff member must be designated as being responsible for the institution's animal record-keeping system. That person must be charged with establishing and maintaining the institution's animal records, as well as with keeping all animal care staff members apprised of relevant laws and regulations regarding the institution's animal collection.

(1.4.7) Animal records must be kept current, and data must be logged daily.

(1.4.5) At least one set of the institution's historical animal records must be stored and protected. Those records should include permits, titles, declaration forms, and other pertinent information.

(1.4.4) Animal records, whether in electronic or paper form, including health records, must be duplicated and stored in a separate location.

(1.4.3) Animals must be identifiable, whenever practical, and have corresponding ID numbers. For animals maintained in colonies or other animals not considered readily identifiable, the institution must provide a statement explaining how record keeping is maintained.

(1.4.1) An animal inventory must be compiled at least once a year and include data regarding acquisitions and dispositions in the animal collection.

(1.4.2) All species owned by the institution must be listed on the inventory, including those animals on loan to and from the institution. In both cases, notations should be made on the inventory.

(2.7.1) The institution must have holding facilities or procedures for the quarantine of newly arrived animals and isolation facilities or procedures for the treatment of sick/injured animals.

(2.7.3) Quarantine, hospital, and isolation areas should be in compliance with standards or guidelines adopted by the AZA.

(2.7.2) Written, formal procedures for quarantine must be available and familiar to all staff working with quarantined animals.

(11.1.2) Training and procedures must be in place regarding zoonotic diseases.

(11.1.3) A tuberculin testing and surveillance program must be established for appropriate staff in order to ensure the health of both the employees and the animal collection.

(2.5.1) Deceased animals should be necropsied to determine the cause of death. Disposal after necropsy must be done in accordance with local/federal laws.

(2.4.1) The veterinary care program must emphasize disease prevention.

(1.5.5) For animals used in offsite programs and for educational purposes, the institution must have adequate protocols in place to protect the rest of the collection from exposure to infectious agents.

(2.3.1) Capture equipment must be in good working order and available to authorized, trained personnel at all times.

(2.4.2) Keepers should be trained to recognize abnormal behavior and clinical symptoms of illness and have knowledge of the diets, husbandry (including enrichment items and strategies), and restraint procedures required for the animals under their care. However, keepers should not evaluate illnesses nor prescribe treatment.

(2.3.2) Hospital facilities should have x-ray equipment or have access to x-ray services.

(1.5.8) The institution must develop a clear process for identifying and addressing animal welfare concerns within the institution.

Chapter 8

(1.6.1) The institution must have a formal written enrichment program that promotes species-appropriate behavioral opportunities.

(1.6.2) The institution must have a specific staff member(s) or committee assigned for enrichment program oversight, implementation, training, and interdepartmental coordination of enrichment efforts.

Chapter 9

(5.3) A written policy on the use of live animals in programs should be on file. Animals in education programs must be maintained and cared for by trained staff, and housing conditions must meet standards set for the remainder of the animal collection, including species-appropriate shelter, exercise, social and environmental enrichment, access to veterinary care, nutrition, etc. Since some of these requirements can be met outside of the primary enclosure, for example, enclosures may be reduced in size provided that the animal's physical and psychological needs are being met.

(1.5.3) If animal demonstrations are a part of the institution's programs, an education and conservation message must be an integral component.

Chapter 10

(5.3) Institutions should maximize the generation of scientific knowledge gained from the animal collection. This might be achieved by participating in AZA TAG/SSP sponsored research when applicable, conducting original research projects, affiliating with local universities, and/or employing staff with scientific credentials.

(5.2) Institutions must have a written policy that outlines the type of research that it conducts, methods, staff involvement, evaluations, animals to be involved, and guidelines for publication of findings.

(5.1) Research activities must be under the direction of a person qualified to make informed decisions regarding research.

Appendix B: Acquisition/Disposition Policy

I. Introduction: The Association of Zoos and Aquariums (AZA) was established, among other reasons, to foster continued improvement in the zoological park and aquarium profession. One of its most important roles is to provide a forum for debate and consensus building among its members, the intent of which is to attain high ethical standards, especially those related to animal care and professional conduct. The stringent requirements for AZA accreditation and high standards of professional conduct are unmatched by similar organizations and also far surpass the United States Department of Agriculture's Animal and Plant Health Inspection Service's requirements for licensed animal exhibitors. AZA member facilities should abide by a Code of Professional Ethics - a set of standards that guide all aspects of animal management and welfare. As a matter of priority, AZA institutions should acquire animals from other AZA institutions and dispose of animals to other AZA institutions.

AZA-accredited zoological parks and aquariums cannot fulfill their important missions of conservation, education and science without living animals. Responsible management of living animal populations necessitates that some individuals be acquired and that others be removed from the collection at certain times. Acquisition of animals can occur through propagation, trade, donation, loan, purchase, capture, or rescue. Animals used as animal feed are not accessioned into the collection.

Disposition occurs when an animal leaves the collection for any reason. Reasons for disposition vary widely, but include cooperative population management (genetic or demographic management), reintroduction, behavioral incompatibility, sexual maturation, animal health concerns, loan or transfer, or death.

The AZA Acquisition/Disposition Policy (A/D) was created to help (1) guide and support member institutions in their animal acquisition and disposition decisions, and (2) ensure that all additions and removals are compatible with the Association's stated commitment to "save and protect the wonders of the living natural world." More specifically, the AZA A/D Policy is intended to:

- Ensure that the welfare of individual animals and conservation of populations, species and ecosystems are carefully considered during acquisition and disposition activities;
- Maintain a proper standard of conduct for AZA members during acquisition and disposition activities;
- Ensure that animals from AZA member institutions are not transferred to individuals or organizations that lack the appropriate expertise or facilities to care for them.
- Support the goal of AZA's cooperatively managed populations and associated programs, including Species Survival Plans® (SSPs), Population Management Plans (PMPs), and Taxon Advisory Groups (TAGs).

The AZA Acquisition/Disposition Policy will serve as the default policy for AZA member institutions. Institutions may develop their own A/D Policy in order to address specific local concerns. Any institutional policy must incorporate and not conflict with the AZA acquisition and disposition standards.

Violations of the AZA Acquisition/Disposition Policy will be dealt with in accordance with the AZA Code of Professional Ethics. Violations can result in an institution's or individual's expulsion from membership in the AZA.

II. Group or Colony-based Identification: For some colonial, group-living, or prolific species, such as certain insects, aquatic invertebrates, schooling fish, rodents, and bats, it is often impossible or highly impractical to identify individual specimens. These species are therefore maintained, acquisitioned, and disposed of as a group or colony. Therefore, when this A/D Policy refers to animals or specimens, it is in reference to both individuals and groups/colonies.

III. Germplasm: Acquisition and disposition of germplasm should follow the same guidelines outlined in this document if its intended use is to create live animal(s). Ownership of germplasm and any resulting animals should be clearly defined. Institutions acquiring or dispositioning germplasm or any animal parts or samples should consider not only its current use, but also future possible uses as new technologies become available.

IV(a). General Acquisitions: Animals are to be acquisitioned into an AZA member institution's collection if the following conditions are met:

1. Acquisitions must meet the requirements of all applicable local, state, federal and international regulations and laws.
2. The Director or Chief Executive Officer of the institution is charged with the final authority and responsibility for the monitoring and implementation of all acquisitions.
3. Acquisitions must be consistent with the mission of the institution, as reflected in its Institutional Collection Plan, by addressing its exhibition/education, conservation, and/or scientific goals.
4. Animals that are acquired for the collection, permanently or temporarily, must be listed on institutional records. All records should follow the Standards for Data Entry and Maintenance of North American Zoo and Aquarium Animal Records Databases®.
5. Animals may be acquired temporarily for reasons such as, holding for governmental agencies, rescue and/or rehabilitation, or special exhibits. Animals should only be accepted if they will not jeopardize the health, care or maintenance of the animals in the permanent collection or the animal being acquired.
6. The institution must have the necessary resources to support and provide for the professional care and management of a species, so that the physical and social needs of both specimen and species are met.
7. Attempts by members to circumvent AZA conservation programs in the acquisition of SSP animals are detrimental to the Association and its conservation programs. Such action may be detrimental to the species involved and is a violation of the Association's Code of Professional Ethics. All AZA members must work through the AZA SSP program in efforts to acquire AZA SSP species and adhere to the AZA Full Participation policy.
8. Animals are only to be acquired from sources that are known to operate legally and conduct their business in a manner that reflects and/or supports the spirit and intent of the AZA Code of Professional Ethics as well as this policy. Any convictions of state, federal, or international wildlife laws should be reviewed, as well as any previous dealings with other AZA-accredited institutions.
9. When acquiring specimens managed by a PMP, institutions should consult with the PMP manager.
10. Institutions should consult AZA Wildlife Conservation and Management Committee (WCMC)-approved Regional Collection Plans (RCPs) when making acquisition decisions.

IV(b). Acquisitions from the Wild: The maintenance of wild animal populations for education and wildlife conservation purposes is a unique responsibility of AZA member zoos and aquariums. To accomplish these goals, it may be necessary to acquire wild-caught specimens. Before acquiring animals from the wild, institutions are encouraged to examine sources including other AZA institutions or regional zoological associations.

When acquiring animals from the wild, careful consideration must be taken to evaluate the long-term impacts on the wild population. Any capture of free-ranging animals should be done in accordance with all local, state, federal, and international wildlife laws and regulations and not be detrimental to the long-term viability of the species or the wild or managed population(s). In crisis situations, when the survival of a population is at risk, rescue decisions are to be made on a case-by-case basis.

V(a). Disposition Requirements – living animals: Successful conservation and animal management efforts rely on the cooperation of many entities, both within and outside of AZA. While preference is given to placing animals within AZA member institutions, it is important to foster a cooperative culture among those who share the primary mission of AZA-accredited facilities. The AZA draws a strong distinction between the mission, stated or otherwise, of non-AZA member organizations and the mission of professionally managed zoological parks and aquariums accredited by the AZA.

An accredited AZA member balances public display, recreation, and entertainment with demonstrated efforts in education, conservation, and science. While some non-AZA member organizations may meet minimum daily standards of animal care for wildlife, the AZA recognizes that this, by itself, is insufficient to warrant either AZA membership or participation in AZA's cooperative animal management programs. When an animal is sent to a non-member of AZA, it is imperative that the member be confident that the animal will be cared for properly.

Animals may only be disposed of from an AZA member institution's collection if the following conditions are met:

1. Dispositions must meet the requirements of all applicable local, state, federal and international regulations and laws.
2. The Director or Chief Executive Officer of the institution is charged with the final authority and responsibility for the monitoring and implementation of all dispositions.
3. Any disposition must abide by the Mandatory Standards and General Advisories of the AZA Code of Professional Ethics. Specifically, "a member shall make every effort to assure that all animals in his/her collection and under his/her care are disposed of in a manner which meets the current disposition standards of the Association and do not find their way into the hands of those not qualified to care for them properly."
4. Non-domesticated animals shall not be disposed of at animal auctions. Additionally, animals shall not be disposed of to any organization or individual that may use or sell the animal at an animal auction. In transactions with AZA non-members, the recipient must ensure in writing that neither the animal nor its offspring will be disposed of at a wild animal auction or to an individual or organization that allows the hunting of the animal.
5. Animals shall not be disposed of to organizations or individuals that allow the hunting of these animals or their offspring. This does not apply to individuals or organizations which allow the hunting of only free-ranging game species (indigenous to North America) and established long-introduced species such as, but not limited to, white-tailed deer, quail, rabbit, waterfowl, boar, ring-necked pheasant, chukar, partridge, and trout. AZA distinguishes hunting/fishing for sport from culling for sustainable population management and wildlife conservation purposes.
6. Attempts by members to circumvent AZA conservation programs in the disposition of AZA SSP animals are detrimental to the Association and its conservation programs. Such action may be detrimental to the species involved and is a violation of the Association's Code of Professional Ethics. All AZA members must work through the AZA SSP program in efforts to deacquisition AZA SSP species and adhere to the AZA Full Participation policy.
7. Domesticated animals are to be disposed of in a manner consistent with acceptable farm practices and subject to all relevant laws and regulations.
8. Live specimens may be released within native ranges, subject to all relevant laws and regulations. Releases may be a part of a recovery program and any release must be compatible with the AZA Guidelines for Reintroduction of Animals Born or Held in Captivity, dated June 3, 1992.
9. Detailed disposition records of all living or dead specimens must be maintained. Where applicable, proper animal identification techniques should be utilized.
10. It is the obligation of every loaning institution to monitor, at least annually, the conditions of any loaned specimens and the ability of the recipient to provide proper care. If the conditions and care of animals are in violation of the loan agreement, it is the obligation of the loaning institution to recall the animal. Furthermore, an institution's loaning policy must not be in conflict with this A/D Policy.
11. If live specimens are euthanized, it must be done in accordance with the established policy of the institution and the Report of the American Veterinary Medical Association Panel on Euthanasia (Journal of the American Veterinary Medical Association 218 (5): 669-696, 2001).
12. In dispositions to non-AZA members, the non-AZA member's mission (stated or implied) must not be in conflict with the mission of AZA, or with this A/D Policy.
13. In dispositions to non-AZA member facilities that are open to the public, the non-AZA member must balance public display, recreation, and entertainment with demonstrated efforts in conservation, education, and science.
14. In dispositions to non-AZA members, the AZA members must be convinced that the recipient has the expertise, records management practices, financial stability, facilities, and resources required to properly care for and maintain the animals and their offspring. It is recommended that this documentation be kept in the permanent record of the animals at the AZA member institution.
15. If living animals are sent to a non-AZA member research institution, the institution must be registered under the Animal Welfare Act by the U.S. Department of Agriculture Animal and Plant Health Inspection Service. For international transactions, the receiving facility should be registered by that country's equivalent body with enforcement over animal welfare.
16. No animal disposition should occur if it would create a health or safety risk (to the animal or humans) or have a negative impact on the conservation of the species.

17. Inherently dangerous wild animals or invasive species should not be dispositioned to the pet trade or those unqualified to care for them.
18. Under no circumstances should any primates be dispositioned to a private individual or to the pet trade.
19. Fish and aquatic invertebrate species that meet ANY of the following are inappropriate to be disposed of to private individuals or the pet trade:
    a. species that grow too large to be housed in a 72-inch long, 180 gallon aquarium (the largest tank commonly sold in retail stores)
    b. species that require extraordinary life support equipment to maintain an appropriate *ex situ* environment (e.g., cold water fish and invertebrates)
    c. species deemed invasive (e.g., snakeheads)
    d. species capable of inflicting a serious bite or venomous sting (e.g., piranha, lion fish, blue-ringed octopus)
    e. species of wildlife conservation concern
20. When dispositioning specimens managed by a PMP, institutions should consult with the PMP manager.
21. Institutions should consult WCMC-approved RCPs when making disposition decisions.

V(b). Disposition Requirements – dead specimens: Dead specimens (including animal parts and samples) are only to be disposed of from an AZA member institution's collection if the following conditions are met:

1. Dispositions of dead specimens must meet the requirements of all applicable local, state, federal and international regulations and laws.
2. Maximum utilization is to be made of the remains, which could include use in educational programs or exhibits.
3. Consideration is given to scientific projects that provide data for species management and/or conservation.
4. Records (including ownership information) are to be kept on all dispositions, including animal body parts, when possible.
5. AZA SSP and TAG necropsy protocols are to be accommodated insofar as possible.

VI. Transaction Forms: AZA member institutions will develop transaction forms to record animal acquisitions and dispositions. These forms will require the potential recipient or provider to adhere to the AZA Code of Professional Ethics, the AZA Acquisition/Disposition Policy, and all relevant AZA and member policies, procedures and guidelines. In addition, transaction forms must insist on compliance with the applicable laws and regulations of local, state, federal and international authorities.

Appendix C: Recommended Quarantine Procedures

Quarantine facility: A separate quarantine facility, with the ability to accommodate mammals, birds, reptiles, amphibians, and fish should exist. If a specific quarantine facility is not present, then newly acquired animals should be isolated from the established collection in such a manner as to prohibit physical contact, to prevent disease transmission, and to avoid aerosol and drainage contamination.

Such separation should be obligatory for primates, small mammals, birds, and reptiles, and attempted wherever possible with larger mammals such as large ungulates and carnivores, marine mammals, and cetaceans. If the receiving institution lacks appropriate facilities for isolation of large primates, pre-shipment quarantine at an AZA or American Association for Laboratory Animal Science (AALAS) accredited institution may be applied to the receiving institutions protocol. In such a case, shipment must take place in isolation from other primates. More stringent local, state, or federal regulations take precedence over these recommendations.

Quarantine length: Quarantine for all species should be under the supervision of a veterinarian and consist of a minimum of 30 days (unless otherwise directed by the staff veterinarian). Mammals: If during the 30-day quarantine period, additional mammals of the same order are introduced into a designated quarantine area, the 30-day period must begin over again. However, the addition of mammals of a different order to those already in quarantine will not have an adverse impact on the originally quarantined mammals. Birds, Reptiles, Amphibians, or Fish: The 30-day quarantine period must be closed for each of the above Classes. Therefore, the addition of any new birds into a bird quarantine area requires that the 30-day quarantine period begin again on the date of the addition of the new birds. The same applies for reptiles, amphibians, or fish.

Quarantine personnel: A keeper should be designated to care only for quarantined animals or a keeper should attend quarantined animals only after fulfilling responsibilities for resident species. Equipment used to feed and clean animals in quarantine should be used only with these animals. If this is not possible, then equipment must be cleaned with an appropriate disinfectant (as designated by the veterinarian supervising quarantine) before use with post-quarantine animals.

Institutions must take precautions to minimize the risk of exposure of animal care personnel to zoonotic diseases that may be present in newly acquired animals. These precautions should include the use of disinfectant foot baths, wearing of appropriate protective clothing and masks in some cases, and minimizing physical exposure in some species; e.g., primates, by the use of chemical rather than physical restraint. A tuberculin testing/surveillance program must be established for zoo/aquarium employees in order to ensure the health of both the employees and the animal collection.

Quarantine protocol: During this period, certain prophylactic measures should be instituted. Individual fecal samples or representative samples from large numbers of individuals housed in a limited area (e.g., birds of the same species in an aviary or frogs in a terrarium) should be collected at least twice and examined for gastrointestinal parasites. Treatment should be prescribed by the attending veterinarian. Ideally, release from quarantine should be dependent on obtaining two negative fecal results spaced a minimum of two weeks apart either initially or after parasiticide treatment. In addition, all animals should be evaluated for ectoparasites and treated accordingly.

Vaccinations should be updated as appropriate for each species. If the animal arrives without a vaccination history, it should be treated as an immunologically naive animal and given an appropriate series of vaccinations. Whenever possible, blood should be collected and sera banked. Either a 70ºC (-94°F) - frost-free freezer or a 20ºC (-4°F) - freezer that is not frost-free should be available to save sera. Such sera could provide an important resource for retrospective disease evaluation.

The quarantine period also represents an opportunity to, where possible, permanently identify all unmarked animals when anesthetized or restrained (e.g., tattoo, ear notch, ear tag, etc.). Also, whenever animals are restrained or immobilized, a complete physical, including a dental examination, should be performed. Complete medical records should be maintained and available for all animals during the quarantine period. Animals that die during quarantine should have a necropsy performed under the supervision of a veterinarian and representative tissues submitted for histopathologic examination.

Quarantine procedures: The following are recommendations and suggestions for appropriate quarantine procedures for large canids

Large Canids:

Required:

1. direct and floatation fecals
2. vaccinate as appropriate

Strongly Recommended:

1. CBC/sera profile
2. urinalysis
3. appropriate serology (FIP, FeLV, FIV)
4. heartworm testing in appropriate species

Appendix D: Program Animal Policy and Position Statement

Program Animal Policy

Originally approved by the AZA Board of Directors – 2003

Updated and approved by the Board – July 2008 & June 2011

The Association of Zoos & Aquariums (AZA) recognizes many benefits for public education and, ultimately, for conservation in program animal presentations. AZA's Conservation Education Committee's *Program Animal Position Statement* summarizes the value of program animal presentations (see pages 42-44).

For the purpose of this policy, a Program Animal is defined as "an animal whose role includes handling and/or training by staff or volunteers for interaction with the public and in support of institutional education and conservation goals". Some animals are designated as Program Animals on a full-time basis, while others are designated as such only occasionally. Program Animal-related Accreditation Standards are applicable to all animals during the times that they are designated as Program Animals.

There are three main categories of Program Animal interactions:

1. On Grounds with the Program Animal Inside the Exhibit/Enclosure:
    i. Public access outside the exhibit/enclosure. Public may interact with animals from outside the exhibit/enclosure (e.g., giraffe feeding, touch tanks).
    ii. Public access inside the exhibit/enclosure. Public may interact with animals from inside the exhibit/enclosure (e.g., lorikeet feedings, 'swim with' programs, camel/pony rides).
2. On Grounds with the Program Animal Outside the Exhibit/Enclosure:
    i. Minimal handling and training techniques are used to present Program Animals to the public. Public has minimal or no opportunity to directly interact with Program Animals when they are outside the exhibit/enclosure (e.g., raptors on the glove, reptiles held "presentation style").
    ii. Moderate handling and training techniques are used to present Program Animals to the public. Public may be in close proximity to, or have direct contact with, Program Animals when they're outside the exhibit/enclosure (e.g., media, fund raising, photo, and/or touch opportunities).
    iii. Significant handling and training techniques are used to present Program Animals to the public. Public may have direct contact with Program Animals or simply observe the in-depth presentations when they're outside the exhibit/enclosure (e.g., wildlife education shows).
3. Off Grounds:
    i. Handling and training techniques are used to present Program Animals to the public outside of the zoo/aquarium grounds. Public may have minimal contact or be in close proximity to and have direct contact with Program Animals (e.g., animals transported to schools, media, fund raising events).

These categories assist staff and accreditation inspectors in determining when animals are designated as Program Animals and the periods during which the Program Animal-related Accreditation Standards are applicable. In addition, these Program Animal categories establish a framework for understanding increasing degrees of an animal's involvement in Program Animal activities.

Program animal presentations bring a host of responsibilities, including the safety and welfare of the animals involved, the safety of the animal handler and public, and accountability for the take-home, educational messages received by the audience. Therefore, AZA requires all accredited institutions that make program animal presentations to develop an institutional program animal policy that clearly identifies and justifies those species and individuals approved as program animals and details their long-term management plan and educational program objectives.

AZA's accreditation standards require that education and conservation messages must be an integral component of all program animal presentations. In addition, the accreditation standards require that the conditions and treatment of animals in education programs must meet standards set for the remainder of the animal collection, including species-appropriate shelter, exercise, appropriate environmental enrichment, access to veterinary care, nutrition, and other related standards. In addition, providing program animals with options to choose among a variety of conditions within their environment is

essential to ensuring effective care, welfare, and management. Some of these requirements can be met outside of the primary exhibit enclosure while the animal is involved in a program or is being transported. For example, free-flight birds may receive appropriate exercise during regular programs, reducing the need for additional exercise. However, the institution must ensure that in such cases, the animals participate in programs on a basis sufficient to meet these needs or provide for their needs in their home enclosures; upon return to the facility the animal should be returned to its species-appropriate housing as described above.

Program Animal Position Statement

Last revision 1/28/03
Re-authorized by the Board June 2011

The Conservation Education Committee (CEC) of the Association of Zoos and Aquariums supports the appropriate use of program animals as an important and powerful educational tool that provides a variety of benefits to zoo and aquarium educators seeking to convey cognitive and affective (emotional) messages about conservation, wildlife and animal welfare.

Utilizing these animals allows educators to strongly engage audiences. As discussed below, the use of program animals has been demonstrated to result in lengthened learning periods, increased knowledge acquisition and retention, enhanced environmental attitudes, and the creation of positive perceptions concerning zoo and aquarium animals.

Audience Engagement

Zoos and aquariums are ideal venues for developing emotional ties to wildlife and fostering an appreciation for the natural world. However, developing and delivering effective educational messages in the free-choice learning environments of zoos and aquariums is a difficult task.

Zoo and aquarium educators are constantly challenged to develop methods for engaging and teaching visitors who often view a trip to the zoo as a social or recreational experience (Morgan and Hodgkinson, 1999). The use of program animals can provide the compelling experience necessary to attract and maintain personal connections with visitors of all motivations, thus preparing them for learning and reflection on their own relationships with nature.

Program animals are powerful catalysts for learning for a variety of reasons. They are generally active, easily viewed, and usually presented in close proximity to the public. These factors have proven to contribute to increasing the length of time that people spend watching animals in zoo exhibits (Bitgood, Patterson and Benefield, 1986, 1988; Wolf and Tymitz, 1981).

In addition, the provocative nature of a handled animal likely plays an important role in captivating a visitor. In two studies (Povey, 2002; Povey and Rios, 2001), visitors viewed animals three and four times longer while they were being presented in demonstrations outside of their enclosure with an educator than while they were on exhibit. Clearly, the use of program animals in shows or informal presentations can be effective in lengthening the potential time period for learning and overall impact.

Program animals also provide the opportunity to personalize the learning experience, tailoring the teaching session to what interests the visitors. Traditional graphics offer little opportunity for this level of personalization of information delivery and are frequently not read by visitors (Churchman, 1985; Johnston, 1998). For example, Povey (2001) found that only 25% of visitors to an animal exhibit read the accompanying graphic; whereas, 45% of visitors watching the same animal handled in an educational presentation asked at least one question and some asked as many as seven questions. Having an animal accompany the educator allowed the visitors to make specific inquiries about topics in which they were interested.

Knowledge Acquisition

Improving our visitors' knowledge and understanding regarding wildlife and wildlife conservation is a fundamental goal for many zoo educators using program animals. A growing body of evidence supports the validity of using program animals to enhance delivery of these cognitive messages as well.

- MacMillen (1994) found that the use of live animals in a zoomobile outreach program significantly enhanced cognitive learning in a vertebrate classification unit for sixth grade students.
- Sherwood and his colleagues (1989) compared the use of live horseshoe crabs and sea stars to the use of dried specimens in an aquarium education program and demonstrated that students made the greatest cognitive gains when exposed to programs utilizing the live animals.
- Povey and Rios (2002) noted that in response to an open-ended survey question ("Before I saw this animal, I never realized that . . . "), visitors watching a presentation utilizing a program animal provided 69% cognitive responses (i.e., something they learned) versus 9% made by visitors viewing the same animal in its exhibit (who primarily responded with observations).
- Povey (2002) recorded a marked difference in learning between visitors observing animals on exhibit versus being handled during informal presentations. Visitors to demonstrations utilizing a raven and radiated tortoises were able to answer questions correctly at a rate as much as eleven times higher than visitors to the exhibits.

Enhanced Environmental Attitudes

Program animals have been clearly demonstrated to increase affective learning and attitudinal change.

- Studies by Yerke and Burns (1991) and Davison and her colleagues (1993) evaluated the effect live animal shows had on visitor attitudes. Both found their shows successfully influenced attitudes about conservation and stewardship.
- Yerke and Burns (1993) also evaluated a live bird outreach program presented to Oregon fifth-graders and recorded a significant increase in students' environmental attitudes after the presentations.
- Sherwood and his colleagues (1989) found that students who handled live invertebrates in an education program demonstrated both short and long-term attitudinal changes as compared to those who only had exposure to dried specimens.
- Povey and Rios (2002) examined the role program animals play in helping visitors develop positive feelings about the care and well-being of zoo animals.
- As observed by Wolf and Tymitz (1981), zoo visitors are deeply concerned with the welfare of zoo animals and desire evidence that they receive personalized care.

Conclusion

Creating positive impressions of aquarium and zoo animals, and wildlife in general, is crucial to the fundamental mission of zoological institutions. Although additional research will help us delve further into this area, the existing research supports the conclusion that program animals are an important tool for conveying both cognitive and affective messages regarding animals and the need to conserve wildlife and wild places.

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Appendix E: Developing an Institutional Program Animal Policy

Last revision 2003
Re-authorized by the Board June 2011

Rationale

Membership in AZA requires that an institution meet the AZA Accreditation Standards collectively developed by our professional colleagues. Standards guide all aspects of an institution's operations; however, the accreditation commission has asserted that ensuring that member institutions demonstrate the highest standards of animal care is a top priority. Another fundamental AZA criterion for membership is that education be affirmed as core to an institution's mission. All accredited public institutions are expected to develop a written education plan and to regularly evaluate program effectiveness.

The inclusion of animals (native, exotic and domestic) in educational presentations, when done correctly, is a powerful tool. CEC's **Program Animal Position Statement** describes the research underpinning the appropriate use of program animals as an important and powerful educational tool that provides a variety of benefits to zoo and aquarium educators seeking to convey cognitive and affective messages about conservation and wildlife.

Ongoing research, such as AZA's Multi-Institutional Research Project (MIRP) and research conducted by individual AZA institutions will help zoo educators to determine whether the use of program animals conveys intended and/or conflicting messages and to modify and improve programs accordingly and to ensure that all program animals have the best possible welfare.

When utilizing program animals our responsibility is to meet both our high standards of animal care and our educational goals. Additionally, as animal management professionals, we must critically address both the species' conservation needs and the welfare of the individual animal. Because "wild creatures differ endlessly," in their forms, needs, behavior, limitations and abilities (Conway, 1995), AZA, through its Animal Welfare Committee, has recently given the responsibility to develop taxon- and species-specific animal welfare standards and guidelines to the Taxon Advisory Groups (TAG) and Species Survival Plan® Program (SSP). Experts within each TAG or SSP, along with their education advisors, are charged with assessing all aspects of the taxons' and/or species' biological and social needs and developing Animal Care Manuals (ACMs) that include specifications concerning their use as program animals.
However, even the most exacting standards cannot address the individual choices faced by each AZA institution. Therefore, each institution is required to develop a program animal policy that articulates and evaluates program benefits. The following recommendations are offered to assist each institution in formulating its own Institutional Program Animal Policy, which incorporates the AZA Program Animal Policy and addresses the following matters.

The Policy Development Process

Within each institution, key stakeholders should be included in the development of that institution's policy, including, but not limited to representatives from:

- the Education Department
- the Animal Husbandry Department
- the Veterinary and Animal Health Department
- the Conservation & Science Department
- the Behavioral Husbandry Department
- any animal show staff (if in a separate department)
- departments that frequently request special program animal situations (e.g., special events, development, marketing, zoo or aquarium society, administration)

Additionally, staff from all levels of the organization should be involved in this development (e.g., curators, keepers, education managers, interpreters, volunteer coordinators).

To develop a comprehensive Program Animal Policy, we recommend that the following components be included:

I. Philosophy

In general, the position of the AZA is that the use of animals in up close and personal settings, including animal contact, can be extremely positive and powerful, as long as:

1. The use and setting is appropriate.
2. Animal and human welfare is considered at all times.
3. The animal is used in a respectful, safe manner and in a manner that does not misrepresent or degrade the animal.
4. A meaningful conservation message is an integral component. Read the AZA Board-approved Conservation Messages.
5. Suitable species and individual specimens are used.

Institutional program animal policies should include a philosophical statement addressing the above, and should relate the use of program animals to the institution's overall mission statement.

II. Appropriate Settings

The Program Animal Policy should include a listing of all settings both on and off site, where program animal use is permitted. This will clearly vary among institutions. Each institution's policy should include a comprehensive list of settings specific to that institution. Some institutions may have separate policies for each setting; others may address the various settings within the same policy. Examples of settings include:

I. On-site programming
   A. Informal and non-registrants:
      1. On-grounds programming with animals being brought out (demonstrations, lectures, parties, special events, and media)
      2. Children's zoos and contact yards
      3. Behind-the-scenes open houses
      4. Shows
      5. Touch pools
   B. Formal (registration involved) and controlled settings
      1. School group programs
      2. Summer Camps
      3. Overnights
      4. Birthday Parties
      5. Animal rides
      6. Public animal feeding programs

II. Offsite and Outreach
   1. PR events (TV, radio)
   2. Fundraising events
   3. Field programs involving the public
   4. School visits
   5. Library visits
   6. Nursing Home visits (therapy)
   7. Hospital visits
   8. Senior Centers
   9. Civic Group events

In some cases, policies will differ from setting to setting (e.g., on-site and off-site use with media). These settings should be addressed separately, and should reflect specific animal health issues, assessment of distress in these situations, limitations, and restrictions.

III. Compliance with Regulations

All AZA institutions housing mammals are regulated by the USDA's Animal Welfare Act. Other federal regulations, such as the Marine Mammal Protection Act, may apply. Additionally, many states, and some cities, have regulations that apply to animal contact situations. Similarly, all accredited institutions are bound by the AZA Code of Professional Ethics. It is expected that the Institution Program Animal Policy address compliance with appropriate regulations and AZA Accreditation Standards.

IV. Collection Planning

All AZA accredited institutions should have a collection planning process in place. Program animals are part of an institution's overall collection and must be included in the overall collection planning process. The AZA Guide to Accreditation contains specific requirements for the institution collection plan. For more information about collection planning in general, please see the Collection Management pages in the Members Only section.

The following recommendations apply to program animals:

1. Listing of approved program animals (to be periodically amended as collection changes). Justification of each species should be based upon criteria such as:
    - Temperament and suitability for program use
    - Husbandry requirements
    - Husbandry expertise
    - Veterinary issues and concerns
    - Ease and means of acquisition / disposition according to the AZA code of ethics
    - Educational value and intended conservation message
    - Conservation Status
    - Compliance with TAG and SSP guidelines and policies
2. General guidelines as to how each species (and, where necessary, for each individual) will be presented to the public, and in what settings
3. The collection planning section should reference the institution's acquisition and disposition policies.

V. Conservation Education Message

As noted in the AZA Accreditation Standards, if animal demonstrations are part of an institution's programs, an educational and conservation message must be an integral component. The Program Animal Policy should address the specific messages related to the use of program animals, as well as the need to be cautious about hidden or conflicting messages (e.g., "petting" an animal while stating verbally that it makes a poor pet). This section may include or reference the AZA Conservation Messages.

Although education value and messages should be part of the general collection planning process, this aspect is so critical to the use of program animals that it deserves additional attention. In addition, it is highly recommended to encourage the use of biofacts in addition to or in place of the live animals. Whenever possible, evaluation of the effectiveness of presenting program animals should be built into education programs.

VI. Human Health and Safety

The safety of our staff and the public is one of the greatest concerns in working with program animals. Although extremely valuable as educational and affective experiences, contact with animals poses certain risks to the handler and the public. Therefore, the human health and safety section of the policy should address:

1. Minimization of the possibility of disease transfer from non-human animals to humans, and vice-versa (e.g., handwashing stations, no touch policies, use of hand sanitizer)
2. Safety issues related to handlers' personal attire and behavior (e.g., discourage or prohibit use of long earrings, perfume and cologne, not eating or drinking around animals, smoking etc.)

AZA's Animal Contact Policy provides guidelines in this area; these guidelines were incorporated into accreditation standards in 1998.

VII. Animal Health and Welfare

Animal health and welfare are the highest priority of AZA accredited institutions. As a result, the Institutional Program Animal Policy should make a strong statement on the importance of animal welfare. The policy should address:

1. General housing, husbandry, and animal health concerns (e.g. that the housing and husbandry for program animals meets or exceeds general AZA standards and that the physical, social and psychological needs of the individual animal, such as adequate rest periods, provision of enrichment, visual cover, contact with conspecifics as appropriate, etc., are accommodated).
2. Where ever possible provide a choice for animal program participation, e.g., retreat areas for touch tanks or contact yards, evaluation of willingness/readiness to participate by handler, etc.)
3. The empowerment of handlers to make decisions related to animal health and welfare; such as withdrawing animals from a situation if safety or health is in danger of being compromised.
4. Requirements for supervision of contact areas and touch tanks by trained staff and volunteers.
5. Frequent evaluation of human / animal interactions to assess safety, health, welfare, etc.
6. Ensure that the level of health care for the program animals is consistent with that of other animals in the collection.
7. Whenever possible have a “cradle to grave” plan for each program animal to ensure that the animal can be taken care of properly when not used as a program animal anymore.
8. If lengthy “down” times in program animal use occur, staff should ensure that animals accustomed to regular human interactions can still maintain such contact and receive the same level of care when not used in programs.

VIII. Taxon Specific Protocols

We encourage institutions to provide taxonomically specific protocols, either at the genus or species level, or the specimen, or individual, level. Some taxon-specific guidelines may affect the use of program animals. To develop these, institutions refer to the Conservation Programs Database.

Taxon and species -specific protocols should address:

1. How to remove the individual animal from and return it to its permanent enclosure, including suggestions for operant conditioning training.
2. How to crate and transport animals.
3. Signs of stress, stress factors, distress and discomfort behaviors.

Situation specific handling protocols (e.g., whether or not animal is allowed to be touched by the public, and how to handle in such situations)

1. Guidelines for disinfecting surfaces, transport carriers, enclosures, etc. using environmentally safe chemicals and cleaners where possible.
2. Animal facts and conservation information.
3. Limitations and restrictions regarding ambient temperatures and or weather conditions.
4. Time limitations (including animal rotation and rest periods, as appropriate, duration of time each animal can participate, and restrictions on travel distances).
5. The numbers of trained personnel required to ensure the health and welfare of the animals, handlers and public.
6. The level of training and experience required for handling this species
7. Taxon/species-specific guidelines on animal health.
8. The use of hand lotions by program participants that might touch the animals

IX. Logistics: Managing the Program

The Institutional Policy should address a number of logistical issues related to program animals, including:

1. Where and how the program animal collection will be housed, including any quarantine and separation for animals used off-site.
2. Procedures for requesting animals, including the approval process and decision making process.
3. Accurate documentation and availability of records, including procedures for documenting animal usage, animal behavior, and any other concerns that arise.

X. Staff Training

Thorough training for all handling staff (keepers, educators, and volunteers, and docents) is clearly critical. Staff training is such a large issue that many institutions may have separate training protocols and procedures. Specific training protocols can be included in the Institutional Program Animal Policy or reference can be made that a separate training protocol exists.

It is recommended that the training section of the policy address:

1. Personnel authorized to handle and present animals.
2. Handling protocol during quarantine.
3. The process for training, qualifying and assessing handlers including who is authorized to train handlers.
4. The frequency of required re-training sessions for handlers.
5. Personnel authorized to train animals and training protocols.
6. The process for addressing substandard performance and noncompliance with established procedures.
7. Medical testing and vaccinations required for handlers (e.g., TB testing, tetanus shots, rabies vaccinations, routine fecal cultures, physical exams, etc.).
8. Training content (e.g., taxonomically specific protocols, natural history, relevant conservation education messages, presentation techniques, interpretive techniques, etc.).
9. Protocols to reduce disease transmission (e.g., zoonotic disease transmission, proper hygiene and hand washing requirements, as noted in AZA's Animal Contact Policy).
10. Procedures for reporting injuries to the animals, handling personnel or public.
11. Visitor management (e.g., ensuring visitors interact appropriately with animals, do not eat or drink around the animal, etc.).

XI. Review of Institutional Policies

All policies should be reviewed regularly. Accountability and ramifications of policy violations should be addressed as well (e.g., retraining, revocation of handling privileges, etc.). Institutional policies should address how frequently the Program Animal Policy will be reviewed and revised, and how accountability will be maintained.

XII. TAG and SSP Recommendations

Following development of taxon-specific recommendations from each TAG and SSP, the institution policy should include a statement regarding compliance with these recommendations. If the institution chooses not to follow these specific recommendations, a brief statement providing rationale is recommended.

Appendix F: Large Canid Ethograms

Mexican gray and red wolf: General *Canis* behavior is well-documented. It has been suggested by numerous researchers that closely-related canids (such as the *Canis spp*.) exhibit similar forms of behavior, though the frequency of the behaviors exhibited may differ significantly. The following ethogram is not comprehensive; instead, it is intended that zoo managers use the following as a descriptive guide to both Mexican grey and red wolf behavior (and as an example for other canid species) to assist them in day-to-day management. The behaviors listed below are grouped by general category. It should be noted, however, that many of these behaviors may be observed in more than one context. Following this ethogram is one for maned wolves to allow for a general comparison.

Mexican grey wolf and red wolf ethogram (adapted from Waddell, 1998; Mexican Wolf Species Survival Plan®. 2009. Mexican Gray Wolf Husbandry Manual: Guidelines for Captive Management.)

Category	Behavior	Description
General	Approach	Wolf approaches another within 3 body lengths. An interaction is not required. Distinguished from threat by lack of an aggressive body posture or vocalizations.
	Follow	Wolf follows another. They do not have to be within 3 body lengths, but actions must be simultaneous, i.e. both are locomoting, one behind the other.
	Pass	Wolves locomote towards each other and pass (one going one direction, one the other) without stopping or interacting.
Elimination	Leg lift urination	Urination with one leg lifted off the ground.
	Squat urination	Urination from a squatting position.
	Over mark	Wolf urinating or defecating almost immediately over the urination or defecation of another.
	Scent rolling	Rub of head, neck, back on a surface; often where another urinated or defecated. May include repeated full rolls in the area.
	Defecate	Wolf discharges fecal material.
	Scrape mark	Wolf uses both front paws and back to tear at the ground (dirt is usually thrown up in the process). May follow the discharge of urine.
Social	Play	Interactive behavior event identified by play initiator (see below) performed by one wolf at some point in the interaction (most often at the beginning, but does not have to be). Play initiator can include any of the following: a) player bow-crouches on forelegs with elevated rear-end and straightened rear legs, b) exaggerated approach-gait is bouncy, or a rush; head and shoulders frequently moved side to side; c) approach/withdrawal can be at different speeds or showing physical intent to move away (e.g., rock back and forth); d) general movements such as head tosses, paw raises, etc. Play types include prone play, one up/one down play, wrestle play, locomotor play, and ambush/stalk play. Wolves can show any of the following: play “grin”, head up and alert, tail wag, mouth open with lips drawn back and tongue out.
	Unreciprocated play	Wolf directs any play type towards another, who subsequently does not respond, actively tries to avoid the initiator, or becomes aggressive towards it.
	Self play	Wolf chasing its own tail, limb biting, etc.
	Sniff or Lick A-G	Sniff or lick another’s ano-genital area.

Category	Behavior	Description
	Present A-G	Female stands or walks with hindquarters oriented to male's face, back slightly arched, base of tail deflected up or to the side.
	Attempted mount	Male attempts to mount female, though mount is unsuccessful (may be from incorrect orientation.)
	Mount	Wolf standing behind another resting upon its back with forepaws clasped around the midsection/pelvic region; may be followed by pelvic thrusts.
	Copulatory tie	Male and female are joined in a 'mount' position that lasts a minimum of 60 seconds. Seen after several short pelvic thrusts followed by 2-3 deep thrusts resulting in the tie. Back to back ties are sometimes seen.
Agonistic	Charge	Wolf locomotes rapidly towards another exhibiting one or more of the following behaviors: ears back, head down, hair is piloerect, forelegs are stiff. Recipient may either react (see fight, passive/active submission) or retreat.
	Threat	Orientation towards another with threat facial expressions (vertical retraction of the lips and baring of the front teeth), stiff legs, ears forward and erect, elevated tail and hair piloerected. May be accompanied by a growl.
	Chase	Wolf runs after another (both are running).
	Passive Submission	Wolf approaches another in crouch or semi-crouch position with body oriented sideways to partner; head typically rolled sideways while looking at partner. May be accompanied by whimper/whine and licking intentions towards partner.
	Active Submission	In presence of another, wolf falls or lies on its side or back, often with hind legs raised and ears back. May be accompanied by whimper/whine. Can follow passive submission.
	Fight	An interaction which is usually initiated by a charge from one individual, followed by both growling, rising up or partially up on their hind legs and batting at each other with the forepaws or grasping each other around the neck or shoulders (sparring), growling, threatening, and attempting to pin the other to the ground.
Vocalization	Whimper/whine	Soft whine, usually emitted while approaching another in a submissive (ears back, somewhat crouched) posture.
	Aggressive	Growl, bark vocalization.
	Distress	High pitched, whining or yelping vocalization. Often associated with submissive vocalization behavior.
	Howl	A sustained vocalization in which the pitch can remain constant or vary smoothly between pitches.

Maned wolf: The following table provides an ethogram for maned wolves. The behaviors listed below are grouped by general category, but many may be observed in more than one context.

Maned wolf ethogram (adapted from AZA Maned Wolf SSP 2007)

Category	Behavior	Description
General	Lying down	Mid-section of body in contact with the ground
	Sit	Back part of body in contact with the ground, usually occurs when animal is scratching
	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
Solitary	Self-groom	Lick own body
	Sniff	Investigate object with nose; nose <10 cm (3.9 in) 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 or marking	Squat	Urination using a squatting posture, sometimes one hind leg may be lifted slightly off the ground
	Leg lift	Urination with hind leg lifted 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	Sniff conspecific	Investigate another wolf anywhere except the anogenital region

Category	Behavior	Description
	Groom conspecific	Lick body of another wolf
	Sniff/lick anogenital	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	Regurgitate	Disgorge partly digested food to mate or pups
	Regurgitation solicit	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

Category	Behavior	Description
	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

Appendix G: Large Canid Necropsy Protocol

(Adapted from Waddell, 1998; AZA Mexican Wolf SSP, 2009)

INSTITUTION/OWNER	
CANID SPECIES	ANIMAL ISIS ID #
STUD BOOK #	SEX
BIRTH DATE/AGE	WEIGHT
REPRODUCTIVE HISTORY:	
SHOWN BEHAVIORAL ESTRUS?	
EVER BRED?	
PRODUCED PUPS?	
EVER HOUSED WITH OPPOSITE SEX?	
DATE OF DEATH DATE OR NECROPSY	
HISTORY: (Briefly summarize clinical signs, circumstances of death.)	

Institution pathologists should perform a histopathology on the tissues. The gross examination worksheets and pathologists report should be sent to the relevant AZA Canid SSP Pathology Advisor, and copies also sent to the appropriate AZA Canid SSP Veterinary Advisor. Contact details can be provided by the AZA Canid TAG and AZA Canid SSP Program Coordinators.

GROSS EXAMINATION (*WORKSHEET)*

Animal ISIS ID#_____

PROSECTOR: _____

GENERAL CONDITION: (Nutritional condition, physical condition)
- NEONATES: Examine for malformations (cleft palate, deformed limbs, etc.)

SKIN: (Including pinna, feet)

MUSCULOSKELETAL SYSTEM: (Bones, joints, muscles)

BODY CAVITIES: (Fat stores, abnormal fluids)
- NEONATES: Assess hydration (tissue moistness)

HEMOLYMPHATIC: (Spleen, lymph nodes, thymus)

RESPIRATORY SYSTEM: (Nasal cavity, larynx, trachea, lungs, regional lymph nodes)
- NEONATES: Determine if breathing occurred (Do the lungs float in formalin?)

CARDIOVASCULAR SYSTEM: (Heart, pericardium, great vessels)

DIGESTIVE SYSTEM: (Mouth, teeth, esophagus, stomach, intestines, liver, pancreas, mesenteric lymph nodes)
- NEONATES: Is milk present in stomach?

URINARY SYSTEM: (Kidneys, ureters, urinary bladder, urethra)

REPRODUCTIVE SYSTEM: (Testis/ovary, uterus, vagina, penis, prepuce, prostate, mammary glands, placenta)

ENDOCRINE SYSTEM: (Adrenals, thyroid, parathyroids, pituitary)

NERVOUS SYSTEM: (Brain, spinal cord, peripheral nerves)

SENSORY ORGANS: (Eyes, ears)

PRELIMINARY DIAGNOSES:

LABORATORY STUDIES: (List bacterial and viral cultures submitted and results, if available.)

FIXED TISSUE CHECK LIST

The following tissues should be preserved in 10% buffered formalin at a ratio of 1 part tissue to 10 parts formalin. Tissues should be no thicker than 1 cm (0.4 in). Sections of all lesions and samples of all listed tissues (see below) should be included. Additional tissues from neonates are also needed, and are listed in the neonatal protocol below. Specific information on specific tissue sectioning procedures is provided below.

TISSUES TO SAMPLE (ALL TISSUES CAN BE PUT IN ONE CONTAINER):

Heart
Trachea
Thyroid/parathyroid glands
Lungs
Thymus
Lymph nodes
Spleen
Liver
Stomach
Small intestines
Pancreas
Spinal cord (if neurological disease)
Large intestines
Adrenal
Kidneys
Urinary bladder
Testis/Ovary
Uterus
Brain
Skin
Skeletal muscle
Bone marrow
Long bone (if bone disease)

FROZEN TISSUE: Frozen tissues should be stored in plastic bags at -70 or -20°C (-94 or -4°F) for toxicology: The liver, brain, kidney, and (if possible) antemortem serum and plasma should also be frozen. If an infectious disease is suspected, samples of the small intestines, lung, spleen, and lymph nodes should be frozen.

NEONATAL NECROPSY PROTOCOL

Please follow the adult protocol in addition to the following:

1. Fix umbilical stump and surrounding tissues.
2. Examine of malformations (cleft palate, deformed limbs, heart defects).
3. Assess hydration (tissue moistness) and evidence of nursing (milk in stomach).
4. Determine if breathing occurred. (Do the lungs float in formalin?)
5. Check foot pads for erosions and ulcers.

Additional tissues for histopathology from neonates:

- All tissues from the adult necropsy check list
- Umbilicus (including external and internal vessels and surrounding skin)
- Foot pads from all feet.
- Extra sections of lung.

RECOMMENDED TISSUE SAMPLING PROCEDURES

Adrenal glands: Entire gland with transverse incision.

Brain: Cut longitudinally along the midline. Submit entire brain and pituitary gland except for frozen sections.

Eye: Both eyes intact. Remove extra-ocular muscles and periorbital tissues.

Gastrointestinal tract: Open carefully along the long axis.

- Esophagus: Take a 3 cm (1.2 in) long section
- Stomach: Take multiple sections from cardia, fundus (body), and atrium of pylorus.
- Small intestines: Duodenum, jejunum, ileum,
- Large intestines: Cecum, colon
- Omentum: 3 cm (1.2 in) square

Heart: Longitudinal section including atrium, ventricle, and valves from (both) right and left heart (include large vessels).

Kidney: Cortex and medulla from each kidney.

Liver: Sections from 3 lobes with capsule and gall bladder.

Lungs: Sections from several lobes including a major bronchus.

Lymph nodes: Cervical, mediastinal, bronchial, mesenteric, and lumbar cut transversely.

Opened rib or longitudinally sectioned half femur: Marrow must be exposed for proper fixation.

Oral/pharyngeal mucosa and tonsil: Plus any areas with erosions, ulcerations, or proliferative lesions.

Pancreas: Representative sections from two areas including central ducts.

Pituitary glands: Entire gland.

Reproductive tract: Ovaries and entire uterus with longitudinal cut into lumens of uterine horns. Both testes (transversely cut) with epididymis. Entire prostate, transversely cut.

Salivary gland:

Sciatic nerve: 3 cm (1.2 in) section.

Skeletal muscle: Cross section of thigh muscle.

Skin: Full thickness of abdominal skin, lip, and ear pinna.

Spinal Cord: If neurological disease, sections from cervical, thoracic, and lumbar cord.

Spleen: Cross-sections including capsule.

Thymus:

Thyroid/parathyroids: Leave glands intact.

Tongue: Cross section near tip including both mucosal surfaces.

Trachea:

Urinary bladder/ureters/urethra: Cross section of bladder and 2 cm (0.8 in) sections of tubular structures.

SHIPPING TISSUES:
After at least 72 hours in fixative, tissues should be shipped in a leak-proof container in adequate formalin to keep tissues moist. Tissues can be shipped by U.S. Mail or UPS to the relevant AZA Canid TAG/SSP Pathology Advisor. Contact the AZA Canid TAG and/or AZA Canid SSP Program Coordinators for contact details.

Appendix H: Maned wolf necropsy protocol

AZA Maned Wolf SSP 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 AZA 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 AZA Maned Wolf SSP Veterinary Advisor and Pathology Advisor listed below. The submitted information will be included in a year-end summary report for 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, DVM, Dipl. ACVP
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 two projects that make use of female reproductive tracts. Please follow directions in #1 and #2 below to provide the appropriate tissues to Drs. Agnew (1 ovary and upper reproductive tract: uterus) and Songsasen (1 ovary and lower reproductive tract: vagina and cervix from female; testis from male).

1. Dr. Dalen Agnew has accepted the responsibility of serving as a pathology advisor to the AZA Wildlife Contraception Center and will continue the projects begun by Dr. Linda Munson. Please provide Dr. Agnew with **upper reproductive tract (uterus), 1 ovary, and mammary gland tissue from female maned wolves regardless of their contraceptive history**. Samples should be fixed in 10% neutral buffered formalin. **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. Dalen Agnew
Attn: Histo Research
Diagnostic Center for Population & Animal Health4125 Beaumont Road
Lansing, MI 48910-8104
agnewd@dcpah.msu.edu (517) 353-1683

2. Dr. Nucharin Songsasen has requested the **lower reproductive tracts (vagina and cervix) and 1 ovary 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 **540-635-0030**

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 animal's medical record and submit as part of the historical information in necropsy requests/reports.

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 (0.2 in)
- 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/1.2-1.6 in 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 –70°C (-94°F) (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 –20°C (-4°F)
  - 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 (1.2-2.0 in) blocks of tissue from major organs (e.g. heart, lung, liver, kidney, spleen, intestines, brain, spleen) in small plastic bags
   (1) Store at –70°C (-94°F) 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.

Necropsy Protocol ADDENDUM I: Instructions & Survey

AZA CAG Contraceptive Pathology Survey Adapted for AZA Maned Wolf SSP

MEMORANDUM

TO: MANED WOLF SSP VETERINARIANS AND MAMMAL CURATORS

FROM:

Dr. Dalen Agnew
Attn: Histo Research
Diagnostic Center for Population & Animal Health4125 Beaumont Road
Lansing, MI 48910-8104
agnewd@dcpah.msu.edu (517) 353-1683

DATE: July 2010

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

The AZA Contraceptive Advisory Group is continuing the pathology surveillance of reproductive tracts from managed 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*** **upper reproductive** tract (uterus), **1 ovary and mammary gland** obtained from necropsy or ovariohysterectomy of any female mammal. (**Reminder**: collect, process and overnight ship *fresh*, *unfixed* lower reproductive tract (cervix and vagina), 1 ovary, and mammary gland in 10 ml of 0.9% saline to Dr. Nucharin Songsasen (follow the instructions on page 1 of AZA MWSSP Necropsy protocol)).

FIXATION: Remove upper reproductive tract (uterus), 1 ovary and mammary gland tissue. Make a *small* incision into one horn (bicornuate uteri). Immerse all tissues in 10% neutral buffered formalin for at least 72 hours (ratio of tissue to formalin = 1:10) prior to shipment for processing.

SHIPPING: For shipment, wrap fixed tissues for the AZA CAG Contraceptive Pathology Survey in formalin-soaked paper towels, enclose in a leak-proof plastic container and ship by ground US mail (Federal Express is not necessary) to Dr. Dalen Agnew at 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.

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)

Appendix I: Diet Analyses

African wild dog diet analysis (Pribyl and Crissey 1999)

Nutrient	Target Nutrient Levels	100% Meat-Based Canine	100% Meat-Based Feline	Feline & Dry Dog Kibble 86/14*	100% Dry Dog Kibble	100% Commercial Canned Cat Food	100% Commercial Dry Cat Food
Energy (Kcal/g)	3.5-5						
Crude Protein (%)	18-24	61	50	44	30	40	34
Fat (%)	5	23	32	26	11	20	9
Fiber (%)	-	5	4	4	5	4	5
Linoleic Acid (%)	.5-1	-	-	0.5	2	-	1
Arachidonic Acid (%)	.02	-	-	-	-	-	-
Vitamin A (IU/g)	3.33-5	8	97	76	20	10	11
Vitamin D (IU/g)	.5	1	1	1	2	.8	2
Vitamin E (mg/kg)	30-50	57	42	34	12	60	11
Thiamin (mg/kg)	1-5	20	15	13	8	-	6
Riboflavin (mg/kg)	2.2-4	15	16	13	5	-	6
Niacin (mg/kg)	11.4-40	160	194	155	53	-	85
Pyridoxine (mg/kg)	1-4	12	10	10	8	-	6
Folacin (mg/kg)	.18-.80	.002	.002	0.41	1.5	-	1.1
Vitamin B12 (mg/kg)	.02	.04	.08	0.07	.03	-	.02
Pantothenic Acid (mg/kg)	5-10	45	51	40	10	-	23
Choline (mg/kg)	1200-2400	5097	5921	4642	1250	-	2500
Biotin (mg/kg)	.07-.10	-	-	.040	.15	-	.09
Vitamin C (mg/kg)	-	-	-	-	-	-	-
Nutrient	Target Nutrient Levels	100% Meat-Based Canine	100% Meat-Based Feline	Feline & Dry Dog Kibble 86/14*	100% Dry Dog Kibble	100% Commercial Canned Cat Food	100% Commercial Dry Cat Food
Magnesium (%)	.04	.16	.05	.08	.17	.10	.16
Potassium	.4-.6	1.3	1.1	1.0	0.7	-	.74
Sodium (%)	.05-.06	.58	.60	.59	.57	.72	.57
Iron (mg/kg)	60-80	227	221	223	227	-	284
Zinc (mg/kg)	50-120	113	96	99	108	-	102
Copper (mg/kg)	5-7.3	9	5	8	15	-	14
Manganese (mg/kg)	5	85	63	63	63	-	51
Selenium (mg/kg)	.10-.11	.23	0.18	0.13	-	-	-
Iodine (mg/kg	.35-1.54	.71	0.58	1.11	2.5	-	1.71

Maned wolf diet analysis

Nutrient requirements of domestic dogs (NRC, 2006) and calculated nutrient composition of diets offered to maned wolves (*Chrysocyon brachyurus*) at 3 representative North American zoos (adapted from Phipps & Edwards, 2009). All data, except moisture, expressed on a dry-matter basis.

NUTRIENT	NRC-DOG[1]	Zoo1[2]	Zoo2[2]	Zoo3[2]
Moisture, %	-	43.2	15.7	17.89
Crude protein, %	10	23.33	29.99	28.62
Lysine, %	0.35	1.48	1.971	1.845
Methionine, %	0.33	0.494	0.635	0.604
Cystine, %	0.32	0.321	0.428	0.4
Taurine, %	-	0.099	0.129	0.129
Fat, %	5.5	14.9	19.76	18.96
CHO, %	-	47.19	32.09	34.4
Crude fiber, %	-	2.48	2.365	2.48
Ash, %	-	6.015	6.975	6.85
ME, kcal/kg	-	4.29	4.325	4.43
Ca (%)	0.3	1.128	1.493	1.453
P (%)	0.3	0.796	1.028	1.004
K (%)	0.4	0.866	0.862	0.89
Na (%)	0.04	0.287	0.385	0.365
Mg (%)	0.06	0.101	0.111	0.109
Cu (ppm)	0.6	14.5	17.3	17.3
Fe (ppm)	3	276.55	345.95	343.2
Mn (ppm)	0.5	65.85	81.8	81.9
Zn (ppm)	6	197.15	255.6	253.7

[1] National Research Council (2006)

[2] adapted from Phipps & Edwards (2009)

Appendix J: ISIS Physiological Values

International **S**pecies **I**nformation **S**ystem (Teare, 2002)
All tables in conventional U.S.A. units

AFRICAN HUNTING DOG, *Lycaon pictus*
Physiological reference ranges calculated for:

- Both sexes combined
- All ages combined

Sample results submitted by 22 member institutions.

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	11.09	3.516	4.180	21.70	277	109
RED BLOOD CELL COUNT	*10^6/µl	8.07	1.21	4.94	15.10	238	90
HEMOGLOBIN	g/dl	15.4	1.9	11.0	20.5	248	94
HEMATOCRIT	%	44.3	5.9	29.3	63.4	292	113
MCV	fL	55.5	4.2	35.8	76.2	235	90
MCH	pg/cell	19.1	1.7	11.3	27.8	232	89
MCHC	g/dl	34.5	2.2	27.9	40.8	246	94
PLATELET COUNT	*10^3/µl	430	181	114	1155	105	54
NUCLEATED RED BLOOD CELLS	/100 WBC	2	1	0	5	66	33
RETICULOCYTES	%	0.4	0.4	0.0	1.2	8	6
SEGMENTED NEUTROPHILS	*10^3/µl	8.331	2.842	3.180	20.80	269	107
LYMPHOCYTES	*10^3/µl	1.757	1.183	0.123	7.480	268	107
MONOCYTES	*10^3/µl	0.490	0.345	0.050	2.416	243	103
EOSINOPHILS	*10^3/µl	0.642	0.499	0.060	3.528	225	100
BASOPHILS	*10^3/µl	0.115	0.109	0.000	0.432	22	18
AZUROPHILS	*10^3/µl	0.000	0.000	0.000	0.000	1	1
NEUTROPHILIC BANDS	*10^3/µl	0.372	0.717	0.000	4.560	57	40
ERYTHROCYTE SEDIMENTATION RATE	mm/Hr	0	0	0	0	4	3
CALCIUM	mg/dl	10.2	0.8	8.1	12.4	222	102
PHOSPHORUS	mg/dl	4.6	1.9	1.8	10.6	206	93
SODIUM	mEq/L	148	5	129	161	200	95
POTASSIUM	mEq/L	4.4	0.7	3.2	9.6	203	95
CHLORIDE	mEq/L	116	7	90	150	203	95

BICARBONATE	mEq/L	18.7	3.9	12.0	33.0	31	17
CARBON DIOXIDE	mEq/L	19.5	3.5	0.0	25.0	79	43
OSMOLARITY	mOsmol/L	305	18	263	331	17	6
IRON	µg/dl	86	20	60	116	8	5
MAGNESIUM	mg/dl	2.49	0.41	1.94	3.28	10	4
BLOOD UREA NITROGEN	mg/dl	23	8	9	58	252	104
CREATININE	mg/dl	1.2	0.3	0.5	2.4	222	103
URIC ACID	mg/dl	0.6	0.3	0.0	1.8	97	49
TOTAL BILIRUBIN	mg/dl	0.2	0.1	0.0	0.7	234	100
DIRECT BILIRUBIN	mg/dl	0.1	0.1	0.0	0.4	81	42
INDIRECT BILIRUBIN	mg/dl	0.1	0.1	0.0	0.3	81	42
GLUCOSE	mg/dl	151	45	73	407	239	103
CHOLESTEROL	mg/dl	259	67	145	537	216	98
TRIGLYCERIDE	mg/dl	71	39	16	231	115	52
CREATINE PHOSPHOKINASE	IU/L	235	207	34	1375	144	72
LACTATE DEHYDROGENASE	IU/L	243	239	32	1270	126	56
ALKALINE PHOSPHATASE	IU/L	48	51	7	324	224	101
ALANINE AMINOTRANSFERASE	IU/L	56	27	14	194	247	103
ASPARTATE AMINOTRANSFERASE	IU/L	39	23	6	188	240	104
GAMMA GLUTAMYLTRANSFERASE	IU/L	6	3	0	15	132	67
AMYLASE	U/L	615	352	259	1742	77	46
LIPASE	U/L	140	71	73	400	24	13
TOTAL PROTEIN (COLORIMETRY)	g/dl	6.0	0.5	4.6	7.3	224	99
GLOBULIN (COLORIMETRY)	g/dl	2.8	0.4	1.7	4.1	198	96
ALBUMIN (COLORIMETRY)	g/dl	3.2	0.4	2.3	4.4	201	98
FIBRINOGEN	mg/dl	121	216	0	500	5	5
GAMMA GLOBULIN (ELECTROPHORESIS)	g/dl	1.6	1.4	0.7	3.2	3	3
ALBUMIN (ELECTROPHORESIS)	g/dl	3.0	0.5	2.7	3.3	2	2
ALPHA-1 GLOBULIN (ELECTROPHORESIS)	mg/dl	330.7	53.4	295.0	392.0	3	3
ALPHA-2 GLOBULIN (ELECTROPHORESIS)	mg/dl	481.3	6.5	475.0	488.0	3	3
BETA GLOBULIN (ELECTROPHORESIS)	mg/dl	583.3	80.4	497.0	656.0	3	3
CORTISOL	µg/dl	8.8	0.0	8.8	8.8	1	1

PROGESTERONE	ng/dl	38.90	0.000	38.90	38.90	1	1
TOTAL TRIIODOTHYRONINE	ng/ml	135.0	7.9	126.0	141.0	3	2
TOTAL THYROXINE	µg/dl	1.7	0.3	1.1	2.0	6	6
Body Temperature:	°F	101.7	1.8	96.8	105.8	151	75

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

COYOTE, *Canis latrans*
Physiological reference ranges calculated for:

- Both sexes combined
- All ages combined

Sample results submitted by 15 member institutions.

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	9.286	3.992	4.400	25.00	59	25
RED BLOOD CELL COUNT	*10^6/µl	13.36	20.60	2.32	72.50	34	22
HEMOGLOBIN	g/dl	14.9	2.4	10.9	20.8	52	27
HEMATOCRIT	%	45.8	7.6	31.0	66.0	63	29
MCV	fL	68.6	37.9	6.9	245.7	34	22
MCH	pg/cell	23.7	14.1	2.3	89.7	33	22
MCHC	g/dl	33.3	3.5	25.5	47.7	52	27
PLATELET COUNT	*10^3/µl	292	66	162	383	14	9
NUCLEATED RED BLOOD CELLS	/100 WBC	0	0	0	1	6	4
RETICULOCYTES	%	1.0	0.6	0.2	1.7	15	6
SEGMENTED NEUTROPHILS	*10^3/µl	6.287	3.033	2.500	17.50	54	23
LYMPHOCYTES	*10^3/µl	1.557	1.110	0.325	7.000	53	22
MONOCYTES	*10^3/µl	0.435	0.323	0.058	1.170	47	20
EOSINOPHILS	*10^3/µl	1.036	0.856	0.150	4.030	51	22
BASOPHILS	*10^3/µl	0.014	0.031	0.000	0.099	15	5
NEUTROPHILIC BANDS	*10^3/µl	0.194	0.291	0.000	1.250	20	9
CALCIUM	mg/dl	9.6	0.8	7.5	11.5	48	24
PHOSPHORUS	mg/dl	3.8	1.4	1.7	7.6	47	25
SODIUM	mEq/L	147	4	138	157	42	21
POTASSIUM	mEq/L	4.6	0.6	3.5	6.6	42	20
CHLORIDE	mEq/L	115	3	108	120	34	18

BICARBONATE	mEq/L	19.5	2.5	15.0	23.0	10	5
CARBON DIOXIDE	mEq/L	20.1	2.4	17.0	27.0	18	12
OSMOLARITY	mOsmol/L	311	6	307	317	3	3
IRON	µg/dl	183	80	87	307	7	3
MAGNESIUM	mg/dl	2.05	0.07	2.00	2.10	2	2
BLOOD UREA NITROGEN	mg/dl	23	10	8	51	53	26
CREATININE	mg/dl	1.2	0.4	0.7	2.2	53	25
URIC ACID	mg/dl	1.3	3.5	0.0	13.4	14	6
TOTAL BILIRUBIN	mg/dl	0.2	0.2	0.0	0.7	44	25
DIRECT BILIRUBIN	mg/dl	0.0	0.1	0.0	0.3	13	7
INDIRECT BILIRUBIN	mg/dl	0.1	0.1	0.0	0.2	13	7
GLUCOSE	mg/dl	122	33	57	231	52	26
CHOLESTEROL	mg/dl	155	53	0	242	41	25
TRIGLYCERIDE	mg/dl	33	13	15	70	18	12
CREATINE PHOSPHOKINASE	IU/L	380	337	82	1264	30	18
LACTATE DEHYDROGENASE	IU/L	161	89	63	451	18	9
ALKALINE PHOSPHATASE	IU/L	43	37	7	209	50	24
ALANINE AMINOTRANSFERASE	IU/L	47	21	15	133	49	24
ASPARTATE AMINOTRANSFERASE	IU/L	55	22	23	114	33	20
GAMMA GLUTAMYLTRANSFERASE	IU/L	4	4	0	14	19	12
AMYLASE	U/L	436	314	182	1497	23	16
LIPASE	U/L	305	226	113	693	8	6
TOTAL PROTEIN (COLORIMETRY)	g/dl	6.2	0.8	4.8	8.2	43	24
GLOBULIN (COLORIMETRY)	g/dl	2.8	0.7	1.5	4.3	40	24
ALBUMIN (COLORIMETRY)	g/dl	3.3	0.4	2.5	4.1	46	25
TOTAL THYROXINE	µg/dl	0.7	0.3	0.4	0.9	3	2
Body Temperature:	°F	100.9	1.1	100.4	104.0	23	15

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

DHOLE, *Cuon alpinus*
Physiological reference ranges calculated for:

- Both sexes combined
- All ages combined

Sample results submitted by 3 member institutions.

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	9.087	2.765	5.650	15.30	12	8
RED BLOOD CELL COUNT	*10^6/µl	10.51	1.60	7.64	12.40	9	8
HEMOGLOBIN	g/dl	15.6	2.0	12.3	18.2	12	8
HEMATOCRIT	%	46.4	7.7	32.5	55.6	12	8
MCV	fL	44.6	7.3	26.2	51.2	9	8
MCH	pg/cell	14.5	1.2	13.1	16.1	9	8
MCHC	g/dl	34.2	5.8	29.3	50.2	12	8
PLATELET COUNT	*10^3/µl	541	0	541	541	1	1
RETICULOCYTES	%	0.6	0.6	0.1	1.0	2	1
SEGMENTED NEUTROPHILS	*10^3/µl	5.784	1.629	4.030	9.110	10	6
LYMPHOCYTES	*10^3/µl	1.990	0.952	0.735	3.430	10	6
MONOCYTES	*10^3/µl	0.231	0.147	0.063	0.452	10	6
EOSINOPHILS	*10^3/µl	0.268	0.145	0.075	0.410	9	6
NEUTROPHILIC BANDS	*10^3/µl	0.086	0.039	0.058	0.113	2	2
ERYTHROCYTE SEDIMENTATION RATE	mm/Hr	0	0	0	0	2	1
CALCIUM	mg/dl	10.0	0.2	9.6	10.4	7	3
PHOSPHORUS	mg/dl	4.7	1.0	3.2	6.2	7	3
SODIUM	mEq/L	149	3	145	153	7	3
POTASSIUM	mEq/L	4.5	0.2	4.3	4.8	7	3
CHLORIDE	mEq/L	115	3	111	121	7	3
BICARBONATE	mEq/L	17.5	2.2	15.1	20.0	5	2
BLOOD UREA NITROGEN	mg/dl	35	12	22	60	9	5
CREATININE	mg/dl	1.1	0.2	0.8	1.5	9	5
URIC ACID	mg/dl	0.3	0.0	0.3	0.3	1	1
TOTAL BILIRUBIN	mg/dl	0.1	0.1	0.0	0.2	7	3
GLUCOSE	mg/dl	136	39	95	193	7	3
CHOLESTEROL	mg/dl	221	58	150	296	7	3

TRIGLYCERIDE	mg/dl	85	13	76	94	2	1
CREATINE PHOSPHOKINASE	IU/L	148	50	75	192	5	2
LACTATE DEHYDROGENASE	IU/L	52	0	52	52	1	1
ALKALINE PHOSPHATASE	IU/L	41	15	25	71	7	3
ALANINE AMINOTRANSFERASE	IU/L	82	45	16	139	9	5
ASPARTATE AMINOTRANSFERASE	IU/L	49	19	25	79	9	5
GAMMA GLUTAMYLTRANSFERASE	IU/L	3	1	2	3	2	1
AMYLASE	U/L	1184	363	654	1556	7	3
LIPASE	U/L	230	84	162	371	5	2
TOTAL PROTEIN (COLORIMETRY)	g/dl	6.3	0.3	5.8	6.7	7	3
GLOBULIN (COLORIMETRY)	g/dl	2.9	0.3	2.6	3.5	7	3
ALBUMIN (COLORIMETRY)	g/dl	3.4	0.3	3.1	4.0	7	3
Body Temperature:	°F	100.8	2.5	96.8	104.0	9	5

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

DOMESTIC DOG, *Canis lupus familiaris*
Physiological reference ranges calculated for:

- Both sexes combined
- All ages combined

Sample results submitted by 15 member institutions.

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	10.36	3.146	4.760	24.20	303	97
RED BLOOD CELL COUNT	*10^6/µl	6.60	0.78	2.48	9.08	272	92
HEMOGLOBIN	g/dl	16.1	1.8	10.8	20.8	267	86
HEMATOCRIT	%	45.6	5.0	31.7	61.0	301	93
MCV	fL	68.4	4.8	56.5	85.4	265	87
MCH	pg/cell	24.3	1.4	19.7	28.5	266	86
MCHC	g/dl	35.6	2.0	27.2	42.3	264	85
PLATELET COUNT	*10^3/µl	287	112	84	706	106	54
NUCLEATED RED BLOOD CELLS	/100 WBC	2	2	0	6	21	19
SEGMENTED NEUTROPHILS	*10^3/µl	6.695	2.586	2.350	20.80	292	96
LYMPHOCYTES	*10^3/µl	2.627	1.302	0.276	9.180	298	96
MONOCYTES	*10^3/µl	0.448	0.379	0.048	4.010	270	92

EOSINOPHILS	*10^3/µl	0.610	0.473	0.052	3.792	286	91
BASOPHILS	*10^3/µl	0.222	0.236	0.011	1.002	18	16
NEUTROPHILIC BANDS	*10^3/µl	0.265	0.287	0.052	1.710	91	50
CALCIUM	mg/dl	10.2	0.6	8.6	12.1	250	85
PHOSPHORUS	mg/dl	4.1	0.9	2.2	7.0	244	84
SODIUM	mEq/L	145	3	134	154	236	82
POTASSIUM	mEq/L	4.5	0.4	3.6	5.6	237	81
CHLORIDE	mEq/L	114	4	102	129	236	82
BICARBONATE	mEq/L	20.3	1.5	19.0	22.0	3	2
CARBON DIOXIDE	mEq/L	23.1	3.2	16.0	31.0	52	22
OSMOLARITY	mOsmol/L	295	0	295	295	1	1
IRON	µg/dl	176	68	68	295	12	10
MAGNESIUM	mg/dl	2.06	0.28	1.58	2.40	8	8
BLOOD UREA NITROGEN	mg/dl	17	6	7	39	254	88
CREATININE	mg/dl	1.1	0.3	0.6	2.0	78	32
URIC ACID	mg/dl	0.6	0.4	0.1	1.8	60	36
TOTAL BILIRUBIN	mg/dl	0.3	0.2	0.1	0.9	137	64
DIRECT BILIRUBIN	mg/dl	0.1	0.1	0.0	0.4	23	16
INDIRECT BILIRUBIN	mg/dl	0.2	0.2	0.1	0.9	17	11
GLUCOSE	mg/dl	96	15	52	145	250	87
CHOLESTEROL	mg/dl	214	58	93	436	246	85
TRIGLYCERIDE	mg/dl	86	77	22	487	186	78
CREATINE PHOSPHOKINASE	IU/L	131	82	27	570	166	61
LACTATE DEHYDROGENASE	IU/L	147	180	24	1211	56	34
ALKALINE PHOSPHATASE	IU/L	67	45	12	352	250	86
ALANINE AMINOTRANSFERASE	IU/L	47	24	13	181	243	84
ASPARTATE AMINOTRANSFERASE	IU/L	36	12	13	89	250	85
GAMMA GLUTAMYLTRANSFERASE	IU/L	7	5	0	19	134	59
AMYLASE	U/L	676	271	231	1804	108	55
LIPASE	U/L	379	379	95	1380	11	8
TOTAL PROTEIN (COLORIMETRY)	g/dl	6.3	0.6	4.7	7.9	253	86
GLOBULIN (COLORIMETRY)	g/dl	3.3	0.6	2.0	5.0	124	64
ALBUMIN (COLORIMETRY)	g/dl	3.2	0.4	1.9	4.4	122	64
FIBRINOGEN	mg/dl	0	0	0	1	56	23

CORTISOL	µg/dl	8.4	13.8	0.6	29.0	4	3
PROGESTERONE	ng/dl	19.80	0.000	19.80	19.80	1	1
TOTAL TRIIODOTHYRONINE	ng/ml	0.5	0.0	0.5	0.5	1	1
FREE TRIIODOTHYRONINE	pg/ml	4.7	1.4	3.7	5.7	2	1
TOTAL THYROXINE	µg/dl	137.8	255.5	1.2	645.0	8	5
Body Temperature:	°F	100.6	1.6	98.6	104.0	14	10

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

GRAY WOLF, Canis lupus
Physiological reference ranges calculated for:

- Both sexes combined
- All ages combined

Sample results submitted by 52 member institutions.

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	9.710	3.177	3.500	24.10	564	183
RED BLOOD CELL COUNT	*10^6/µl	7.04	1.34	3.43	9.99	391	141
HEMOGLOBIN	g/dl	16.4	3.3	7.9	25.2	416	143
HEMATOCRIT	%	48.1	8.4	23.0	68.0	572	184
MCV	fL	70.0	6.6	40.2	100.3	363	136
MCH	pg/cell	24.1	2.0	10.2	35.0	355	132
MCHC	g/dl	33.6	3.3	17.6	51.6	390	138
PLATELET COUNT	*10^3/µl	260	89	102	595	140	75
NUCLEATED RED BLOOD CELLS	/100 WBC	1	1	0	3	79	40
RETICULOCYTES	%	0.7	0.7	0.0	2.1	24	14
SEGMENTED NEUTROPHILS	*10^3/µl	7.023	2.703	1.890	20.70	542	178
LYMPHOCYTES	*10^3/µl	1.599	0.830	0.074	5.670	560	182
MONOCYTES	*10^3/µl	0.481	0.361	0.000	2.381	490	167
EOSINOPHILS	*10^3/µl	0.702	0.767	0.000	5.973	501	169
BASOPHILS	*10^3/µl	0.072	0.073	0.000	0.336	62	39
NEUTROPHILIC BANDS	*10^3/µl	0.292	0.378	0.000	1.950	128	68
ERYTHROCYTE SEDIMENTATION RATE	mm/Hr	0	0	0	0	1	1
CALCIUM	mg/dl	9.9	0.8	7.1	12.5	489	168

PHOSPHORUS	mg/dl	3.8	1.6	1.1	10.1	427	152
SODIUM	mEq/L	148	5	128	182	428	148
POTASSIUM	mEq/L	4.6	0.5	2.9	6.8	435	149
CHLORIDE	mEq/L	115	5	96	134	402	134
BICARBONATE	mEq/L	19.2	3.2	13.0	25.0	45	20
CARBON DIOXIDE	mEq/L	19.3	3.5	11.0	30.0	130	63
OSMOLARITY	mOsmol/L	305	8	292	322	42	23
IRON	µg/dl	140	44	43	264	102	27
MAGNESIUM	mg/dl	1.68	0.21	1.20	2.40	48	28
BLOOD UREA NITROGEN	mg/dl	22	8	6	72	500	174
CREATININE	mg/dl	1.1	0.4	0.3	4.2	494	170
URIC ACID	mg/dl	0.4	0.4	0.0	3.3	158	52
TOTAL BILIRUBIN	mg/dl	0.2	0.2	0.0	1.6	423	148
DIRECT BILIRUBIN	mg/dl	0.1	0.1	0.0	0.5	139	60
INDIRECT BILIRUBIN	mg/dl	0.2	0.2	0.0	1.6	138	60
GLUCOSE	mg/dl	123	31	47	305	496	171
CHOLESTEROL	mg/dl	181	63	56	447	472	163
TRIGLYCERIDE	mg/dl	55	44	10	381	231	77
CREATINE PHOSPHOKINASE	IU/L	245	196	32	1244	241	109
LACTATE DEHYDROGENASE	IU/L	160	152	14	778	206	71
ALKALINE PHOSPHATASE	IU/L	42	44	0	232	471	167
ALANINE AMINOTRANSFERASE	IU/L	55	28	13	189	484	167
ASPARTATE AMINOTRANSFERASE	IU/L	48	24	13	182	454	154
GAMMA GLUTAMYLTRANSFERASE	IU/L	4	3	0	28	244	93
AMYLASE	U/L	374	187	100	1190	189	92
LIPASE	U/L	218	217	22	1271	122	62
TOTAL PROTEIN (COLORIMETRY)	g/dl	6.1	0.7	4.1	8.7	471	165
GLOBULIN (COLORIMETRY)	g/dl	2.7	0.7	1.2	5.1	421	148
ALBUMIN (COLORIMETRY)	g/dl	3.4	0.4	2.2	4.7	424	150
FIBRINOGEN	mg/dl	67	58	0	100	3	2
ALPHA-1 GLOBULIN (ELECTROPHORESIS)	mg/dl	0.2	0.0	0.2	0.2	1	1
ALPHA-2 GLOBULIN (ELECTROPHORESIS)	mg/dl	0.4	0.0	0.4	0.4	1	1
CORTISOL	µg/dl	12.5	0.0	12.5	12.5	1	1

PROGESTERONE	ng/dl	1.600	0.424	1.300	1.900	2	2
TOTAL TRIIODOTHYRONINE	ng/ml	91.6	48.1	31.0	197.0	19	11
FREE TRIIODOTHYRONINE	pg/ml	3.0	0.0	3.0	3.0	1	1
TOTAL THYROXINE	µg/dl	1.6	1.1	0.3	6.3	35	20
Body Temperature:	°F	102.0	2.2	96.8	108.7	248	91

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

MANED WOLF, *Chrysocyon brachyurus*
Physiological reference ranges calculated for:

- Both sexes combined
- All ages combined

Sample results submitted by 27 member institutions.

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	10.36	4.008	3.700	26.50	556	127
RED BLOOD CELL COUNT	*10^6/µl	5.43	0.95	2.89	9.73	472	113
HEMOGLOBIN	g/dl	13.6	2.3	7.6	23.2	452	119
HEMATOCRIT	%	40.9	6.5	23.5	68.5	579	132
MCV	fL	75.3	7.4	42.1	109.0	467	113
MCH	pg/cell	25.5	2.4	16.7	34.4	437	111
MCHC	g/dl	33.5	2.4	21.9	46.8	449	119
PLATELET COUNT	*10^3/µl	219	86	78	690	100	46
NUCLEATED RED BLOOD CELLS	/100 WBC	1	1	0	3	46	33
RETICULOCYTES	%	1.3	0.8	0.0	2.7	15	9
SEGMENTED NEUTROPHILS	*10^3/µl	7.296	3.251	0.083	23.10	516	119
LYMPHOCYTES	*10^3/µl	2.150	1.115	0.005	6.540	524	121
MONOCYTES	*10^3/µl	0.333	0.280	0.000	2.049	462	117
EOSINOPHILS	*10^3/µl	0.622	0.454	0.000	3.120	484	117
BASOPHILS	*10^3/µl	0.109	0.119	0.000	0.582	78	49
NEUTROPHILIC BANDS	*10^3/µl	0.419	0.834	0.000	6.210	134	65
ERYTHROCYTE SEDIMENTATION RATE	mm/Hr	0	0	0	1	12	7
CALCIUM	mg/dl	9.7	0.7	6.6	12.5	517	124
PHOSPHORUS	mg/dl	5.8	2.6	2.4	12.7	423	108

SODIUM	mEq/L	145	4	134	156	447	112
POTASSIUM	mEq/L	4.8	0.5	3.4	6.9	450	115
CHLORIDE	mEq/L	114	4	87	130	416	99
BICARBONATE	mEq/L	19.4	3.7	14.0	24.0	10	7
CARBON DIOXIDE	mEq/L	18.7	2.7	13.0	26.0	77	39
OSMOLARITY	mOsmol/L	297	13	275	322	9	7
IRON	µg/dl	121	47	43	278	40	14
MAGNESIUM	mg/dl	3.80	4.06	1.30	12.50	7	5
BLOOD UREA NITROGEN	mg/dl	23	9	6	58	517	128
CREATININE	mg/dl	1.3	0.4	0.4	2.6	380	118
URIC ACID	mg/dl	0.3	0.3	0.0	1.5	130	59
TOTAL BILIRUBIN	mg/dl	0.3	0.2	0.0	0.9	401	113
DIRECT BILIRUBIN	mg/dl	0.1	0.1	0.0	0.6	93	33
INDIRECT BILIRUBIN	mg/dl	0.2	0.1	0.0	0.6	71	28
GLUCOSE	mg/dl	114	25	51	207	518	128
CHOLESTEROL	mg/dl	289	82	0	526	464	107
TRIGLYCERIDE	mg/dl	42	32	0	167	310	77
CREATINE PHOSPHOKINASE	IU/L	318	317	33	1698	189	65
LACTATE DEHYDROGENASE	IU/L	156	140	24	880	258	76
ALKALINE PHOSPHATASE	IU/L	137	191	5	2046	511	123
ALANINE AMINOTRANSFERASE	IU/L	78	72	0	605	456	120
ASPARTATE AMINOTRANSFERASE	IU/L	43	21	9	214	498	121
GAMMA GLUTAMYLTRANSFERASE	IU/L	5	3	0	12	231	67
AMYLASE	U/L	416	331	74	2218	277	77
LIPASE	U/L	173	77	12	367	32	18
TOTAL PROTEIN (COLORIMETRY)	g/dl	6.2	0.8	4.0	8.9	472	120
GLOBULIN (COLORIMETRY)	g/dl	3.2	0.7	1.8	5.0	353	100
ALBUMIN (COLORIMETRY)	g/dl	3.0	0.4	1.9	4.5	353	100
FIBRINOGEN	mg/dl	96	110	0	400	78	30
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	99.9	1.4	96.8	104.0	371	90

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

RED WOLF, Canis rufus
Physiological reference ranges calculated for:

- Both sexes combined
- All ages combined

Sample results submitted by 17 member institutions.

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	11.08	3.422	4.630	24.00	333	93
RED BLOOD CELL COUNT	*10^6/µl	6.51	1.06	3.92	9.75	464	96
HEMOGLOBIN	g/dl	16.5	2.5	9.5	21.9	472	100
HEMATOCRIT	%	48.7	8.0	27.7	67.6	489	100
MCV	fL	74.7	5.9	42.4	133.6	461	96
MCH	pg/cell	25.4	1.5	14.9	38.0	461	96
MCHC	g/dl	34.1	2.0	28.2	50.0	467	100
PLATELET COUNT	*10^3/µl	280	84	101	630	283	56
NUCLEATED RED BLOOD CELLS	/100 WBC	1	1	0	5	100	44
RETICULOCYTES	%	2.1	3.1	0.5	9.6	8	5
SEGMENTED NEUTROPHILS	*10^3/µl	7.159	2.335	0.248	16.20	324	90
LYMPHOCYTES	*10^3/µl	2.442	1.629	0.044	9.210	329	90
MONOCYTES	*10^3/µl	0.534	0.385	0.007	4.032	305	80
EOSINOPHILS	*10^3/µl	0.938	0.828	0.002	5.580	319	88
BASOPHILS	*10^3/µl	0.091	0.181	0.002	1.044	52	31
NEUTROPHILIC BANDS	*10^3/µl	0.157	0.095	0.064	0.370	14	11
ERYTHROCYTE SEDIMENTATION RATE	mm/Hr	1	2	0	8	95	17
CALCIUM	mg/dl	9.7	1.1	0.0	12.9	470	100
PHOSPHORUS	mg/dl	4.2	1.8	0.0	10.0	445	98
SODIUM	mEq/L	148	8	0	166	436	91
POTASSIUM	mEq/L	5.1	0.6	0.0	6.8	431	90
CHLORIDE	mEq/L	115	8	0	131	362	78
BICARBONATE	mEq/L	19.5	2.6	15.0	24.0	11	5
CARBON DIOXIDE	mEq/L	18.0	5.7	0.0	70.0	256	49
OSMOLARITY	mOsmol/L	289	3	284	293	5	5

IRON	µg/dl	120	60	36	306	31	12
MAGNESIUM	mg/dl	1.42	0.38	0.53	1.90	42	13
BLOOD UREA NITROGEN	mg/dl	21	6	0	45	466	100
CREATININE	mg/dl	1.2	0.3	0.0	2.1	466	99
URIC ACID	mg/dl	0.4	0.3	0.0	1.2	46	21
TOTAL BILIRUBIN	mg/dl	0.2	0.2	0.0	0.9	423	98
DIRECT BILIRUBIN	mg/dl	0.0	0.0	0.0	0.1	39	19
INDIRECT BILIRUBIN	mg/dl	0.2	0.1	0.0	0.5	39	19
GLUCOSE	mg/dl	125	31	0	221	465	100
CHOLESTEROL	mg/dl	141	48	0	336	387	85
TRIGLYCERIDE	mg/dl	44	25	10	177	112	35
CREATINE PHOSPHOKINASE	IU/L	268	230	0	1743	231	48
LACTATE DEHYDROGENASE	IU/L	320	273	0	987	58	31
ALKALINE PHOSPHATASE	IU/L	52	62	0	275	438	97
ALANINE AMINOTRANSFERASE	IU/L	54	25	0	161	438	99
ASPARTATE AMINOTRANSFERASE	IU/L	56	32	0	365	415	85
GAMMA GLUTAMYLTRANSFERASE	IU/L	4	2	0	10	266	54
AMYLASE	U/L	398	119	0	828	293	65
LIPASE	U/L	222	95	67	529	205	36
TOTAL PROTEIN (COLORIMETRY)	g/dl	6.1	0.9	3.6	8.4	310	88
GLOBULIN (COLORIMETRY)	g/dl	2.9	0.6	1.5	5.1	409	90
ALBUMIN (COLORIMETRY)	g/dl	3.2	0.5	1.7	4.5	411	91
FIBRINOGEN	mg/dl	159	79	0	400	76	13
PROGESTERONE	ng/dl	20.26	13.06	1.010	36.90	5	4
TOTAL TRIIODOTHYRONINE	ng/ml	90.0	45.3	58.0	122.0	2	2
TOTAL THYROXINE	µg/dl	1.2	0.5	0.7	1.8	3	3
Body Temperature:	°F	101.8	1.8	98.6	107.6	123	48

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

Appendix K: Sample Animal Acquisition and Disposition Forms

Sample Animal Acquisition Form

Date Acquired: (dd/mm/yy)	Date of Pre-Acquisition: (dd/mm/yy)	Accession # (*filled in by Registrar*)

Species Information

Scientific Name: **Common Name**:

Sex:0.0.0 **Age**: **Parent ID # - Dam**: **Sire**:

Acquisition Information

Acquisition Type: _____ Birth _____ Donation _____ Purchase _____ Trade

_____ Breeding Loan _____ Loan Transfer _____Wild Caught

_____ Other (Please Note)

Comments:

Acquired From: (Please fill in **all** lines)

Name:

Address:

City: State: Zip:

Phone: USDA # or
State Drivers License if private vendor

Authorization/Administrative Approval

Submitted by: Date:
(Area. Keeper)

Registrar: _____ Date: _____
Veterinarian: _____ Date: _____
General Curator: _____ Date: _____
Deputy Director: _____ Date: _____
Director: _____ Date: _____

Sample Animal Disposition Form

Date of Disposition: (dd/mm/yy)	Date of Pre-Disposition: (dd/mm/yy)	Accession #

Species Information

Scientific Name: Common Name:

Sex: 0.0.0 Age: Parent ID # - Dam: Sire:

Disposition Information

Disposition Type: ____ Death _____ Donation ____ Sale _____ Trade

____ Breeding Loan _____ Breeding Returned _____ Loan Transfer

____ Euthanize _____ Escape _____ Other (please note)

Comments:

Shipped To: (Please fill in **all** lines)

Name:

Address:

City: State: Zip:

Phone: USDA# or
State Drivers License Number

Authorization/Administrative Approval

Submitted By: Date:
(Area. Keeper)

Registrar: _____ Date: _____
Veterinarian: _____ Date: _____
General Curator: _____ Date: _____
Deputy Director: _____ Date: _____
Director: _____ Date: _____

Appendix L: Maned wolf pup development and hand-rear protocol (Maned Wolf SSP, 2007)

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 second 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 begin 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 AZA MWSSP recommends that pups be separated from parents by 10 months of age, prior to the subsequent breeding season.

Hand-Rearing

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 unsuccessful with regard to both reproduction and neonatal mortality. By 1989, the age structure of the managed 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 AZA 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 in 1989. Several members of the AZA MWSSP assisted in preparing updates and revisions. 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 (Lincoln Park Zoological Society, 1993). In addition to the Lincoln Park litter, the booklet also summarizes growth and feeding information for maned wolves hand-reared at six AZA 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 AZA 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 AZA 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 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 AZA 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 AZA SSP Coordinator. The AZA SSP 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 AZA Red wolf and AZA 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 AZA 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. 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 should 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 AZA 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 domestic dog replacement formula is the recommended formula for hand rearing maned wolves. See AZA SSP Coordinator for specific product recommendations. It is also recommended that an enzyme 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). See AZA SSP Coordinator for specific product recommendations. Formula should be diluted with sterile water or an electrolyte replacement for the first several days. Liquid formula 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 100 g of formula, mix 10 g powder with 90 g 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. See the AZA SSP Coordinator for specific product recommendations.

First feeding: It is recommended that the first bottle offered to pups contain 5% dextrose or other electrolyte replacer 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 Maned Wolf Diaries: Growth and Development of Hand-Reared Pups (Lincoln Park

Zoological Society, 1993) 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, commercial brand beef baby food, and pureed calf liver have all been successfully added to bottled formula. See the AZA SSP Coordinator for specific product recommendations.

Weaning: The AZA 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 29.4°C (85°F) 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.

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Appendix M: Mexican wolf hand-rearing guidelines (Mexican Wolf SSP, 2009)

As a rule, wolf pups are only removed for hand-rearing in extraordinary circumstances and with prior approval of the USFWS. The genetic value of the pups should greatly outweigh the domesticating influences of hand-rearing.

Whenever possible, cross-fostering of pups would be preferred to pulling pups for hand-rearing. In cross-fostering, pups would be placed with those being cared for by a competent proven female at the same institution or another institution with a suitable match. Consultation with and approval from the USFWS Mexican Wolf Recovery Coordinator and AZA MWSSP Coordinator are required as with hand-rearing.

If the pups are removed before they have suckled, they will not have received any passive immunity from their mother's first milk. The pups need to be provided with passive immunity, and this can be done in two ways. First, the mother can be milked for her colostrum which can then be fed directly to the pups. Second, 3cc to 5cc of mother's blood serum can be injected subcutaneously to each pup. The serum can be administered a few days after they have received their colostrum or twice the first week if colostrum is not available.

The pups should be fed an unmodified domestic dog milk replacer formula. See the AZA SSP Coordinator for specific product recommendations. The amount of formula fed per twenty-four hour period should total about 20% of the pup's body weight. For example, a 700 g (24.5 oz) pup should receive 140 g (4.9 oz) or 140 ml (4.2 oz) of formula, divided into several evenly spaced feedings, per twenty-four hour period. A regular human infant nipple works well. Heat only the amount measured for each feeding to body temperature. At three weeks of age begin to offer milk-soaked puppy chow, and then wean them from the bottle.

Until the pups are at least twenty-one days old, they should be kept in an environment above 29°C (85°F). A pup's rectal temperature should be about 37.8°- 38.3°C (100°-101°F).

Document the feeding schedule, the amounts of formula offered and taken at each feeding, the stool condition, and daily weights. Vaccinate according to schedule.

On the few occasions when hand-rearing was necessary in the AZA Mexican Wolf SSP, the pup(s) were placed with a suitable wolf companion at as early an age as possible. Socialization to humans is very undesirable and not compatible with the goals of the recovery program. Recommendations may include shipping pups to a facility where they could be housed with age mates or a suitable older foster parent wolf. Close communication with the USFWS Mexican Wolf Recovery Coordinator and AZA MWSSP Coordinator is required throughout this process.

APPROXIMATE BODY WEIGHTS

AGE	♀ (kg)	♀ (lb)	♂ (kg)	♂ (lb)
5 days	0.9 kg	1.98 lb	0.9 kg	1.98 lb
21 days	1.8 kg	3.97 lb	1.8 kg	3.97 lb
45 days	3.9 kg	8.6 lb	3.9 kg	8.6 lb
2 months	5 kg	11.0 lb	5 kg	11.0 lb
3 months	10 kg	22.0 lb	10 kg	22.0 lb
4 months	15 kg	33.1 lb	15 kg	33.1 lb
10 months	27 kg	59.5 lb	30 kg	66.1 lb
2+ years	28 kg	61.7 lb	34 kg	75.0 lb